CRC

IMAGE ANALYSIS *in* BIOLOGY

Donat-P. Häder

CRC PRESS

Image Analysis in Biology

Editor

Donat-P. Häder, Dr.rer.nat.
Professor of Botany
Department of Botany and Pharmaceutical Biology
Friedrich-Alexander University
Erlangen, Germany

CRC Press
Boca Raton Ann Arbor London

Library of Congress Cataloging-in-Publication Data

Image analysis in biology / editor. Donat-P. Häder.
p. cm.
Includes bibliographical references and index.
ISBN 0-8493-6033-1
1. Microscope and microscopy. 2. Image processing. I. Häder, Donat-Peter.
QH205.2.I43 1991
578'.4—dc20 91-3778
CIP

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida 33431.

International Standard Book Number 0-8493-6033-1

Library of Congress Card Number 91-3778

Printed in the United States of America 1 2 3 4 5 6 7 8 9 0

PREFACE

Automatic image analysis has become an important tool in many fields in biology, medicine, and other sciences. The availability of powerful hardware and the development of specialized algorithms have dramatically improved the versatility of digital image analysis. Depending on the application, the techniques can be fully automatic or interactive with a human operator; they can operate in real time or require long calculation times dictated by the complexity of the task. The image can be either black and white or color, or a pseudocolor representation of gray values can be used.

The applications range from the submicroscopic (transmission and scanning electron microscopy) and even atomic level (scanning tunnel microscopy) to the macroscopic scale. Simple techniques include counting and quantitating objects such as leaf areas or sizes of microorganisms. Specific mathematical filters have been developed to enhance the quality of the original image or to extract specific features of interest. More complex programs attempt to analyze the form of objects in order to discriminate, e.g., cancer cells from normal tissue cells. Three-dimensional analysis of proteins, organelles, or macroscopic objects is even more involved since the number of calculations multiplies with the number of layers in the third dimension.

Real-time applications include cell and organism tracking used to analyze motility, velocity, and the direction of movement of motile organisms. Several techniques have been developed to detect the position of individuals and to follow these objects during a predetermined time interval.

As editor I very much appreciate the contributions of the authors to this book. Their specialized and diverse expertise with the various aspects of hardware, software, and biological application is reflected in each chapter, all of which meet a high quality standard.

Donat-P. Häder
Erlangen, Germany

THE EDITOR

Donat-P. Häder, Dr.rer.nat., is Professor of Botany, Department of Botany and Pharmaceutical Biology, at the Friedrich-Alexander University at Erlangen, Germany.

He received his doctoral degree and his habilitation from the University of Marburg. He has held a research associate position in the Department of Energy, Michigan State University, East Lansing, Michigan, and was visiting scientist in the Chemistry Department, Texas Tech University, Lubbock, Texas, CNR Institute of Biophysics, Pisa, Italy, and the National Research Laboratory, Okazaki, Japan.

Professor Häder has worked on the photomovement of microorganisms and the effect of solar ultraviolet radiation on phytoplankton and is involved in space biology, studying the effect of microgravity on motility in flagellates. He is a member of the Committee on Ecology for the German Ministry for Science and Technology, an expert for an Enquete Commission of the German Parliament, and a member of a United Nations Environment Program Commission on the Effects of Ozone Destruction.

One of the tools for his research activities is a real-time image analysis system developed over the last eight years. He has published over 130 original papers, coauthored 3 books, and translated 2 others.

CONTRIBUTORS

Shanti J. Aggarwal, Ph.D.
Department of Biomedical Engineering
Biomedical Engineering Program
The University of Texas at Austin
Austin, Texas

Norio Baba, Ph.D.
Department of Electrical Engineering
Kogakuin University
Tokyo, Japan

Egbert J. Boekema, Ph.D.
Biochemistry Laboratory
Rijksuniversiteit Groningen
Groningen, The Netherlands

Alan C. Bovik, Ph.D.
Department of Electrical and Computer Engineering
Biomedical Engineering Program
The University of Texas at Austin
Austin, Texas

Santos Carvajal-Gonzalez, M.Sc.
Laboratory of Quantitative Microscopy in Histopathology
INSERM U.263
University of Paris 7
Paris, France

Giuliano Colombetti, Ph.D.
CNR Institute of Biophysics
Pisa, Italy

Primo Coltelli, Ph.D.
CNR, CNUCE Institute
Pisa, Italy

Judith G. Croxdale, Ph.D.
Department of Botany
University of Wisconsin-Madison
Madison, Wisconsin

Kenneth R. Diller, Sc.D.
Department of Mechanical Engineering
Biomedical Engineering Program
The University of Texas at Austin
Austin, Texas

David B. Dusenbery, Ph.D.
School of Biology
Georgia Institute of Technology
Atlanta, Georgia

Günther Galfe, Dipl.-Ing.
Institut für Zoologie
Technische Universität München
Garching, Germany

David Gauthier, M.Eng.
McGill Research Centre for Intelligent Machines
McGill University
Montreal, Quebec, Canada

Paolo Gualtieri, Ph.D.
CNR Institute of Biophysics
Pisa, Italy

Donat-P. Häder, Dr.rer.nat.
Department of Botany and Pharmaceutical Biology
Friedrich-Alexander University
Erlangen, Germany

George Harauz, Ph.D.
Department of Molecular Biology and Genetics
University of Guelph
Guelph, Ontario, Canada

