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Instrumentation Platform for Imaging Cell Membrane Dynamics

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14. ABSTRACT
In this DURIP grant, a microscope that is compatible for wavelengths ranging from the visible to the infrared regime was developed, thus combining fluorescence imaging and differential interference contrast microscopy with infrared vibrational spectroscopy. Electrophysiology measurements can be simultaneously performed while fluorescence measurements or vibrational spectroscopy is conducted. The imaging platform is custom-designed to support visible, near-infrared and infrared light sources that span the whole fingerprint region with a tunable quantum cascade laser. Different key building blocks that are critical for the custom-designed spectroscopic imaging system were purchased and tested: A tunable quantum cascade laser was acquired for identification of protein and lipids via their vibrational absorption signatures. In order to resolve temporal dynamics at high speeds and with a strong signal to noise ratio, a high-speed lock-in amplifier was purchased and integrated into the system. The imaging resolution for the vibrational imaging offers sub-diffraction limited spatial resolution with regards to the mid-infrared beam, enabling the investigation of subcellular features in biological samples. Hence, morphological and structural details can be simultaneously acquired with chemical information and thermal diffusion dynamics, making this an attractive tool to study the mechanisms associated with infrared nerve stimulation and blocking and cell membrane dynamics. The imaging platform is versatile and can be extended to study tissue sections and to provide chemical identification and thermal diffusion analysis for sensors, various materials characterization and trace and impurity detection. Studies utilizing the novel imaging platform are ongoing (due to the pandemic and the restricted lab time available), which will result in several paper manuscripts to be submitted

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In this DURIP grant, the acquisition of equipment for microscopy from visible to infrared wavelengths in one imaging platform was proposed, thus combining fluorescence imaging with infrared vibrational spectroscopy. This allows detection of fluorescently labeled probes in biological specimens, identification of label-free bond-specific inherent spectral signatures, characterization of phase transitions and of the underlying structural order of molecules. The development of such a technology platform aims to advance the scientific development and technology progress for external nerve stimulation and inhibition. Specifically, novel insights into the biophysical mechanisms during infrared nerve stimulation and other external neuromodulation schemes will be gained by resolving the structure and dynamics of the cell membrane during different neurostimulation challenges and to analyze cell membrane dynamics.

To build the proposed instrumentation platform, a high speed lock-in amplifier, higher numerical aperture infrared objective and a tunable quantum cascade laser system were purchased. The UHFLI lock-in amplifier (Zurich Instruments) covers a frequency range up to 600 MHz and box car functionality to resolve temporal dynamics with high signal-to-noise. A refractive infrared objective with 0.4 numerical aperture from Pike Technologies was acquired to enhance the overall spatial resolution. A tunable quantum cascade laser from Daylight solutions was purchased since this allows wavelength coverage between 3-12 µm that offers more versatility for spectroscopic characterization in the fingerprint region. The wavelength modules were chosen specifically so that detailed protein, lipid and nucleic acid studies can be performed with the laser source. A customized microscope from Olympus has been tested, which can simultaneously capture fluorescence, differential interference contrast (DIC) and infrared vibrational imaging in one combined platform. The whole setup has been shielded so that low-noise electrical measurements as needed for electrophysiology can be conducted successfully. Overall, the platform enables a large diversity of sample studies from extracted neurons from various animal models to dissociated cells and brain tissue slides where electrophysiology measurements can be pursued simultaneously with imaging. Thus, the proposed instrumentation will provide an important platform to advance research on neuromodulation and the associated cell membrane dynamics during infrared nerve stimulation and blocking. The equipment will be critical to train the next generation of interdisciplinary researchers and students at the interface of engineering, photonics, biophysics and electrophysiology.

The completed microscope showing the full functionality of the electrophysiology setup is shown below in Figure 1. The multi-functionality of the stage and different light pathways allow for easy switching between fluorescence, DIC and photothermal microscope imaging while keeping the sample in position. The electrophysiology measurements can be conducted with extremely low noise level in this configuration. The resolution for the vibrational imaging offers sub-diffraction limited spatial resolution with regards to the mid-infrared beam, enabling the investigation of subcellular features in biological samples. Hence, morphological and structural details can be simultaneously acquired with chemical information and thermal diffusion dynamics, making this an attractive tool to study the mechanisms associated with infrared nerve stimulation and blocking and cell membrane dynamics. The imaging platform is versatile and can be extended to study tissue sections and to provide chemical identification and thermal diffusion analysis for sensors, various materials characterization and trace and impurity detection.

The availability of this novel instrumentation has led to one conference presentation and one submitted manuscript at this point. Several papers are in preparation (on infrared inhibition, photothermal spectroscopy of fibroblast cells, on hydrogel studies) that will highlight the impact of the imaging platform.

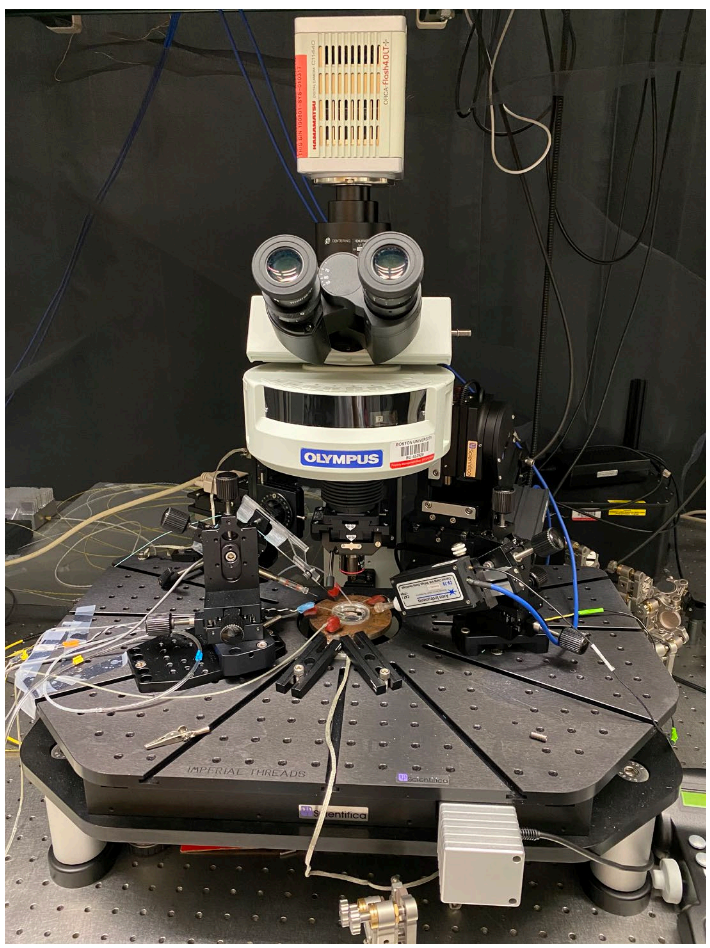

Figure 1: Multi-modular microscope integrating electrophysiology with photothermal chemical imaging. The setup shows the configuration optimized for electrophysiology recordings with various micromanipulators.