Book Review

Microscopy AND Microanalysis

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***Electron Crystallography of Biological Macromolecules***, R. M. Glaeser, K. Downing, D. DeRosier, W. Chiu, J. Frank. Oxford University Press; 2007, 476 pages. ISBN 0195088719 (Hardcover)

Science is witnessing an explosive growth in the use of transmission electron microscopy (TEM) to record images of biological macromolecules, preserved in near-native form, from which three-dimensional (3D) reconstructions are computed. Three-dimensional electron microscopy (3DEM) nicely complements traditional methods such as X-ray crystallography or nuclear magnetic resonance spectroscopy in structure determination work, and more frequently is now providing the only means by which biochemists, cell and molecular biologists, biophysicists, and structural biologists are able to investigate the 3D structures of large and often complex, macromolecular machines to gain insights about how they function.

This book fills an important void especially for those wishing to understand and practice 3DEM methods at more than a superficial level. Indeed, the book is a rigorous, A–Z encyclopedia of the theory and practice of how to prepare specimens, record and process images, compute 3D density maps, and extract and interpret structural information from the maps. The book is a valuable resource for serious students as well as more seasoned practitioners and would serve as an excellent text for an advanced, graduate-level course. The depth, breadth, and rigor of the book, which also contains many new insights, are simply not available in any other text focused on 3DEM studies.

Success in 3DEM requires a firm grasp of the fundamental principles by which 3D structure is gleaned from noisy images of unstained, vitrified samples. A staggering number of parameters and variables ultimately dictate the detail or resolution that one might attain when determining the structure of a particular macromolecule. The book vigilantly introduces, defines, and discusses each of these, often in multiple contexts, and more than likely may illuminate a few concepts that self-proclaimed experts missed or misunderstood. If nothing else, the dedicated reader will emerge with an appreciation for just how truly remarkable it is that, when properly performed, the techniques are capable of extracting exquisite 3D details from two-dimensional (2D) images with horrible contrast and that appear to contain nothing but random noise.

The bulk of the text was written by Glaeser (Chapters 1–8 and 15) and Downing and Glaeser (Chapters 9–11), with Chapters 12–14 authored by DeRosier, Chiu, and Frank, respectively. All five authors are highly regarded for their keen insights and pioneering contributions to the 3DEM field over several decades. Despite the challenges inherent with coauthored books, the uniformity of presentation and style is excellent and topics are presented in a logical order.

*Chapter 1 (Introduction)* provides a broad overview of the scope of the book and identifies the types of specimens and techniques to be covered. The book mainly focuses on the analysis of highly-ordered, 2D crystalline specimens, where the ultimate goal is to reach "very high resolution" (~0.35 nm). Despite the primary emphasis on studies of 2D crystals, much of the theoretical and practical aspects of examining such specimens are directly applicable to the investigation of "single particles," which include periodic and aperiodic macromolecular complexes.

Chapters 2–5 provide the theoretical underpinnings upon which the remainder of the book rests. They define and introduce much of the language used in structure determination and with which anyone new to the field must become familiar and comfortable. Readers with limited mathematical skills will likely struggle with Chapters 2–4 and may fail to gain full benefit from the book unless they first familiarize themselves with the subject of X-ray crystallography, as Glaeser suggests in Chapter 1.

*Chapter 2 (Structure Determination as It Has Been Developed through X-Ray Crystallography)* covers numerous topics: crystals and crystallization; data collection; Fourier transforms; Fourier series; Fourier synthesis; unit cells, periodicity, and motifs; structure factor amplitudes and phases; convolution theorem; Miller indices; short- and long-range crystal disorder; Ewald sphere; spatial frequency; reciprocal space; Bragg's law (curiously, the definition provided differs from that presented in crystallography books); phase problem; fast Fourier transform (FFT) algorithm; density map interpretation and modeling; model refinement; and R-factor and free R-factor.

*Chapter 3 (Fourier Optics and the Role of Diffraction in Image Formation)* introduces numerous topics: Abbe's theory of images; lens aberrations and defocus; phase contrast microscopy; Fraunhoffer diffraction; diffraction limited optics; point spread function or impulse response; Airy pattern; image restoration; weak phase object; projection theorem; Friedel symmetry; phase contrast transfer function; spatial and temporal coherence; envelope function; amplitude contrast; single side-band image; and beam tilt.

*Chapter 4 (Theoretical Foundation Specific to Electron Crystallography)* provides in-depth discussions of several relevant topics including: kinematic (single) scattering; Ewald sphere; proof of the projection theorem; use of specimen tilting to sample the 3D Fourier transform; 3D reconstruction resolution; Fourier versus Rayleigh criterion of resolution; effects of cone of missing data; Shannon sampling theorem; radiation damage; Rose equation; spatial (Fourier and correlation) averaging; and cross-correlation function.

