

Summary

Our understanding of the nervous system depends critically on our ability to develop means to study it. This is not to say that a clever application of a method or fortuitous discovery is without merit. Often, one sees a convergence of both, as the development of the Golgi histological staining technique enabled Cajal to study the intricate morphology of neurons. The main focus of the present work was to develop and improve upon optical methods that are suitable to study the morphology and electric signaling of neurons.

The first chapter gives an overview of key methods used to study the form and function of the nervous system, discussing their strength and weaknesses. The second chapter presents a new class of membrane potential sensitive fluorescent dyes. In conjunction with two-photon laser scanning microscopy, these are used to measure electrical signals occurring in neuronal dendrites that are otherwise inaccessible to the traditional patch-clamping technique. In the third chapter I describe the design and construction of a high quality two-photon laser scanning microscopes and show how various lens designs can affect its performance. The fourth chapter demonstrates the use of third-harmonic generation, a well known nonlinear optical phenomenon, in the context of laser scanning microscopy to visualize neuronal tissue morphology without the need of applying exogenous fluorescent labels. In the fifth chapter I describe a new data acquisition software, DAQLab, that can flexibly adapt to ever changing experimental needs and use it to perform two-photon glutamate uncaging to stimulate multiple synapses and demonstrate supralinear dendritic integration. Finally, this work concludes with an overview and expected future developments.