Preface

Investigations into fundamental questions in cell biology have long benefited from experiments that utilize neuronal systems. Neurons have proven particularly useful model systems for enhancing our understanding of intracellular transport. Their long thin axons, which can comprise 95% of the cellular volume, cannot be maintained by diffusion alone, and thus they may be regard as specialized for transport. In addition, their morphology makes axons ideal systems to image motor protein-based movement with live cell microscopy. These properties render axonal transport an effective model for investigating the cytoskeleton, motor proteins, and organelle transport.

The chapters in this volume describe methods that utilize live cell imagining, genetic, molecular, biochemical, and proteomic approaches in neuronal systems to characterize and explore fundamental questions related to intracellular motility, especially axonal transport. The contributors employ a wide variety of culture systems including sympathetic, cortical, hippocampal, dorsal root ganglion, and Purkinje neurons as well as in vitro slice cultures and axoplasm from the squid giant axon; as well as model organisms *Drosophila*, zebrafish, and mice. The first chapter introduces the basic paradigm for the mechanism(s) of movement in the axon. It reviews in vivo pulse labeling experiments which identified the movement of three distinct sets of structural components from the cell body down the axon; membrane-bounded organelles, microtubule, and neurofilaments, and actin with the over 200 remaining axonal proteins. The chapter continues by discussing recent live cell imaging data, utilizing the excellent optical properties of long thin axons, to define the mechanisms for the moment of the structures. The volume is then organized into three overlapping areas with methods chapters that focus on (1) cytoskeletal protein dynamics and filament transport, (2) the motor proteins responsible for transport, and (3) the transport of membrane-bounded organelle cargos.

Procedures are given for the live imaging of neurofilament transport and actin dynamics and transport in cultured neurons. In addition, methods are described to image tubulin dynamics in cultured hippocampal slices and single molecule resolution of tubulin and microtubule plus-end-tracking proteins in cultured neurons. Techniques for live imaging of the movement of cytoplasmic dynein and the initiation of retrograde organelle transport in axons of cultured neurons are also presented. Assays to probe kinesin motor domain function and the role of a kinesin family member in cytokinesis in neuroprogenitors are reviewed. Genetic and imaging approaches to analyze motor protein function and organelle motility and neuroprogenitor migration are provided using zebrafish, *Drosophila,* and mouse models.

A variety of approaches to image and analyze membrane-bounded organelle and other cargo motility (including endosomes, lysosomes, autophagosomes, mitochondria, signaling endosomes, viruses, and ribonucleoprotein particles) in axons, dendrites, and squid axoplasm are discussed. These include utilizing microfluidics chambers for culturing neurons; labeling the membrane-bounded organelle cargos with dyes or fluorescent-tagged proteins; tracking internalized transmembrane

proteins with quantum dot- or fluorochrome-labeled ligands or antibodies; and investigating effect of the Alzheimer's disease peptide β-amyloid on organelle transport.

Several chapters take advantage of molecular and biochemical methods to analyze cytoskeletal and motor protein activity. The squid axoplasm system is utilized to investigate kinase pathways of phosphorylation of filament subunits and motor proteins. A proteomics method is presented to probe the effects of mouse mutations on the cytoskeleton and motor proteins; and affinity chromatography is used to investigate motor proteins association with ribonucleoprotein particle transport in axons. Two contributions discuss methods for knocking down the expression of neuronal proteins using RNAi, one focuses on using siRNA in sympathetic and hippocampal neurons; the second describes a plasmid-based approach to reduce myosin Va levels in cultured Purkinje cells.