*Chapter 5 (Instrumentation and Experimental Techniques)* introduces the reader to the basic design, alignment, and use of the TEM. Notable topics include: electron gun; electron beam accelerating voltage, energy spread, and temporal and spatial coherence; condenser and objective lens apertures; spherical and chromatic aberration; depth of field; objective lens defocus; axial astigmatism; energy filtering; low temperature stages and anticontamination; recording images and diffraction patterns; detective quantum efficiency, dynamic range, and modulation transfer function of recording media; low dose imaging; spot-scan imaging and dynamic focus correction; and specimen charging.

*Chapter 6 (Specimen Preparation)* gives a broad yet very thorough introduction to the most widely used methods for preparing macromolecules for imaging by TEM. This includes: selection of TEM grids; preparation of support films of various types (e.g., continuous carbon and holey or lacy films); negative staining; metal shadowing/decoration; embedment in glucose and other "sustains"; vitrification; and cryo-negative staining. Also addressed are issues involving specimen charging and flatness, use of anticontaminators, and use of spot-scan and time-resolved imaging.

Chapters 7–14 provide detailed discussions of the theoretical and practical aspects of studying macromolecular specimens organized as 2D crystals (Chapters 7–11), helices (Chapter 12), icosahedra (Chapter 13), and asymmetric, single particles (Chapter 14).

*Chapter 7 (Symmetry and Order in Two Dimensions)* is a comprehensive, didactic discourse on symmetry and related concepts that are fundamental to the processing and analysis of images of 2D crystals. Two critical concepts, reciprocal lattice lines and types of short- and long-range disorder, round out the discussion.

*Chapter 8 (Two-Dimensional Crystallization Techniques)* is an excellent, up-to-date exposé on a variety of methods for inducing macromolecules to form highly-ordered 2D crystals or helical arrays so they might yield structural results at moderate to high resolution.

*Chapter 9 (Data Processing: Diffraction Patterns of 2D Crystals)* and *Chapter 10 (Data Processing: Images of 2D Crystals)* provide nuts and bolts, detailed descriptions of how data from 2D crystals are recorded and processed to produce a final, 3D density map of the unit cell contents. The pros and cons of recording diffraction intensity data photographically (film) or digitally (CCD) are thoroughly discussed as is the need to convert analog (film) data to digital form. Pattern indexing, intensity measurements, background subtraction, data merging, lattice-line fitting and refinement, and data quality measures complete the Chapter 9 topics. Chapter 10 describes how optical diffraction is used to evaluate image quality; how information transfer is limited by image digitization; how FFTs provide efficient computations, indexing, and extraction of amplitudes and phases from diffraction patterns; how data from separate images are merged; how data quality are evaluated; how quasi-optical filtering is used to reduce noise and facilitate corrections ("unbending") for electron optical or specimen distortions; and how corrections are made to compensate for the effects of the microscope contrast transfer function and when the electron beam or specimen is tilted.

*Chapter 11 (High-Resolution Density Maps and Their Structural Interpretation)* provides brief overviews of several topics, including: displaying features in density maps, handling the "missing cone" problem with 2D crystals, building a known chemical structure into a high-resolution density map, fitting atomic models into density maps, model refinement, and use of difference Fourier maps. Use of color provides a powerful means to highlight features in complex structures that might otherwise remain obscure, but unfortunately no color reproductions are included in the book.

Chapters 12–14 discuss the analyses of single particles that exhibit helical, icosahedral, or no symmetry. *Chapter 12 (Electron Crystallography of Helical Structures)* is noteworthy for an extensive tutorial on the analysis of real helical structures, i.e., those that exhibit various types of disorder and hence imperfect symmetry. The mature Fourier-Bessel methods are thoroughly described, but it would be helpful to include more expanded discussions of newer approaches that have been developed to analyze images of imperfect helical particles (e.g., with seams or that are highly flexible). As an example of just how fast the field is moving, some of the challenges identified in *Chapter 13 (Icosahedral Particles)* at the time of writing have now recently been addressed successfully and virus structures at better than 0.4 nm resolution are appearing in the literature. *Chapter 14 (Single Particles)* provides a comprehensive review of the specialized methods that have been developed to handle images of asymmetric particles, with the ribosome serving as the prime example of a complex that has been studied exhaustively and recently at sub-nanometer resolution.

*Chapter 15 (Special Considerations Encountered with Thick Specimens)* deals with three primary, "under the rug issues." Since real specimens are not infinitely thin and thus not pure "phase objects" as was assumed in previous chapters, the effects that multiple elastic scattering, inelastic scattering, and curvature of the Ewald sphere have in images are discussed. Practical tips on how these effects might be treated to enhance the reliability and resolution achieved in 3D reconstructions are offered.

Of course, no book is perfect nor will it fulfill the need of every reader. The number of typos and minor inconsistencies is remarkably small for a book as comprehensive as this one. However, the primary disappointment, and one likely echoed by the authors, resides in the poor reproduction quality of many illustrations. This is surprising given modern publishing practices but even more so because images and their inherent quality form the heart and soul of 3DEM. Most continuous tone figures are irregular and exhibit poor contrast and consequently do an injustice to the data. In some instances they render the accompanying text ineffective. Also, many figures, particularly in Chapter 11, would be significantly enhanced by judicious use of color. On balance however, the book is a must have resource for anyone wishing to become a 3DEM expert.

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