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## Preface

Cell biology is the study of the structure and function of cells, cellular organelles, and sub-cellular structures. Originally, cellular functions were studied without a detailed knowledge of the structures involved and structures were described without an understanding of their function. Imaging techniques that simultaneously studied structure and function or at least correlated function with structure eventually closed this disparity. Several technologies have been greatly responsible for this progress by providing high-resolution images rich in functional information. Over the past decades, these technologies and their combinations have provided possibilities previously unthinkable. This book contains a collection of timely techniques and methods that have been instrumental in the evolution of microscopy from a purely descriptive technique to one of four-dimensional imaging in living organisms.

The biochemist and molecular biologist determines the functions of the molecules and macromolecular complexes found within cellular structures. They isolate individual cellular constituents and reconstruct vital cellular processes. These *in vitro* experiments provide a valuable understanding of cellular function. But, biochemistry lacks the potential to place this knowledge into the cellular context of cell types and cellular compartments.

Electron microscopy (EM) was the first technique to bridge the gap between biochemistry, molecular biology, and cellular context by localizing macromolecules to cellular structures. EM collected valuable information about proteins contained within a structure and their spatial relationships with other proteins and structures. The combination of immunolabeling with EM made it even possible to localize specific proteins of known function to subcellular structures. Immunoelectron microscopy can be used to study virtually every unicellular and multicellular organism. The only requirements are suitable protocols and the availability of an antibody to the molecule whose structural location is to be determined. Although introduced decades ago, this technology is far from obsolete because of its nanometer precision in localizing proteins in cells and tissues. More recently, labeling methods for scanning EM and for serial sections and electron tomography were developed and used to visualize specific biomolecules within the three-dimensional structure of organelles and subcellular compartments. Computer-assisted image acquisition and analysis greatly contributed to this development.

Advances in light microscopy soon made it a competitive alternative to EM for studies correlating structure and function. The introduction of video cameras marked a breakthrough adding a new dimension, time, to microscopic observations of structure in a living cell. The development of fluorescent dyes that could be conjugated to antibodies and dyes localized to specific subcellular compartments further advanced live cell imaging. To fully utilize the potential of these probes confocal and two-photon microscopes were designed. These microscopes overcame the limitations of standard fluorescent microscopes by increasing the localization accuracy in tissue and the resolution in the *z*-axis. The next innovation that boosted functional live cell imaging was the discovery of the green fluorescent protein (GFP). The ability to use DNA cloning methods to create constructs encoding a protein of interest fused to GFP opened the door to using live cells for studying the function of specific proteins. The development of a diverse color pallet of fluorescent proteins

and of methods to make fluorescence dependent upon the interaction of two proteins as well as photocontrollable fluorescent tags and the constant advances in designing ever faster and more sensitive cameras have greatly expanded the structure function information that can be obtained from live cell imaging.

It did not take long before correlative methods were developed in which the distribution of specific proteins was examined first by confocal microscopy and then by EM. An example from our own work demonstrates how confocal and immunoelectron microscopy provide unexpected insights into structure-function relationships. Thus, immunoelectron microscopy first demonstrated that the *Euglena* light harvesting chlorophyll a/b binding protein of photosystem II (LHCPII) is present in the Golgi apparatus prior to its presence in the chloroplast. This finding was the impetus for detailed biochemical studies that elucidated a new mechanism for chloroplast protein import, namely transport from the ER to the Golgi apparatus to the chloroplast.

This volume takes into account the increasingly multidisciplinary nature of microscopy by presenting three toolboxes. The molecular toolbox focuses on the development of molecular tools for microscopy. It will present methods for the expression of epitope-tagged proteins in animal cells. Methods for the production of antipeptide and polyclonal antibodies and how to conjugate colloidal gold to these proteins will also be presented. A molecular toolbox would be incomplete without the discussion of genetic tools that exploit viral vectors to optimize the transfer of genes into living cells. This technology is also addressed in the following toolboxes together with light and electron microscopic imaging.

From the fluorescent microscopy toolbox, this section presents methods that span diverse applications based on the use of conventional fluorophores and expressed fluorescent proteins such as GFP in plants, parasites, and animal cells. Fluorescence microscopy also enables monitoring protein-protein interactions in real time and bimolecular complementation methods enabling this feat will be presented. A pH-sensitive GFP variant is used to monitor exocytosis and endocytosis of synaptic vesicles in real time. How the trafficking of proteins or organelles can be monitored by Fluorescence Recovery after Photobleaching and Fluorescence Redistribution after Photoactivation is presented. Finally one chapter presents the labeling of brain cells and the imaging of these cells in the living brain.

From the EM toolbox, this section details methods for cryo-ultramicrotomy and rapid freeze-replacement fixation which have the advantage of retaining protein antigenicity but at the expense of ultrastructural integrity as well as chemical fixation methods that maintain structural integrity while sacrificing protein antigenicity. The toolbox also includes a protocol for immunogold labeling of freeze-fracture replicas. This technique is known for its high sensitivity and its capability of localizing proteins to nanoscale protein assemblies like ion channels. Plants and algae contain cell walls, vacuoles, and other structures which present barriers to antibody penetration and complicate fixation. Due to these problems, separate chapters will discuss fixation and immunolabeling protocols for animals, plants, and yeast. Pre- and post-embedding immunogold labeling protocols will be presented. Pre-embedding methods perform immunogold labeling before ultrathin sections are prepared from resin-embedded samples resulting in greater sensitivity and better microstructure preservation. Post-embedding methods perform immunolabeling after ultrathin sections are prepared from resin-embedded samples resulting in decreased antigenicity. The detailed methods and notes will facilitate choosing the best method for the antibody and biological material to be studied. Extending these approaches, methods will be presented for immunogold labeling of two antigens for protein colocalization studies, for glycan localization, for nanogold enhancement allowing immunogold labeling using smaller gold particles

which more easily enter cells, and for immunogold scanning EM. For many years, the advances in genetics and functional imaging were not used to advance EM. Recently however, these advances have been used to develop powerful EM techniques. Reporter genes suitable for EM and fixation techniques that capture structure at a defined time point have been developed. Furthermore, these techniques are suitable for correlative light-electron microscopy. The volume presents two examples of these advances; the use of genetically engineered horseradish peroxidase as a genetically encoded label for electron microscopy and superfast fixation for monitoring cellular processes second by second.

It is our hope that the toolboxes created by this volume will be used by cell biologists interested in understanding structure-function relationships at the fundamental level as well as by cancer biologists, toxicologists, and microbiologists interested in understanding disease mechanisms as a foundation to developing new therapies.

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