Wavefront shaping systems for deep high throughput brain imaging

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Optical imaging techniques hold great promise in neuroscience due to their ability to monitor neuronal activity with high spatiotemporal resolution using calcium or voltage indicators. However, these techniques encounter considerable difficulties because of light scattering. The first barrier is the skull, a highly scattering layer that impedes light penetration. Consequently, most brain research today involves highly invasive craniotomy procedures. Even after opening the skull, the effectiveness of imaging methods is largely limited to the brain's superficial layers. Within these accessible regions, scattering limits both spatial and temporal resolutions.

Brain imaging using low-power single-photon (1P) fluorescent excitation is currently confined to the first 200um of brain tissue. Expensive two-photon (2P) excitation lasers, which operate at longer wavelengths, can penetrate up to 700um before scattering becomes a problem, but they still cannot access very deep brain layers. Moreover, 2P excitation presents several significant drawbacks. Firstly, deep tissue imaging with 2P lasers requires sequential scanning, which limits the temporal resolution of recordings. Secondly, even for shallow layers, as 2P excitation demands very high laser power; safety considerations restrict simultaneous excitation to no more than 20 neurons. Since voltage activity must be tracked at high kilohertz frame rates, this makes it nearly impossible to reliably monitor large neuronal populations.

Our research is dedicated to enhancing the penetration depth of brain imaging and improving signal-to-noise ratios through two innovative non-invasive approaches: **skull clearing** and **wavefront shaping**. Skull clearing methods, such as the Through-Intact-Skull (TIS) chronic window technique, employ chemical agents that match the refractive index variations in the skull, rendering it transparent. To image through the brain tissue itself, we will integrate spatial light modulators (SLM). These SLMs will be employed to reshape both the incoming and outgoing wavefronts, thereby correcting aberrations caused by tissue inhomogeneity as well as remaining aberrations in the cleared skull. We will leverage new mirror-based SLMs recently introduced by Texas Instruments. These SLMs work at a kilohertz refresh rate and are not sensitive to polarization.

In this research, we will apply wavefront shaping techniques to both 1P and 2P imaging. For 2P imaging, correcting the emitted wavefronts will eliminate the need for sequential scanning, enabling us to simultaneously image the voltage activity of multiple neurons at a high frame rate. Additionally, correcting the excitation wavefronts will allow us to extend 2P imaging to depths currently accessible only by unstable 3P processes. Correcting 1P wavefronts will enable this technology to reach depths comparable to those achieved with expensive 2P lasers, but at higher frame rates as we can eliminate the need for sequential scanning. Furthermore, since 1P excitation requires four orders of magnitude less power than 2P, we can image large neuronal populations without heat concerns.

Wavefront shaping is an old research idea which carries a very large potential for revolutionizing biomedical imaging, carrying the potential for very deep tissue imaging with very high

signal-to-noise ratios (SNR). However, practical application has been hindered by the difficulty of estimating the modulation using guide-star free, non-invasive feedback. Additionally, early wavefront shaping systems required high SNRs that realistic biological fluorescent sources could not provide. These obstacles were recently overcome by PI Levin's lab, who demonstrated ex-vivo non-invasive imaging of weak fluorescent neurons using 1P excitation. This project aims to utilize these innovative techniques for in-vivo mice brain imaging.

Aims and milestones.

The project would include the following aims and milestones:

Aim 1: skull clearing. The initial step towards non-invasive brain access involves the application of Through-Intact-Skull (TIS) chronic window techniques. This method utilizes chemical agents that align with the refractive index variations in the skull, making it transparent. This technique is well-documented, and we are confident in our ability to implement it within the first months of the project.

Aim 2: correcting outgoing wavfronts in 2P excitation. To enable rapid detection of voltage neural activity from multiple neurons, we employ a holographic illumination pattern. This technique can simultaneously excite a sparse set of target neurons and measure the resulting fluorescent emission using a 2D sensor. Due to the shorter wavelength of the emitted fluorescent light, it undergoes heavy scattering, limiting previous systems to measuring neurons no deeper than 200um into the tissue. Our objective is to correct the emitted light using a SLM. By converging all photons emitted from a neuron into the same sensor pixel, we can enhance the signal-to-noise ratio (SNR).

Evaluation metrics: For the 200um depths considered in previous studies using a standard 2D sensor, we anticipate our correction to achieve a tenfold improvement in SNR. Additionally, our correction is expected to extend the depth of measurable neurons to up to 500um.

Milestones**:**

- Build the wavefront shaping system by the end of year 1.
- Apply the system to in-vivo voltage imaging by the end of year 2.

Aim 3: correcting outgoing and incoming wavfronts in 1P excitation. The main drawback of 2P excitation is the significant power required, which leads to heat and safety concerns. As a result, it is not feasible to simultaneously excite more than 20 neurons with 2P excitation. To address these issues, we plan to utilize 1P excitation. However, the primary challenge with 1P excitation is that the excitation light, which uses shorter wavelengths, is also scattered. To overcome this, we will correct both the excitation and emission wavefronts. With this system, we expect to extend the depths at which 1P imaging is applicable, enabling its use in brain layers that are currently only accessible with costly 2P imaging. This approach will also benefit slower calcium imaging, which presently relies on expensive 2P lasers. Furthermore, our

aberration-corrected 1P imaging system will be employed for high-frame-rate voltage imaging. The use of 1P lasers in this context will allow us to target a much larger set of neurons compared to 2P excitation.

