

Introduction

In this wide-ranging review, Olympus microscopy experts draw on their extensive knowledge and many years of experience to provide readers with a highly useful and rare overview of the combined fields of microscopy and imaging. By discussing both these fields together, this review aims to help clarify and summarise the key points of note in this story. Encompassing the creation of highly detailed specimen views through to final image capture and processing, the story will also be backed up by useful insights, examples, as well as handy hints and tips along the way.

To make it easy to dip in and dip out of this comprehensive overview, the review is clearly structured into several chapters. These take the reader from explanations of the physics of light, colour and resolution, through details of contrast and fluorescence techniques, to finish up with discussion on 3D imaging. Highlighting and summarising key points made within this review are a series of information boxes. These also aim to provide useful insight on techniques, as well as expert advice on microscope set up.

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Dear Reader

“A Huge Number of Techniques in a Relatively Short Space”



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Although microscopes are becoming more and more easy to use it still remains important to have an appreciation of the fundamental principles that determine the resolution and contrast seen in microscope images. This series of articles, by authors from Olympus, aims to provide an overview of the physical principles involved in microscope image formation and the various commonly used contrast mechanisms together with much practically oriented advice. Inevitably it is impossible to cover any of the many aspects in great depth. However their approach, which is to discuss applications and provide practical advice for many aspects of modern day microscopy, will prove attractive to many.

The articles begin by setting the scene with a discussion of the factors that determine the resolving power of the conventional microscope. This permits the introduction of important concepts such as numerical aperture, Airy disc, point spread function, the difference between depth of focus and depth of field and the concept of parfocality. Common contrast mechanisms such as darkfield and phase contrast are then introduced followed by differential interference contrast and polarisation contrast imaging. Throughout the discussion the importance of digital image processing is emphasised and simple examples such as histogram equalisation and the use of various filters are discussed.

The contrast mechanisms above take advantage of the fact that both the amplitude and phase of the light is altered as it passes through the specimen. Spatial variations in the amplitude (attenuation) and/or the phase are used to provide the image contrast. Another extremely important source of image contrast is fluorescence whether it arise naturally or as a result of specific fluorescent labels having been deliberately introduced into the specimen. The elements of fluorescence microscopy and techniques of spectral unmixing are discussed and brief mention is made of more advanced techniques where spatial variations in, for example, fluorescence lifetime are used to provide image contrast. Finally techniques to provide three-dimensional views of an object such as those afforded by stereo microscopes are discussed together with a very brief mention of the confocal microscope.

The authors have attempted the very difficult task of trying to cover a huge number of techniques in a relatively short space. I hope you enjoy reading these articles.

“Unique presentation of technical aspects in connection with image processing”



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As a regular reader of “Imaging & Microscopy,” the title of this issue, “Basics of Light Microscopy & Imaging,” must certainly seem familiar to you. From March 2003 to November 2005, we had a series of eleven articles with the same name and content focus.

In collaboration with the authors, Dr. Manfred Kässens, Dr. Rainer Wegerhoff, and Dr. Olaf Weidlich of Olympus, a lasting “basic principles” series was created that describes the different terms and techniques of modern light microscopy and the associated image processing and analysis. The presentation of technical aspects and applications in connection with image processing and analysis was unique at that time and became the special motivation and objective of the authors.

For us, the positive feedback from the readers on the individual contributions time and again confirmed the high quality of the series. This was then also the inducement to integrate all eleven articles into a comprehensive compendium in a special issue. For this purpose, the texts and figures have been once more amended or supplemented and the layout redesigned. The work now before you is a guide that addresses both the novice and the experienced user of light microscopic imaging. It serves as a reference work, as well as introductory reading material.

A total of X0,000 copies of “Basics of Light Microscopy & Imaging” were printed, more than double the normal print run of “Imaging & Microscopy.” With this, we would like to underscore that we are just as interested in communicating fundamental information as in the publication of new methods, technologies and applications.

Enjoy reading this special issue.

“You Can Get Keywords for Expanding Your Knowledge”



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There is a great merit for publishing “Basics of LIGHT MICROSCOPY and IMAGING: From Colour to Resolution, from Fluorescence to 3D imaging”. It is a great merit since it is very important to define, to clarify, and to introduce pivotal elements for the comprehension and successive utilization of optical concepts useful for microscopical techniques and imaging. The niche still occupied by optical microscopy, within a scenario where resolution “obsession” plays an important role, is mainly due to the unique ability of light-matter interactions to allow temporal three-dimensional studies of specimens. This is of key relevance since the understanding of the complicated and delicate relationship existing between structure and function can be better realized in a 4D (x-y-z-t) situation. In the last years several improvements have pushed optical microscopy, from confocal schemes [1-6] to multiphoton architectures, from 7-9 folds enhancements in resolution to single molecule tracking and imaging allowing to coin the term optical nanoscopy [7]. Advances in biological labelling as the ones promoted by the utilization of visible fluorescent proteins [8-12] and of overwhelming strategies like the “F” techniques (FRAP - fluorescence recovery after photobleaching, FRET – Fluorescence Resonance Energy Transfer, FCS – fluorescence correlation spectroscopy, FLIM – fluorescence lifetime imaging microscopy) [4, 13-15] collocate optical microscopy in a predominant position over other microscopical techniques. So it becomes mandatory for primers, and more in general for all those researchers looking for answers about their biological problems that can be satisfied by using the optical microscope, to have a good starting point for finding the optimal microscopical technique and for understanding what can be done and what cannot be done. This long note on Basics can be a good point for starting. It brings the reader through different concepts and techniques. The reader can get the keywords for expanding her/his knowledge. There are some important concepts like the concept of resolution and the related sampling problems, spectral unmixing and photon counting that are introduced for further readings. This article report some interesting examples and a good link between the different mechanisms of contrast from DIC to phase contrast until fluorescence methods. Treatment is not rigorous but it keeps the audience interested and is sufficiently clear. I read it with interest even if I would prefer to have more bibliographic modern references to amplify the achievable knowledge.

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Imprint

Published by
GIT VERLAG GmbH & Co. KG

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Printed by
Frotscher Druck
Riedstrasse 8, 64295 Darmstadt, Germany

Circulation
20,000 copies

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