BOOK REVIEW

Live Cell Imaging, A Laboratory Manual

Editors, Robert D. Goldman, David L. Spector, 631 pages+xvi, ISBN 0-87969-683-4, illus., appendices, index, DVD. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (2005), $159.00 paperback.

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In the late 1600s, Antoni van Leeuwenhoek developed his single lens microscope and began to report his observations to the Royal Society in London. In the course of these reports, he described the mobility of insect and human spermatozoa. Thus began live cell microscopy with the sun as the light source, and the human eye as the detector.

Once termed *intravital fluorochroming,* live cell imaging's long history coincides with many advances in biology and medicine. For instance, in the 1930s intravital microscopy was used to study the microcirculation of the conjunctiva, skin, kidney, and the liver. However, in recent decades advances in microscope design and molecular probes have revolutionized the field.

Live cell imaging technology serves to overcome two critical problems: how to keep the cells alive in their normal physiological and morphological state, and how to mitigate and control myriad external factors (specimen preparation and maintenance; photodamage, thermal damage, optical aberrations to the microscope; image analysis and faulty interpretation). For example, *in vivo* imaging of animal tumors and pathology requires that probe and instrument artifacts do not alter the typical development and regression of tumors.

The combination of epifluorescence microscopes with advances in immunofluorescence has resulted in greatly enhanced specificity and sensitivity for live cell imaging. *Live Cell Imaging* is a comprehensive laboratory guide and textbook that covers, in detail, topics related to these advances.

Several features of *Live Cell Imaging* are exemplary and can be attributed to the excellent editing of Goldman and Spector. It is clear that the editors have extensive experience in teaching students the intricacies of live cell imaging—the emphasis is on safety, caution, and compliance with university, state, and federal regulations; the book is both comprehensive and clear in its exposition; and the physical basis of each instrument or technique is described at the level of a high-quality research publication with sufficient physical and mathematical details. There is a very good balance between the detailed description of the protocols and the validation and calibration of the resulting images and mathematical analysis. As a much welcomed balance, the authors frequently discuss disadvantages, artifacts, and problems in an open manner; the sections on troubleshooting serve to provide the necessary solutions. Overall, from a pedagogical viewpoint, *Live Cell Imaging* successfully integrates a well-written text with numerous tables of disparate data, together with full colored images, illustrations, movies on a DVD, and photographs of actual laboratory equipment.

Live Cell Imaging is divided into two parts. Part 1 contains chapters on the technical approaches to live cell imaging—how to construct and express green fluorescent proteins, and how to use microinjection methods. Individual chapters then introduce various microscopic techniques: polarization, confocal, multiphoton, deconvolution, multispectral, and structured illumination methods. Part 2 covers the imaging of live cells and organisms. Examples range from single molecules, cells, tissues, and whole organisms.

Each chapter includes an introduction with references for further insight and, next, a section that provides a general overview of the technique. With the appropriate background each chapter contains a very detailed set of protocols that can be easily followed. Typically the authors include images and data from specific experiments. The emphasis is on the correct experimental realization of the various techniques and therefore a great effort is made to discuss practical problems and their suggested solutions, sources of materials and instruments, validation and calibration of the techniques, as well as numerous pitfalls and suggestions on how to minimize them.

The mere title of the text, *Live Cell Imaging, A Laboratory Manual,* accurately suggests the book's primary use and audience. Perusal of the book demonstrates that its format, detailed protocols, and movies all provide an excellent manual to accompany a laboratory course on live cell imaging. However, these same attributes also make the book a very good textbook for undergraduate and graduate courses on live cell imaging. It could be a precious resource for research groups centered on live cell imaging. In addition, I suggest the book to individual researchers who wish to use these techniques in their investigations of cells in culture, organs culture, tissues, and whole animals.

A major strength of the text is a rare emphasis on the validation and calibration of the techniques of live cell imaging, as well as the propensity of the authors to discuss the disadvantages, artifacts, and problems in an open manner. For example, many investigators use multiple fluorescent probes and the images are acquired with specific sets of filters and dichroic mirrors. The important problem of filter cross-talk is carefully addressed and mathematical solutions to fluorochrome separation are described in detail.

