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Thesis Title: Development of Magnetometry Based Functional Single Neuron Resolution Imaging

Thesis Abstract:

Development of new functional neuronal imaging techniques, over time, has provided new windows to understand information processing in mammalian neuronal microcircuits. Action potentials (APs), typical all or none neuronal membrane potential fluctuations, occurring in a single neuron or in each of an ensemble of inter-connected neurons are the means of processing, storage and retrieval of information in the mammalian brain. Current techniques to probe APs, for example in-vivo electrophysiology, multi-photon-imaging and functional magnetic resonance imaging, have complementary advantages. Each methodology has its own spatial-resolution (ideal-case resolve single-neurons), temporal resolution (ideal-case sufficiently sample single AP event), ability to identify neuronal types (like differential information from excitatory and inhibitory neurons) and limitations in terms of accessible areas. The state of the art in terms of spatio-temporal resolution is multi-photon-imaging; however, it suffers from inability to access deep regions like the frontal cortex or subcortical regions of the brain, without being invasive and hence leading to tissue damage.

In a search for alternative neuronal imaging techniques, recent advent of ultra-sensitive magnetometers based on electron spins in diamond has enabled detection of action potential associated magnetic fields (APMFs) from large axons of worms. Specifically, this magnetometer comprises of negatively-charged nitrogen vacancy defect centers (NV) in diamond acting as ultra-sensitive detectors of external fields like magnetic fields, at ambient temperature. Biocompatible NV based magnetometers can be operated as single-point bulk magnetometers or as a widefield magnetic field microscope, probing diffraction-limited spatially resolved 2D magnetic field features of a sample.

The research in this thesis is directed towards the initial steps of developing a novel functional imaging technique to probe AP activity at single-neuron resolution from a 3D volume of mammalian neurons using the NV-based magnetic field microscope. In the first part, we perform computational analysis of the feasibility of probing AP events from mammalian pyramidal neurons using NV-based magnetic microscopes. We show that axon-hillock APMF signatures are two-orders of magnitude larger than other locations on the neuron. Expected 2D magnetic field maps of naturalistic spiking activity of a volume of neurons, via widefield NV-magnetometry, have been simulated. We propose a dictionary-based matching pursuit type algorithm, comprising of dominant axon-hillock APMF signatures, to reconstruct 3D volumetric neuronal activity from simulated 2D NV-based magnetic field maps. We show spatiotemporal reconstruction of action potentials in the volume of brain tissue at single cell resolution. In the second part, we design and build an improved NV magnetic field microscope. Previously, magnetic field microscopes have been static in nature, requiring few to several minutes of acquisition time per frame and hence, limiting imaging of millisecond-scale APMF dynamics. We demonstrate that the magnetic field imaging frame rate can be significantly enhanced by performing lock-in detection of NV

photo-luminescence (PL), simultaneously over multiple pixels of a lock-in camera. A detailed protocol for synchronization of frequency modulated PL of NV centers with fast camera frame demodulation, at few kilohertz frequencies, has been experimentally demonstrated. This experimental technique allows magnetic field imaging of sub-second varying microscale currents in planar microcoils with imaging frame rates in the range of 50–200 frames per s (fps). This novel dynamic magnetic field microscope enables a small step towards imaging of millisecond-scale AP dynamics in-vitro. Finally, we discuss how enhancement of neuronal APMF signals coupled with NV-magnetometry advances and ability to reconstruct 3D neuronal activity from 2D maps can potentially evolve into an alternate magnetometry-based functional brain imaging technique.