BOOK REVIEWS

The Science of Biological Specimen Preparation for Microscopy. Scanning Microscopy Supplement 10, 1996.

M. Malecki and G. M. Roomans, Editors, Published by Scanning Microscopy International, AMF O'Hare, Chicago, IL.

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Advance in our knowledge of the molecular organization of living material rests on two kinds of technological development: (1) microscope instrumentation and (2) specimen preparation. This includes new techniques appropriate for a specific mode of microscopy, and information on any structural alterations produced during such specimen preparation.

The present volume contains the proceedings of the 14th Pfefferkorn Conference organized by Scanning Microscopy International and dedicated to the late Prof. G. E. Pfefferkorn.

The organizers and editors of this conference brought together 31 leading scientists in this field from the USA, Europe, and Japan to present and discuss new techniques to prepare biological specimens for microscopy. Discussions with a panel of reviewers are added to each paper. There is not space for a detailed discussion of each paper. I shall try to indicate the general substance of the contributions.

In general, three unique features of this book are worth stressing. The first reflects current trends in science towards interdisciplinary approaches in solving important biological questions. An essential part of incorporating microscopy into these interdisciplinary approaches is development of reporter molecules which will have features precisely determined in biochemistry and molecular biology labs and which will remain stable in changing environments of living cells being labeled for other modes of microscopy. Chapters by Heinfeld (nanogold), Kessels (boronated antibodies), Malecki (organo-metallic ligands), and Swartz (fluorescent derivatives of proteins) are concerned with the development of such new probes which are based upon covalent bonds and therefore can serve as reliable markers for structures of interest. Moreover, chemically defined features can be translated into their functional architecture, e.g., as shown for AFM in the chapter by Woodward and Zasadzinski. The detailed protocols will allow an investigator to have them easily modified for a particular new application. This book also demonstrates how fruitful this interdisciplinary approach can be, e.g., as demonstrated in chapters by DeBault and Gu, Malecki, and Nuovo. Polymerase chain reaction developed for amplification in molecular biology labs can be modified to determine morphological localization of selected sequences. The book clearly demonstrates that in order to solve a biological question it is nearly impossible to categorize approaches and restrain an investigator to traditional research techniques. To the contrary, the interdisciplinary approaches create backgrounds and precedents for breaking the boundaries of traditional and specialized ares of science and opening new possibilities.

The second feature is an attempt toward integrated microscopy. While many published books are dedicated to specialized areas of one kind of microscopy, this one stands out as an eclectic (in a positive sense) but comprehensive review of the most recent developments in many areas of microscopy. This is particularly close to my scientific preferences, since I have promoted this approach for many years. Integrated microscopy allows us to overcome technical limitations of one kind of microscopy alone. For instance, light microscopy of a living cell is limited by resolution to ~250 nm, while electron microscopy with an atomic level of resolution can be pursued only on frozen or fixed cells. Therefore studying the same cells with both types of microscopy creates an opportunity to study living cell phenomena at the molecular level. This approach is elegantly exemplified in the chapters by Malecki, Peachey et al., Ralston and Ploug. It also demonstrates how beneficial and cross-fertilizing this integrating approach can be, e.g., in the chapter by Lyubchenko et al., where a technique of functional modifications of substrate surface used in TEM and SEM, now finds new applications in AFM. These chapters create not only an excellent starting point for a reader, but by collecting many other techniques in one book, open for the investigator a compendium of choices. The newest developments in specimen preparation for two-photon excitation fluorescence microscopy, atomic force microscopy, energy filtered transmission electron microscopy, etc., are all covered in this one volume. Therefore, scientists attempting to solve a biological problem with tools of modern microscopy can make a choice from the vast variety of techniques and examples presented in this book. The detailed hands-on specimen preparation protocols will guide them reliably. The discussions with the reviewers will provide them with a critical evaluation of the choices.

The third feature paves the road for future developments in microscopy. The chapters and discussions with the reviewers fairly define current technical limiations of various modes of microscopy and suggest possible ways towards overcoming these difficulties. In many cases the authors also clearly state future directions in their research.

Specifically, the first group of papers deals with methods to study chromatin and nucleic acids. M. Malecki describes new methods for efficient gene transfer. Development of these methods was based upon incorporation of reporter molecules allowing imaging of cellular pathways in living cells, and in cryo-immobilized cells by energy filtered TEM from the cell surface to the chromatin. G. V. Childs describes methods to identify mRNA and proteins in the same cell, in tissue sections. L. E. DeBault and J. Gu present detailed protocols for *in situ* hybridization, *in situ* transcription, and *in situ* polymerase chain reactin. M. Thiry developed the *in situ* terminal deoxynucleotidyl transferase (TdT)-immunogold technique for DNA to pinpoint specific nucleic acid regions in thin sections.

Scanning probe microscopy is repre-

sented by five contributions. Hydration scanning tunneling microscopy (Heim et al.) is based on conductivity of surface adsorbed water molecules and can image hydrophilic insulators and biological specimens such as collagen IV molecules, TMV, and cryosectioned bovine tendon.

For atomic force microscopy imaging of macromolecules, a strong attachment to the substrate is essential. Lyubchenko et al. show that treatment of mica with aminopropyltriethoxysilane will hold DNA in place for imaging even in water. They also introduced other chemically reactive mica surfaces, hydrophobic or charged Mueller-Reichert and Gross discuss DNA and DNA-protein assembly analyzed by TEM, scanning tunneling microscopy (STM) and atomic force microscopy (AFM). An interesting new application of STM is imaging of freeze-fracture replicas (Woodward and Zasadzinski). It can also examine interior interfaces and provides quantitative information about the vertical dimension of interior structures.

Four contributions discuss light microscope techniques for the imaging of living cells. The first of these by N. S Allen and M. N. Bennet use Alfalfa root hairs before and after treatment with Nod factors (produced by Rhizobia) to study in live and fixed cells the role of actin and endoplasmic reticulum in growth form change. Imaging was with a confocal laser scanning microscope. The paper by Peachey et al. describes techniques to image cultured cells by phase, epifluorescence, and confocal microscopy, and after fixation and critical point drying imaging the same cell as a whole mount with the Jeol 400 kV-EX intermediate voltage TEM, thus correlating structures seen in the living cell with the EM image.

The paper by D. R. Swartz on covalent labeling of proteins with fluorescent compounds for imaging applications beautifully illustrates with alpha–actinin how a protein can be covalently linked to a fluorophor without interfering with its normal chemical interactions in the living cell. This paper is important for all who need fluorescently labeled cell proteins for imaging applications.

L. Edelman and A. Ruf describe a simple treatment to stabilize freeze-dried cells during or after low temperature embedding in Lowicryl, to prevent loss of material from thin sections during wet cutting.

J. F. Hainfeld describes the techniques for labeling with Nanogold, Undecagold and FluoroNanogold. This paper is essential for anybody who requires gold labeling.

The increasing availability of energy filtered TEMs will facilitate immunocytochemistry by providing electron spectroscopic imaging. Kessels et al. describe the development of organo–boron compounds which can be incorporated into organic molecules such as Fab′-boronated peptide conjugates for immunochemistry.

The last paper by H. Sitte contains a masterful critical review of cryofixation, and blueprints on how to build a cryomicrotome that will provide useful cryosections. I think that these 70 pages contain the most valuable part of this volume.

To summarize, this volume obviously contains a wealth of stimulating and useful information. It bears testimony that microscopy is alive and well.