## Biomedical Optics

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## **Quantitative Phase Imaging of Cells and Tissues**

Barry R. Masters



## BOOK REVIEW

## Quantitative Phase Imaging of Cells and Tissues

Gabriel Popescu, 384 pages; ISBN 978-0-07-166342-7, McGraw-Hill, New York (2011), \$150 hardcover.

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Gabriel Popescu, an eminent educator and researcher, has provided the biomedical optics community with a concise overview of quantitative phase microscopy. It was first implemented by the author in the spectroscopy laboratory at the Massachusetts Institute of Technology and subsequently developed by the author and his colleagues in the Department of Electrical and Computer Engineering, and the Beckman Institute for Advanced Science & Technology at the University

of Illinois at Urbana-Champaign.

The audience for this book consists of graduate students and researchers with prior study of physical optics, electromagnetic fields, and digital signal processing. The book's appendices contain brief summaries of some subsets of these topics; however, the careful reader requires a more comprehensive background in order to understand the book's mathematical development. I strongly suggest that the reader first become familiar with the standard textbooks that the author cites following the appendices: the textbooks by Born and Wolf; Mandel and Wolf; Papoulis, Bracewell, Gaskill, and Goodman. In the first six chapters the author presents a very concise summary of topics that range from the first-order Born approximation to the coherent properties of light and holography. This summary and the two appendixes at the end of the book are too condensed for independent study by a reader without a background in physical optics, electromagnetic fields, and mathematics. For example, on page 9 the author briefly introduces Green's function and then uses it to develop wave propagation on the subsequent pages.

Quantitative Phase Imaging of Cells and Tissues provides a summary of the current state of these interferometric microscopic techniques and their applications to problems in the domain of biomedical optics. The author provides an excellent set of references at the end of each chapter and that is very useful for the new investigator who wishes to work with these imaging techniques. The reader will appreciate the discussions of the previous works of Abbe on image formation in the optical microscope; Zernike on phase microscopy; Gabor, Leith and Upatniek on holography; and the works of Mandel and Wolf on optical coherence.

The field of biomedical optics is in a phase of active development in the areas of new instrumentation, new probes, and new algorithms. The biomedical optics community envisions that these advances will increase our understanding of biological processes and have a major impact on the diagnosis and the treatment of disease. Therefore, the continued development and applications of emerging techniques in optical imaging are both desirable and important. What is important for the development of the field is a diminution of the hype for a particular instrumental approach in cellular and tissue imaging, and in its place a critical evaluation of the new device or application and a comparison with other imaging modalities. For example, is the new optical technique more suitable to a particular cell type and structure; e.g. thin and flat cells, or is it only applicable for erythrocytes? Is the mathematical analysis specific to flat cells, or is it also applicable to thick cells? What are the assumptions that are required to analyze the experimental data and produce the phase images? Have these assumptions been adequately validated; and for which specimens? What are the limitations of a specific technique and its image analysis? Another important question is how does a specific technique compare and contrast with alternative methods in biomedical imaging? These questions are important and should be adequately addressed in the book. Unfortunately, I conclude that these important omissions diminish the pedagogical value of the book.

Quantitative Phase Imaging of Cells and Tissues describes several probeless optical imaging techniques. Therefore they are devoid of the problems of the overexpression of genetically expressed fluorescent proteins. It also avoids the problems of photobleaching that occur with fluorescent probes. What is lacking is the high sensitivity of imaging techniques that are based on probes. For a relatively simple cell such as the erythrocyte the interpretation of the phase images is not a formidable task. But for more complex cells and tissues the validation of the phase images and their correlation with cellular structures and specific cell functions requires extensive studies with correlative microscopy. In the book there are many examples of what the author denotes as reasonable assumption in the interpretation of phase images, but this is very different from a careful validation. The specificity of phase imaging is low in comparison to alternative imaging techniques. This in no way diminishes the usefulness of the technique when assumptions and models to interpret the data are properly validated. However, the reader is typically pointed to the numerous references for further details. In this aspect the book is not self-contained in its exposition.

But there are other probeless optical imaging techniques that are based on low-coherence optical tomography or on pump-probe imaging techniques. The book fails to adequately compare the phase-imaging techniques with other optical techniques. With all the limitations of fluorescent probe techniques that are described elsewhere these methods do have a high specificity and are capable of detecting single-fluorescent molecules. Imaging techniques that are based on fluorescent probes also have a major role in molecular biology and its associated technologies, for example, their use in sequencing the genome. *Quantitative Phase Imaging of Cells and Tissues* fails to place these techniques in the context of other optical imaging techniques.

The author has introduced, albeit in a very concise manner with constant pointers to the references for further details, a wide variety of topics that relate to phase imaging microscopies. I conclude following my perusal of the book that *Quantitative Phase Imaging of Cells and Tissues* is more suitable as a text to accompany a short course (which the author organizes) than as a textbook for independent study.

The production of the book is less than adequate. The atypical mathematical type setting results in symbols that are printed with a size that is too small and that makes reading them difficult. Similarly, the line spacing is too small and that also makes the reading difficult. While the line drawings of the instruments are very good in general the figures are inadequate. The color bars should always have labels and units. The figure legends should be self-contained and define all the terms uses in the figures. In many cases the reader must search in the text for the understanding of the terms in the figures.

In summary, *Quantitative Phase Imaging of Cells and Tissues* provides the reader with an overview of the various imaging techniques and biological applications that are related to quantitative phase imaging. The author's survey of the literature is very well done. This book is useful when it accompanies a course and the reader has an adequate preparation in physical optics, electromagnetic fields, and mathematics.