All-Optical Imaging & Stimulation Tools for In Vivo Neuroscience

Introduction

A long-standing objective in neuroscience has been elucidating how neural activity relates to diverse brain functions in vivo. Researchers have attempted to address this by developing a wide-range of all-optical tools for imaging and manipulating neural activity simultaneously in freely-moving animals\(^1\). To be viable for in vivo neuroscience research, these techniques necessitate high temporal resolution to image and manipulate neuronal firing at physiological speed, and high spatial resolution to image and manipulate specific neurons or regions\(^1,2\). These are the technical standards set for these tools.

Researchers initiated in vivo recording using a brain implanted electrode to monitor detailed changes in electrical signals with high temporal resolution\(^2\). However, the drawback of this tool is the inability to differentiate between or identify cell types. Calcium imaging has since emerged to supply researchers with a unique tool to image the activity of many individual cells or subcellular processes\(^3,4\) (see Figure 1). Calcium indicators detect the influx of calcium by emitting a fluorescent signal, which is used as an indirect indicator of cellular activity\(^3,4\). Genetically encoded calcium indicators (GECIs) have allowed researchers to image the activity of select cells, and recently developed GECIs can detect single action potentials in low-spiking cells, such as excitatory pyramidal cells\(^3,4\). Thus, calcium imaging provides an optical method for imaging cellular and sub-cellular activity in relation to specific events with high spatial resolution.

On the front of manipulating neural activity, pharmacological methods have traditionally been a key tool for upregulating or downregulating activity by targeting explicit neurotransmitter systems\(^5\). However, this method does not provide sufficient temporal or spatial resolution for well-tuned manipulations due to long-lasting effects and non-specific cellular binding\(^5\). Designer Receptors Exclusively activated by Designer Drugs (DREADDs) enables exclusive activation of specific cell types by genetically expressing ligand-specific excitatory or inhibitory receptors to provide cell-specific targeting\(^6\). Although, this method also suffers from minimal temporal control and is more suited for long-term excitation or inhibition. Alternatively, the emergence of optogenetics has allowed researchers to turn select cells on or off with millisecond precision and high spatial resolution\(^7\). Specific cells are activated or inhibited by genetically expressed light-sensitive channels that are stimulated by specific wavelengths of light\(^7\). Optogenetics has revolutionized neuroscience by providing researchers with the ability to optically control explicit neural activity during behavioural events (e.g. lever pressing).

Calcium imaging and optogenetics have begun to provide researchers with meaningful insight into explicit brain functions related to cell-specific activity. The unification of these techniques for all-optical imaging and stimulation in vivo will help characterize the precise involvement of specific neurons in sensory, behavioural, cognitive, and/or cortical functions\(^1,8\). Significantly, all-optical systems have the potential for real-time closed-loop control of neural activity\(^1,9\). Currently, there are only a few tools available in the market that can perform simultaneous calcium imaging and optogenetics in vivo, these include (1) two-photon imaging, (2) head-mounted microscopes, (3) fiber photometry, and (4) optical fiberscopes.

This white paper will provide an overview of the all-optical tools mentioned above, and compare the advantages and disadvantages of these techniques. As well, this paper will discuss how these tools utilize calcium imaging and optogenetics for in vivo neuroscience research and how these tools can help researchers understand the direct relationship between specific neural activity and diverse brain functions.

Figure 1. GCaMP6 images taken from the striatum of a mouse with Mightex’s OASIS Implant.
Two Photon Imaging

Two-photon microscopy initiated the ability to image neural activity in the brain of a live head-fixed animal with high resolution \(^{10-12}\) (see Figure 2). The high resolution of two-photon microscopy allows for sub-cellular processes (e.g. dendritic spines) to be imaged \(^{10-12}\). Two-photon microscopy provides better penetration and less scattering in deeper tissue (~800 µm), compared to one-photon microscopy \(^{12}\). The imaging depth of two-photon imaging is limited to shallow brain regions and cannot image deep brain regions. However, the coupling of a brain implanted gradient index (GRIN) lens can overcome this limitation, enabling two-photon microscopy to relay light deep brain regions and image activity \(^{13}\).

