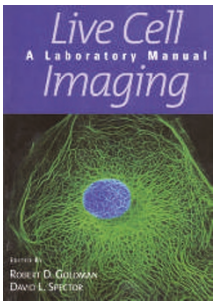
Becoming a cell voyeur



Live Cell Imaging: A Laboratory Manual

Edited by Robert Goldman and David Spector

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If a picture paints a thousand words, the information contained in a time-lapse image series should provide enough text for several volumes. Before the 1990s, most micrographs taken of cells were of fixed specimens. Many cellular phenomena of interest, however, are highly dynamic processes and much information is lost when cells are fixed. Furthermore, cell fixation is prone to artefacts in both localization patterns and gross morphology. Live cell imaging techniques have circumvented these issues and now scientists can observe molecules *in vivo* over time in cells, tissues, or whole organisms, thus greatly increasing our understanding of cellular and developmental events. At first, live cell imaging was limited to a few laboratories that had self-assembled microscopes complete with shuttering and filter wheel devices to adapt the light path for specific experimental needs. Now, most institutes are equipped to perform high-resolution imaging in living specimens, and thus more laboratories than ever before want to gather dynamic experimental information through time-lapse imaging. The growth of this technology has created a need for an authoritative and comprehensive manual on the subject; a need that is addressed by *Live Cell Imaging: A Laboratory Manual*, edited by Robert Goldman and David Spector. This book, written by knowledgeable and experienced microscopists from around the world, provides a collection of methods for modern microscopic techniques.

The first step in designing a live cell imaging experiment is to determine which probes are best suited for addressing the question at hand. This new book devotes several chapters to fluorescent probe selection and delivery methods as well as imaging requirements for excitation and emission of these probes. For example, there are two ways to image RNA distribution and movement in living cells: either by injecting fluorescently labelled nucleotides, or by using a fluorescent protein — such as enhanced green fluorescent protein (eGFP) — fused to a viral protein that binds to a sequence engineered into the RNA of interest. Likewise, the actin cytoskeleton can be imaged either with actin/actin-binding protein–eGFP fusions or with purified, chemically labelled proteins introduced into cells by microinjection. Deciding which fluorescent probes should be used for a given experiment ultimately depends on the microscope setup and the researcher's needs. For example, a sample that is highly autofluorescent when excited with 488-nm light would preclude the use of eGFP, and consequently a fluorophore with a different excitation spectrum would be a better choice in this system. Owing to the fundamental importance of probe selection in designing an experiment, microscopists will benefit greatly from these chapters.

Also critical to live cell imaging is determining the optimum microscopic conditions — without damaging the specimen — for a given time-lapse experiment, the source of illumination, lens elements, filters and detectors all being important considerations. To this end, the manual is well designed to facilitate streamlining a microscope to suit your needs. It covers many examples of how, for each experiment and biological system, settings and components must be tailored to address the user-defined questions and experimental designs. For instance, thin specimens within a few micrometres of the coverslip are often best imaged with either spinning-disc confocal microscopy or wide-field epifluorescence microscopy combined with deconvolution, whereas neurons in a living mammal may only be resolvable using multi-photon laser scanning microscopy (MP-LSM), which would minimize both out-of-focus light and scatter from such a thick specimen.

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Similarly, high-magnification and high-numeric aperture objectives are essential for the resolution demands of fluorescent speckle microscopy (FSM), but low-magnification and -numeric aperture optics may be better for bleaching large areas of a cell during whole-cell photobleaching experiments. In this way, the manual is useful even to expert microscopists, as it covers many aspects of experimental setup for numerous imaging techniques. In addition, readers with all levels of experience will appreciate the accompanying DVD, which contains many beautiful movies taken using the microscopes described in the text.

For the novice reader, however, one weakness of this book is the omission of a basic chapter on light microscopy and the fundamentals of microscope alignment. When imaging living cells, light levels are especially important, as too much light will damage the specimen. Microscopists must first understand how to align and maintain the optics to maximize the signal from the microscope. For this, the inexperienced microscopist may wish to consult a more basic reference before beginning experimentation.

Overall, this new book is a superb resource for imaging techniques commonly used to improve time-lapse data, and a valuable addition to any laboratory's library. It emphasizes the multitude of model systems that can be studied using time-lapse imaging and will surely motivate many more to join the live cell imaging revolution.

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