Clearly, the goal of live cell imaging—whether the sample is cells in culture, or whole organisms—is to answer carefully posed questions and investigate normal and pathological processes. The field's most common questions include "How does this growth factor affect stem cell differentiation?" "What proteins are expressed by this gene?" "On which chromosome and which position is a given gene located?" "What is the interaction between vascular growth and tumor

growth?" "What are the dynamics of cell division?" "How to visualize single RNA molecules in cells?" There is agreement that live cell imaging is important for studies of cell division, differentiation, and apotosis, neuronal plasticity and remodeling, cellular pathology, membrane traffic, and gene expression among many other topics. The text comments on many of these intricacies and provides answers to these questions.

Most of the live cell imaging techniques involve some type of linear or nonlinear fluorescence microscopy: acquiring sequences of images of time, fluorescence resonance energy transfer, photobleaching studies, fluorescence lifetime imaging. The specimen may be single molecules, single cells, tissues, or an organism, but in every case there is some degree of photobleaching and phototoxicity that affects the normal cellular and physiological processes to various degrees. *Live Cell Imaging* is replete with helpful hints and tips to minimize these deleterious processes, though they can never be eliminated. The key to validate and calibrate the live cell studies is to be able to assess the degree and distribution of artifacts. The book describes unique solutions for each of the specimens that are studied. For example, in the study of live mammalian cells it is critical to use the proper medium, and to control the temperature, the *p*H, the oxygen levels, and the osmolarity. The book is very through in its description of chambers for live cell imaging, devices for temperature control, and tips for selecting cell media for mammalian cells.

Relative to live cell imaging processes, the topic of computer image analysis is often subject to pitfalls. This is especially true when the source code of commercial software is not provided. There is the possibility that assumptions made in the writing of the software compromise the interpretation of the results. Relevant chapters in *Live Cell Imaging* provide an overview of many commercial and free software packages, emphasizing that the critical user should consult with experienced users and study the Web sites of the vendors.

There are several deficits in this otherwise well-conceived book. Though the chapter on charge-coupled device (CCD) cameras for fluorescence imaging provides the reader with a qualitative and quantitative overview of CCD cameras, it lacks a discussion of back-illuminated CCDs, which can have quantum efficiencies of over 90%. In addition, a table containing the operational parameters from a variety of CCD cameras from various commercial sources (including their Web sites) would be extremely useful for the reader.

It is also surprising that two important topics of live cell imaging were not included in the volume: dermal imaging and ocular imaging. In 1947 Leon Goldman published a paper on the use of light microscopy to image cells in living human skin. Since that publication the field of *in vivo* skin imaging has developed and three-dimensional imaging of cells can now be obtained with a variety of optical instruments: laser scanning confocal microscopes, optical low-coherence tomography, and multiphoton excitation microscopy.

Furthermore, recent developments in optical microscopy with applications to live cell imaging are not discussed. An appendix could be added that provides annotated references for key advances that could not be included in the text.

A comprehensive chapter on the genesis of live cell microscopy would be a welcome addition in a new edition. There is a long and fascinating history of live cell imaging that starts with Leeuwenhoek and that history should be included in order that students may be aware of the past triumphs and failures. It would also be useful to include a chapter on live cell imaging of complex plants.

In spite of its few deficits, *Live Cell Imaging* is a highly recommended and a very practical book. The production quality of the book is very high. The multicolor illustrations are very sharp and clearly illustrate the text. They are complemented by easily understandable and complete figure legends.

What's more, the chapters are augmented by over 50 movies contained on the DVD, which present the dynamic aspects of live cell imaging. These movies are typically about 30 seconds in duration, although they represent hours of time-lapse image acquisition, and many of them are in pseudocolor. They serve to remind the viewer that proteins and organelles in living cells are dynamic.

In summary, *Live Cell Imaging* is well written, clear, comprehensive, well illustrated, accurate, and extremely useful as a laboratory manual or a textbook.