The high resolution of two-photon imaging is associated with a small field of view (~1mm\(^2\)) and slower image acquisition rates that affect overall imaging capabilities \(^{12}\). However, a recent publication expanded the field of view (>9mm\(^2\)) of two-photon imaging using a custom setup with two imaging beams, and this provided the ability to simultaneously image two brain regions \(^{13}\). Furthermore, two-photon imaging also has simultaneous optogenetic and imaging capabilities \(^{11,15}\). Targeted optogenetic stimulation is possible, but laser scanning limits this with slow frame rates and point to point stimulation, rather than simultaneous multiple region stimulation which is often preferred when manipulating multiple regions of interest \(^{12}\).

Two-photon imaging is restricted to a head-fixed setup and does not allow a rodent to be freely-moving. This technique can be sufficient for certain applications, but not for freely-moving behavioural examinations. This limitation can be partially overcome with the use of virtual reality technology to mimic a natural environment \(^{11,16}\). Although, whether virtual reality can reliably replicate freely-moving behaviour is not well studied and this may induce unnecessary stress for the animal that can interfere with behaviour.

This significant tool has provided researchers with a well-established method for studying the activity of minute cellular processes related to diverse brain functions in head-fixed animals.

Head-Mounted Microscope

The construction of the head-mounted microscope unlocked the ability to image the activity of thousands of neurons in freely moving animals (see Figure 3). This tool integrates all the necessary technology for in vivo imaging into a miniaturized one-photon microscopy unit that can be worn by a behaving rodent \(^{17,18}\). The coupling of the head-mounted microscope with a brain implanted GRIN lens allows this device to relay light to and image deep brain regions \(^{17,18}\).

This technology provides users with the ability to image the activity of numerous neurons simultaneously during specific behaviours. The head-mounted microscope field of view is determined by the size of the implanted GRIN lens (ranging 0.5mm to 1mm diameter) \(^{19}\). Furthermore, these microscopes do not have dendritic imaging resolution, due to low-level cameras with low-sensitivity and high noise, and the number of wavelengths they can illuminate is currently restricted to one or two \(^{17,18,20,21}\). The integrated components are constrained by the miniaturized design of the system, which allows the animal to be freely-moving. This design partially limits the capabilities of this system and the flexibility for future updates, such as additional wavelengths. Moreover, a recent development in head-mounted microscope technology enables this tool to perform dual-colour imaging or optogenetic stimulation, but this is restricted to wide-field optogenetics with no targeted stimulation capabilities \(^{20,21}\).

The major benefit of the introducing the head-mounted microscope is freely-behaving imaging capabilities, which is not possible with two-photon imaging, to capture more natural behaviour. Freely-moving capabilities are supported due to the reduced weight of the head-mounted microscope (~2g), relative to a two-photon system \(^{17,18}\). As well, the head-mounted microscope is not permanently attached to the animal and can be removed once experimentation is completed.

Figure 2. Schematic of a two-photon system.
Head-mounted microscopes have advanced our understanding of neural activity related to memory, hunger, and other specialized functions\textsuperscript{22-24}. This system can provide further insight into the activity of large neuronal populations for calcium imaging in freely-behaving animals.

**Fiber Photometry**

Fiber photometry is an all-optical approach to simultaneously perform calcium imaging and optogenetic stimulation in the brain of freely-moving animals (see figure 4). An implanted optical cannula coupled with an optical fiber sends light to the brain and simultaneously retrieves light to an externally positioned photodetector/camera to provide both stimulation and photodetection capabilities\textsuperscript{8,25}. This tool detects average fluorescence intensity changes from population neural activity within a select region of a freely-behaving animal\textsuperscript{8,25}.

