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Three-Dimensional Electron Microscopy of Macromolecular Assemblies

Joachim Frank

Published in print: 2006 Published Online: April 2010 Publisher: Oxford University Press
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Item type: book

In this book, the mathematical principles and working methods of single-particle reconstruction are described; a method designed to retrieve three-dimensional structural information from electron micrographs showing thousands of “copies” of biological molecules trapped in a thin layer of ice. This technique is uniquely suited to obtain three-dimensional images of molecular machines in different functional states, as it dispenses with the need for crystals. The book starts with an introduction of image formation in the electron microscope, which includes the definition of the contrast transfer function. Next, averaging techniques and tools for image alignment, multivariate data analysis, and classification are described. An introduction into the mathematical principles underlying reconstruction of an object from its projections is followed by detailed accounts on how projection angles are determined, and how reconstruction is done in practice. The book concludes with a chapter on interpretation of density maps reconstructed, including methods for segmentation as well as fitting and docking of atomic coordinates.

Introduction

Joachim Frank

in Three-Dimensional Electron Microscopy of Macromolecular Assemblies: Visualization of Biological Molecules in Their Native State

Published in print: 2006 Published Online: April 2010 Publisher: Oxford University Press
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Item type: chapter

This introductory chapter begins with an appreciation of the unique position of electron microscopy in biological research as it bridges a wide

gap between X-ray crystallography and light microscopy. The scope of this book is defined to cover three-dimensional imaging of molecular assemblies that exist in an in vitro sample in large numbers with identical or near-identical structure. Only with such samples it is possible to collect large numbers of projection images suitable for averaging and three-dimensional reconstruction. The fact that molecules can be imaged as single, isolated particles embedded in ice ("crystallography without crystals") makes the techniques described in this book uniquely suited to image molecular machines in their various processing states. In sharp contrast, electron tomography, not covered in this book, is concerned with the three-dimensional imaging of "unique" objects that may be an organelle or slice of a cell. The vision of a unified structural analysis of macromolecules is articulated, which would lead to an integration of results from cryo-EM, X-ray crystallography and NMR, and a cross-fertilization among these disciplines. The chapter concludes by making the point that the development of single-particle reconstruction would not have been possible without the vast increase in computer power seen in the past decades.

Electron Microscopy in Three Dimensions

Peter B. Moore

in Visualizing the Invisible: Imaging Techniques for the Structural Biologist

Published in print: 2012 Published Online: May 2015
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Item type: chapter
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This chapter emphasizes that the ultimate goal of structural biology is to define the three-dimensional structure of biological specimens at atomic resolutions. To meet this goal, three-dimensional reconstruction strategies such as tomography, single particle reconstruction, and electron crystallography are suggested. the chapter also lays down the procedure and loopholes for transforming two-dimensional micrographs into three-dimensional images.

Digital Reconstructions of Sauropod Dinosaurs and Implications for Feeding

Kent A. Stevens and J. Michael Parrish

in The Sauropods: Evolution and Paleobiology

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This chapter discusses the method for three-dimensional reconstructions of sauropod skeletons, focusing on the pose of the neck and its implications for sauropod herbivory. It determines that most sauropods as medium to low browsers. Neutral pose reconstructions suggest that most sauropods would have their necks held horizontally or subhorizontally when not actively feeding or otherwise raising their heads. The feeding envelope for a given taxon can be visualized as the extremes of head reach allowed by the flexibility of its neck. It is clear from the cranial and dental studies that significant differences existed among sauropod feeding mechanisms. Brachiosaurus and, perhaps, Camarasaurus (at the upward extent of its feeding envelope) appear to have been the only Jurassic sauropods clearly capable of feeding as “high browsers” on arborescent gymnosperms.