[redacted]MAGING LIFE

Biological Systems from Atoms to Tissues

Edited by
GARY C. HOWARD
WILLIAM E. BROWN
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Imaging Life

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Imaging Life

William E. Brown

During the preparation of this book and far too early, we lost Bill Brown. He was an extraordinary scientist, teacher, and leader, who influenced the lives and careers of scores of young scientists at Carnegie Mellon University for over thirty years. Bill looked for the very best in every experiment and every student. He was amazingly generous with his time, help, and encouragement. To me, he was a great mentor, role model, and friend.

Gary C. Howard

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Imaging Life

MANFRED AUER AND GARY C. HOWARD ■

> And in Physical Enquires, we must endeavor to follow nature in the more plain and easy the treads in the most simple and uncompounded bodies, to trace her steps, and be acquainted with her manner of walking there, before we venture ourselves into the multitude of meanders she has in bodies of a more complicated nature.
>
> —Robert Hooke, *Micrographia or Some Physiological Descriptions of Minute Bodies Made by Magnifying Glasses with Observations and Inquiries thereupon*

Humans have been wondering about life for centuries. Mesmerized by the living world around them, early biologists were driven by the basic, yet still relevant question, "what is life?" Early scientists, such as Alcmaeon and Croton in the fifth century B.C. and Erasistratus and Herophilos in the third century B.C., began to describe the human body in remarkable detail, illuminating function through the description of form. Despite these early accomplishments, another two thousand years passed with little progress. Dissections of human and animal bodies were constrained by the visual abilities of our eyes, and our understanding was limited to the level of organs and tissues.

In the late seventeenth century, the invention of the optical microscope began a revolution in biology that merged our curiosity with our understanding of physics and light. When Antonie van Leeuwenhoek looked through his primitive microscope for the first time, he saw a world of microbes that no one even knew existed. Although his claims of single-cell organisms were greeted with great skepticism (as many important discoveries often are), it quickly became clear that "seeing was believing." Even today, few things are more powerful than an imagefor convincing skeptics.

The light microscope dominated biological discovery for a hundred years. Improvements to contrast-generating chemistry and better knowledge of the physical instrumentation improved biological imaging and allowed scientists to more

fully exploit their potential. Clever selective chemical staining procedures enhanced imaging and laid the foundation for our understanding of life at the level of cell organelles, cells, and tissues.

At the turn of the twentieth century, scientists became more aware of the full range of the electromagnetic spectrum, including X-rays, electrons, neutrons and positrons, and gamma rays. Novel forms of imaging were based on the traditional optical, radio, and microwave window of the electromagnetic spectrum, but, more recently, were expanded to include approaches that provide chemical information, such as FTIR, Raman, and mass spectrometry imaging. In second part of the twentieth century, most of the modern imaging technologies were invented or substantially refined. Besides optical light microscopy, the use of X-ray and electrons in diffraction and microscopy, as well as radio waves and microwaves for NMR and fMRI, revolutionized biological imaging and led to exquisite insights into the sophisticated organization and biological functionality of tissues, cells, organelles, and proteins.

Through advances in imaging, the basic building blocks of life, such as proteins, nucleic acids, lipids, and carbohydrates, were described in increasing detail. The oldest discipline for structure determination is X-ray crystallography. Although it is not strictly an imaging method—a diffraction pattern is interpreted and refined by using an atomic model—it quickly became the "gold standard" for structural imaging. For example, it yielded molecular mechanisms that accurately explained protein function. The DNA double helix immediately suggested a mechanism of faithful replication of the genetic information, and enzymatic functions could be explained by ingenious yet simple stereochemical macromolecular organization.

Twentieth century biologists gathered around the idea that cells could be viewed as bags of enzymes, organelles as mere reaction vessels for enzymatic function. Hence, using the "divide and conquer" strategy of biochemistry made sense for determining the function of one protein at a time. As a result of this reductionist approach, we purified each component and used X-ray and electron crystallography or nuclear magnetic resonance (NMR) spectroscopy to determine its atomic or near-atomic structure. The success rate was substantially enhanced by genetic engineering to improve protein stability, reduce conformational heterogeneity, and introduce primary sequence amino acids that facilitate the process of subsequent X-ray structural analysis. Structural analysis combined with site-directed mutagenesis allowed mechanistic insights and prediction of protein function and into protein-protein interaction. Biochemistry was transformed from a discipline that merely sought to enrich and cleverly test enzyme activity to a powerhouse that, when combined with structural analysis, could explain in detail protein function, fueled by high-resolution structures of increasingly complex proteins and small (and not so small) protein complexes. The field of structural biology was born. System complexity was low (often one pure protein species), but mechanistic insight was extremely precise.

Biochemistry's sibling discipline was cell biology, in which the system was often messy and ill-defined, but the observations were tightly linked to actual cell biological phenomena. Within cell biology, electron microscopy has a unique place. Our understanding of cellular function is intimately linked to the advancement in electron microscopic imaging with a number of cellular macromolecular components, such as actin, microtubule, and intermediate filament networks, constituting the cytoskeleton, a variety of organelles and intracellular compartments, as well as