Multiple calcium indicators and/or optogenetic constructs can be detected or stimulated using fiber photometry since the light sources are external from the animal. Therefore, dual-colour calcium imaging or simultaneous calcium imaging and stimulation are possible with fiber photometry\textsuperscript{8}. Furthermore, a recent publication used a custom multi-fiber patchcord to detect average fluorescent intensity changes or stimulate multiple/projection-defined brain regions simultaneously in the same rodent for measuring brain-wide activity\textsuperscript{8,26}.

Like head-mounted microscopes, fiber photometry does not require a rodent to be head-fixed, and thus this method can more accurately capture activity elicited by a rodent’s natural behaviour. The light weight fiber optic cannula and cable required for fiber photometry can help extend the length of experiments and reduce extraneous factors (e.g. stress) allowing for more natural animal behavior to be observed during experiments.

The limitation of fiber photometry is low spatial resolution that restricts photodetection to changes in population activity within a select region\textsuperscript{19,27}, and it is also limited to wide-field optogenetic stimulation and lacks the capability to target individual neurons. Therefore, the distinction between cellular activity in select neurons is not possible with fiber photometry. However, the low cellular resolution provides small data files, fast acquisition, and easy data interpretation, unlike other current calcium imaging tools.

Fiber photometry is a useful tool that can provide us with a better understanding of low level circuitry in the brain. The simplistic design and data output of this tool provides simultaneous calcium imaging and optogenetic stimulation of neural activity.

**Optical Fiberscope**

The optical fiberscope, such as Mightex’s OASIS implant, is an all-optical system that enables simultaneous imaging and manipulation of neural activity with single-cell resolution in the deep brain of freely behaving animals (see figure 5). A removable imaging fiber, coupled with a GRIN lens implanted in the brain, provides both imaging and targeted stimulation in the deep brain.

The imaging fiber consists of thousands of individual micro-fibers (~3 μm diameter each), which can be independently addressed with light, to offer precise patterned (when combined with Mightex’s Polygon400) and/or wide-field optogenetic stimulation. Thus, stimulation and imaging can be targeted to specific cells or regions of interest with single-cell in the brain of freely-moving animals. This capability of simultaneous single-cell imaging and targeted stimulation is not possible with any other current technology. In addition, an optical fiberscope can also be used to perform population level imaging,
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identical to fiber photometry. Moreover, like the head-mounted microscope, the optical fiberscope’s field of view is determined by the size of the implanted GRIN lens.

A wide-range of calcium imaging and optogenetic applications can be executed with the optical fiberscope, such as dual-colour imaging, dual optogenetic targeted stimulation, and simultaneous imaging and targeted stimulation. Furthermore, since both deep-brain imaging and stimulation are delivered through the flexible light imaging fiber and the weight of the head-mounted fixture is very low (as little as 0.7g), the length of experiments can be extended and extraneous factors (e.g. stress) can be reduced allowing for more natural animal behavior to be observed.

A vital benefit of the optical fiberscope is the unique flexible design that is scalable and reconfigurable, making it a generic deep-brain imaging and stimulation platform that can be adapted for different applications, unlike many other single-purpose systems. Two illumination paths allow researchers to attach multiple wide-field and/or patterned light sources with different wavelengths, and to insert different optical filters (e.g. dichroics etc.) suitable for different imaging and/or illumination needs. As well, this system is compatible with high-quality scientific cameras for capturing better quality images (e.g. with better signal-to-noise ratios and better linearity) for data analysis.

With simultaneous calcium imaging and targeted optogenetic stimulation capabilities, the optical fiberscope is an ideal tool to help understand how single-cell interactions are involved in advanced brain functions, which is not possible with other current technology.

