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Methods and Protocols

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Cover illustration: Color Plate 1, following p. 180. Scanning electron micrograph showing a population of fission yeast *S. pombe. See* discussion in Chapter 12 and full caption on p. 184.

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Preface

Electron Microscopy Methods and Protocols is designed for the established researcher as a manual for extending knowledge of the field. It is also for the newcomer who wishes to move into the field. A wide range of applications for the examination of cells, tissues, biological macromolecules, molecular structures, and their interactions are discussed. We have tried to gather together methods that we consider to be those most generally applicable to current research in both cell and molecular biology. Each chapter contains a set of related practical protocols with examples provided by experts who have first-hand knowledge of the techniques they describe. The individual chapters are grouped according to similarities in their specimen preparation and methodology. Methods are presented in detail, in a step-by-step fashion, using reproducible protocols the authors have personally checked.

During the last decade, the scientific literature describing the use of colloidal gold as an immunocytochemical marker has increased at an exponential rate, and this trend is expected to continue. We have included a large number of variations on the immunogold labeling technique. In both the negative staining and cryo chapters, authors emphasize the "immunological applications" in order to correlate as fully as possible with the emphasis on immunogold labeling in the other chapters.

Electron Microscopy Methods and Protocols commences with the routine preparation of biological material for classical transmission electron microscopy involving tissue fixation, embedding, and sectioning (Chap. 1). Chapters from Robin Harris and Marc Adrian deal with negative staining of thinly spread biological particulates and the preparation of thin-film frozen-hydrated/vitrified specimens (Chaps. 2 and 3) . The production of cryosections from fixed, cryoprotected biological material and their use in immunocytochemistry is covered by Paul Webster in Chapter 4. Ken McDonald in Chapter 5 describes high-pressure freezing and freeze-substitution with respect to morphology and antigenicity in immunolabeling (Chap. 5). Since the method of specimen embedding influences labeling with colloidal gold, a detailed protocol for the use of LR Gold and Lowicryl resins is presented in Chapters 6 and 7, respectively. Immunogold labeling following the progressive-lowering-of-temperature method is also detailed. Catherine Rabouille deals with quantitative aspects of immunogold labeling in embedded and nonembedded

sections in Chapter 8. Microwave processing techniques are covered in Chapter 9, followed by enzyme cytochemistry (Chap. 10) and *in situ* molecular hybridization (Chap. 11).

Our knowledge of the genetics, cell biology, and molecular biology of yeast continues to advance at an encouraging rate and with considerable excitement. In Chapter 12 we have emphasized the preparation of fission yeast for ultrastructure and immunocytochemistry in order to demonstrate the value of electron microscopy in yeast biomedical research. The preparation and analysis of structures, such as nucleic acids and proteins, as well as the binding of proteins to nucleic acids and protein-to-protein interaction are discussed in detail in Chapters 13 and 14. The emphasis on cryotechniques continues in this volume, in Chapter 15, with a detailed account of the procedures for quantitative biological X-ray microanalysis by A. John Morgan and his colleagues.

Electron Microscopy Methods and Protocols has been developed through the efforts of twenty-three scientists, representing seven countries. All the contributors are eminent authorities in their respective fields. I hope this volume will prove useful both for the novice and experienced worker carrying out high resolution microscopy in their research. My thanks are owed the authors for their excellent contributions and professional cooperation. Thanks also to my staff—Steve Gschmeissner, Carol Upton, and Ken Blight—for their valuable comments on some of the chapters.

M. A. Nasser Hajibagheri

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Color Plates

The Color Plates appear after p. 180. The images were edited with Adobe Photoshop®.

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