Microscopy 1

This section describes the background in microscopy in the neurosciences, and also how it relates to imaging in healthcare and electrophysiology in neuroscience. It will also describe the basic elements necessary for the construction of a microscope in a laboratory where calcium imaging in an animal is available. It will also refer to later sections which cover the design and construction of mechanical elements for animal handling and optical access (i.e. the headplate and a chronic optical window).

Optical imaging has traditionally involved wide-field imaging or two photon imaging, each with their own distinctive advantages and disadvantages

In recent years, two photon microscopy has been a preeminent choice for imaging in tissue, because of its high spatial resolution, and tissue penetrating features

Two photon calcium imaging has been broadly applied to individual cells or subcellular components of neurons including spines and axons

Because two photon microscopy uses a scanning mechanism, the signal to noise ratio is influenced by the time spent imaging each point, and the spatial resolution is determined by the number of points scanned to obtain each image. As a result, the size of the imaging field is inversely correlated with the overall temporal resolution while maintaining a relatively high signal-to-noise ratio, thus, two photon calcium imaging is often performed on a small area or on a sparse network of cells, when dynamic responses with high temporal fidelity is necessary.

Wide-field, or single photon imaging has been in use for several decades and was first used to characterize the functional architecture and hemodynamic responses in brain tissue

However, this technique has seen a renaissance recently due to its simple instrumentation, relatively inexpensive cost, and the improvements in neural signal indicators. Optical imaging and two photon microscopy have traditionally been performed in head-fixed preparations, but recent advances have also made it possible to perform wide-field calcium imaging in freely moving animals, through miniaturized and wearable microendoscope systems

While wide-field imaging lacks the spatial resolution to resolve fine subcellular structure or the penetrating properties available with two-photon, it is possible to obtain clear neurites and somatic features, including spike detection

Because a single photon microscope does not rely on scanning features, it can be used to sample a larger field of view without sacrificing sampling rates. Additionally, recording sessions may be less sensitive to fluorophore bleaching than other techniques, which makes it possible to perform sustained illumination and subsequent imaging for an extended period of time - a desired feature for analyzing neural networks during some behavior paradigms (*e.g.,* repeated trial learning paradigms). Thus, wide-field imaging offers an advantage if the objective is to simultaneously recording hundreds of neurons in the brain of a living and behaving animal with high temporal fidelity.

Background: Brain Imaging and Microscopy in Neuroscience

Fluorescent Proteins Background

- GCamP
    - vs dyes

Fluorescence Microscope Types Background

- 2p, confocal, wide-field
    - tissue scattering
        - depth comparison
    - cost & complexity
    - (address scalability later)

Widefield Microscope COnfiguration

- historical trend/shift from *finite* to *infinite* conjugate type
    - infinite type uses _infinity corrected lenses_

Filters

- excitation
- emission
- for epifluourescent microscope configuration: dichroic beamsplitter

Lenses

The simplest configuration of infinite wide-field microscope requires an excitation

Excitation

Emission

Emitted Light Collection and Image Formation (emission/collection)

Mechanics

Microscopy and Functional Imaging Two core innovations in available

- technology 1. Synthetic bio (i.e. GCaMP) 2. Cameras
- scientific CMOS