All-Optical Imaging and Stimulation Applications

All-optical imaging and stimulation tools can provide researchers with new capabilities in the field of neuroscience. First, optical imaging using GECIs, such as GCaMP, has already progressed neuroscience by allowing researchers to image the activity of explicit neuronal populations in the brain of freely-moving rodents. All current technologies described in this white paper can perform calcium imaging of a single GECI; however, each technology differs in imaging resolution, field-of-view, and freely-moving capabilities. Two-photon imaging has high cellular resolution, but this is associated with a small field of view, and this imaging modality is limited to a head-fixed setup. Fiber photometry has no cellular resolution to identify cells and instead is limited to population recordings. Head-mounted microscopes and optical fiberscopes have a large field of view, but this is limited by the size of the GRIN lens. As well, these two tools have high cellular resolution to identify single-cells, but optical fiberscopes are compatible with high-grade scientific cameras to acquire higher quality imaging. Calcium imaging is a well-established method for measuring in vivo activity; however, calcium dynamics are much slower than action potentials, and thus, are not directly related. Genetically encoded voltage sensors (GEVIs) measure the change in voltage within neurons and this is directly related to action potentials. GEVIs are better suited for optical imaging with high spatial and temporal resolution; however, GEVI’s require further work for efficient in vivo imaging.

Second, dual-colour imaging of either two GECIs or one GECI and one fluorophore (to identify specific cell types) in freely-moving animals is possible with these tools. This will allow for identification of different cell types and imaging of multiple or interacting cell types in vivo. Dual-colour imaging is possible with all currently available technologies discussed above. However, head-mounted microscopes are restricted to one or two wavelengths with no future flexibility and, due to the lack of cellular resolution of fiber photometry, this tool cannot identify static fluorophores. The main biological hurdle of dual-colour imaging is imaging two different colour indicators, such that the excitation and emission wavelengths are far enough apart to differentiate between them. An example of this is using GECIs GCaMP (image green) and RCaMP (image red). The development of other indicators will help circumvent this issue.

Third, simultaneous imaging and stimulation will enable manipulation of real-time activity in freely-moving animals and how activity is directly related to specific brain

Figure 5. Photo of Mightex’s OASIS Implant, an optical fiberscope system
functions. All four tools have this capability; however, only two-photon imaging and optical fiberscopes offer single-cell targeted optogenetic stimulation. Specifically, the optical fiberscope is the only tool which can perform targeted stimulation of multiple cells simultaneously in freely-moving animals, which is not possible with point to point laser scanning and head-fixed capabilities provided by two-photon imaging. Furthermore, the ability to image activity and stimulate neurons online has the potential for real-time closed-loop control of activity to understand the direct relation between activity and behaviour\(^1,9\). Importantly, to properly image and stimulate simultaneously there must be little cross-talk between biological constructs, such that optogenetic constructs and calcium indicators do not share the same excitation wavelength\(^1\). For example, to image GCaMP and perform optogenetic stimulation simultaneously researchers are using red-shifted channelrhodopsins to alleviate this issue\(^1,8,11\). Future development of new optogenetic constructs and imaging indicators will help resolve this problem. These are a few of the possible applications, but as research progresses the applications these tools can be used for will expand.

**Conclusion**

In conclusion, currently available all-optical technologies for in vivo calcium imaging and optogenetic stimulation each have their own strengths and weaknesses, depending on the target applications, as illustrated in Table 1. Two-photon imaging has high spatial resolution for imaging the activity of small cellular processes in live head-fixed animals. Head-mounted microscopes provide a compact one-photon microscopy unit for imaging numerous neurons simultaneously in the brain of freely moving animals. Fiber photometry images population activity changes in freely moving animals with fast and simple data output. Optical fiberscope is the only tool that provides single-cell optogenetic stimulation and calcium imaging in freely-moving animals. These advanced all-optical tools will help researchers comprehend the direct relationship between specific neural activity and time-stamped sensory, behavioural, cognitive, and/or cortical events with high temporal and high spatial resolution (see Table 1 for a summary comparing these tools).

**Summary Table**

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References


