Preface

One of the primary aims of biology is to understand how expression, activation and inactivation of genes and proteins is orchestrated to generate the wide variety of cells, organs and tissues that comprise a living organism. Ultimately, elucidation of regulatory networks controlling these processes will provide key mechanistic and molecular insight into normal development and have the potential to lead to the discovery of cellular factors that can be targeted in attempts to combat numerous diseases that affect cell and tissue architecture and function. Regardless of where the work starts, at some point the need to study specific genes and proteins within the cellular milieu, becomes central toward achieving this goal.

The chapters included in this volume, taken from *Cell Biology: A Laboratory Handbook, 3rd edition*, describe an array of cell biological assays that can be used to define and characterize experimentally the machinery that regulates events critical to the function of diverse cell types. Broadly defined, these encompass methods to analyze translocation of proteins between sub-cellular compartments, intracellular signaling and cytoskeletal organization. Each of these processes is key to the definition of cell shape, function and responsiveness to both intracellular and extracellular cues.

The methods described in these chapters can be separated into three general categories. The first group of assays lays out the rationale and techniques to measure protein transport between sub-cellular compartments or the plasma membrane *in vitro*, in semi-intact cells and in intact cells. Each of these assays have been used to gain fundamental insight into the mechanisms regulating exocytosis and endocytosis, nuclear-cytoplasmic shuttling and translocation of proteins (including co-translational transport into the endoplasmic reticulum) between intracellular compartments. In combination, these techniques provide researchers with a full arsenal of approaches with which to identify, characterize and determine the minimal essential machinery for protein transport in cells. The second group of assays provides in depth methods to study intracellular signaling and localized protein activity within single, living cells. These assays take advantage of vital fluorescent sensors to measure local changes in intracellular calcium, protein conformation, protein phosphorylation and nucleotide triphosphate occupancy, all read-outs of protein activity. In addition, these assays incorporate methods to control and analyze signaling events with tremendous spatial and temporal resolution in cells. The third group of methods detail *in vitro* and cell-based assays to evaluate the regulation and organization of actin and microtubule networks in response to individual proteins, defined cytosol, signaling cascades and environmental insult by bacteria. These assays also provide researchers with the ability to identify molecular effectors of cytoskeletal dynamics and organization that play key roles in how cells respond physically to a variety of physiological stimuli. Results obtained in any one groups of assays can be fruitfully incorporated into experiments using the others to gain more complete