Evaluation metrics:

- We aim to extend the depth at which 1P imaging is applicable, achieving calcium imaging at depths of 500-700um into the tissue. Currently, these ranges are only accessible using expensive 2P lasers.
- For voltage imaging, we will demonstrate that at the 200um depth range considered in previous studies, wavefront shaping can provide a tenfold improvement in SNR and extend the measurable depth range to 500um.
- Compared to 2P excitation, which cannot target more than 20 neurons simultaneously, our system will measure hundreds of neurons.

Milestones:

- Year 1: Build the wavefront shaping system.
- Year 2: Apply the system to in-vivo calcium imaging.
- Year 3: Apply the system to in-vivo voltage imaging.

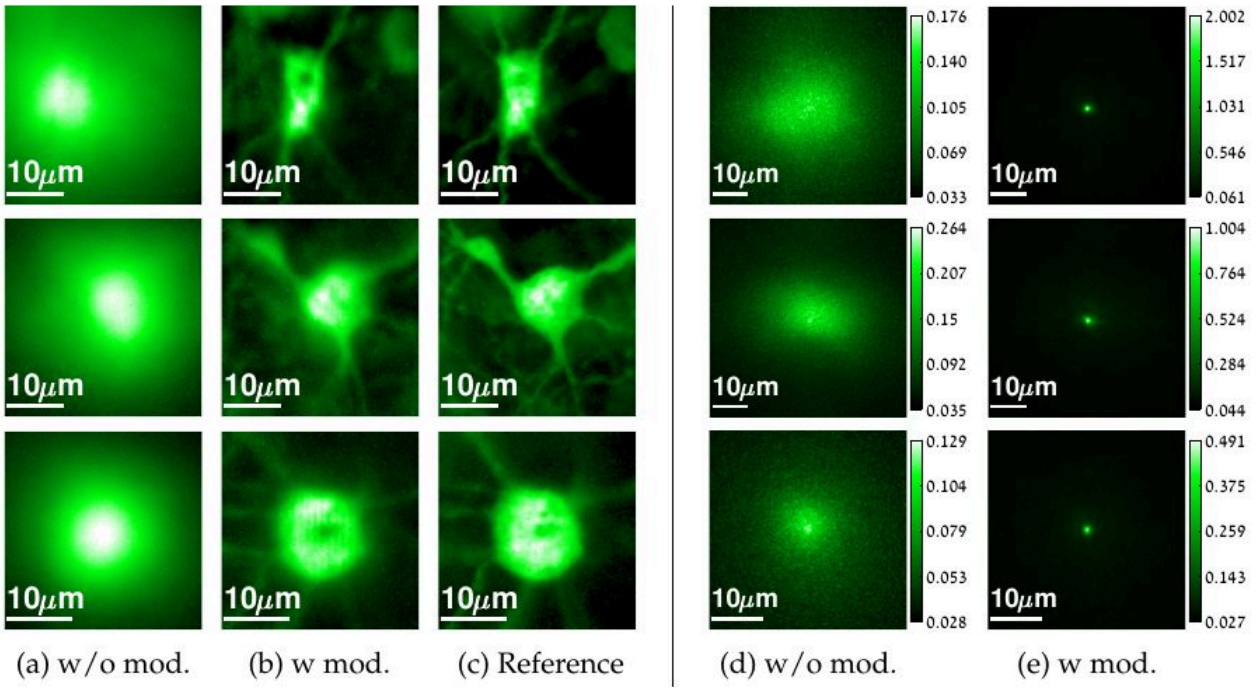
Aim 4 (long term): correcting incoming wave fronts in 2P excitation.

Upon successfully correcting the emitted light, we can extend wavefront shaping to correct the two-photon (2P) excitation wavefronts. This reshaping can potentially push the depth at which we can excite neurons beyond 1mm—ranges currently only accessible via unstable three-photon (3P) processes. As this represents a long-term objective, pinpointing exact milestones is challenging.

Preliminary results:

Below we show preliminary results from the wavefront shaping system recently developed in PI Levin's lab. We used a confocal wavefront shaping system (correcting both emission and excitation) and a 1P laser to image EGFP neurons inside ex-vivo slices of scattering brain tissue. In (a-c) we scanned the area of the neuron to generate an image. Without correction the confocal-scanned neuron is imaged as a wide aberrated blob. However, using our correction the neuron shape can clearly be revealed, including thin axons.

To evaluate the actual aberration corrected, the right part of the figure visualizes the image of a single point. By comparing columns (d,e) we see that our correction can increase the signal in the central pixel by a factor x4--x12 (depending on the actual amount of scattering present in the sample), thus we anticipate that our proposed aim of achieving a tenfold improvement in SNR is realistic.



Confocal scan of neuron area

Single point image

Team:

This project will be led by Prof. Laura Waller from the Berkeley EECS department, in collaboration with Prof. Hillel Adesnik from the Berkeley Neurobiology department and Prof. Anat Levin from the department of ECE at the Technion, Israel. Profs. Waller and Adesnik have a long history of successful collaboration, and Prof. Levin has been a frequent visitor and is planning a year-long sabbatical at the Waller lab starting in the summer of 2025.

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