Tsuyoshi Hayakawa
Central Research Laboratory
Hamamatsu Photonics K. K.
Hamakita, Japan

Michael E. J. Holwill, Ph.D.
Physics Department
King's College
London, England

Nak H. Kim, Ph.D.
Department of Electrical and Computer Engineering
Biomedical Engineering Program
The University of Texas at Austin
Austin, Texas

Martin D. Levine, Ph.D.
Department of Electrical Engineering and
McGill Research Centre for Intelligent Machines
McGill University
Montreal, Quebec, Canada

Roberto Marangoni, Ph.D.
Institute of Biophysics
Faculty of Medicine
University of Genova
Genova, Italy

Peter B. Noble, Ph.D.
Department of Oral Biology
Faculty of Dentistry
McGill University
Montreal, Quebec, Canada

Kenji Omasa, Ph.D.
Environmental Plant Science Department
The National Institute for Environmental Studies
Ibaraki, Japan

Michael Radermacher, Dr.rer.nat.
Wadsworth Center for Laboratories and Research
New York State Department of Health
Albany, New York

Jean Paul Rigaut, Sc.D., M.D., Ph.D.
Laboratory of Quantitative Microscopy in Histopathology
INSERM U.263
University of Paris 7
Paris, France

Peter Satir, Ph.D.
Department of Anatomy and Structural Biology
Albert Einstein College of Medicine
Bronx, New York

Nico A. M. Schellart, Ph.D.
Laboratory of Medical Physics and Informatics
University of Amsterdam
Amsterdam, The Netherlands

Tetsuo Takahashi, Ph.D.
Suntory Institute for Bioorganic Research
Osaka, Japan

Jany Vassy, Sc.D., Ph.D.
Laboratory of Developmental Biology and Cell Differentiation
University of Paris 13
Bobigny, France

Kurt Vogel, Dipl.Biol.
Department of Botany and Pharmaceutical Biology
Friedrich-Alexander University
Erlangen, Germany

Dieter G. Weiss, Dr.rer.nat.
Institut für Zoologie
Technische Universität München
Garching, Germany

TABLE OF CONTENTS

Introduction

Chapter 1

INTRODUCTION

Donat-P. Häder

Vision by man and machine differ in many respects and certainly the human visual apparatus and brain are still far superior to machine vision.[1] Both the spatial and gray or color shade resolutions of the human eye surpass those of high resolution video cameras and the image processing of the human brain far exceeds the speed and complexity of even the fastest computers.[2] However, using image technologies facilitates quantitative analysis and objective interpretation of selected parameters.

Since its first developmental stages in the 1970s, computerized image analysis has witnessed a rapid growth and enormous progress. Digitization of analog images lends itself to image enhancement and to the extraction of specific features of interest such as dimensions, areas of a distinct color or gray shade,[3,4] or movement of individual elements in a sequence of images.[5,6]

The advent of more powerful hardware and the development of robust and efficient algorithms[7,8] have laid the ground for highly specialized and automatic image analysis which has found applications in all fields of biology and medicine.[9-11] Early in the development of image analysis, researchers found interest in the digitization of cellular structures.[12-15] Both light microscopy[16,17] and electron microscopy[18,19] are important fields for image enhancement and analysis, and even holograms have been used as image sources.[20] Especially in medical samples, it is a declared goal to develop automated image analysis systems which allow the recognition and identification of specific features[21] in order to reliably determine quantitative distributions of, e.g., blood cells or sediments[22,23] and to identify abnormal cells such as cancer cells.[24-26] Reliable cell identification requires a high spatial resolution.[27] In addition to morphometric methods,[28] image analysis and reconstruction are used for three-dimensional (3-D) reconstruction of biological structures.[29-32]

This volume is divided into three sections, the first of which describes the methods of image analysis, including 3-D analysis, quantitation by laser scanning confocal microscopy and quantitative area determination. This section is intended to give a more general introduction into the techniques, includes a chapter on digital filters, and describes the principles of object detection in digital images as well as single photon imaging.

The second section focuses on the applications of image analysis in microscopy including contrast enhancement in video images, automatic detection and recognition of live cells, as well as image analysis of bending shapes of eukaryotic flagella and cilia. This section is also devoted to the analysis of subcellular structures in electron micrographs and protein analysis as well as the reconstruction of crystals and 3-D histological objects from serial section images.

The final section describes the techniques used for movement analysis of microorganisms, bacteria and animals, which require fast routines to track the organisms in real time in a sequence of video frames.

REFERENCES

1. **Poggio, T.,** Vision by man and machine, *Sci. Am.*, 250, 68, 1984.
2. **Blake, A.,** Real-time seeing machines?, *Nature*, 328, 759, 1987.
3. **McMillian, P. J., Yakush, A., Frykman, G., Nava, P. B., and Ras, V. R.,** Minima equalization: a useful strategy in automatic processing of microscopic images, *J. Microsc.*, 148, 253, 1987.
4. **Russ, J. C. and Russ, J. C.,** Automatic discrimination of features in grey-scale images, *J. Microsc.*, 148, 263, 1987.
5. **Turano, T. A., D'Arpa, P., Clark, W. L., and Williams, J. R.,** A time-lapse, image digitization videomicroscope system based on a mini computer with large peripheral memory, *Comput. Biol. Med.*, 15, 177, 1985.
6. **Thurston, G., Jaggi, B., and Palcic, B.,** Cell motility measurements with an automated microscope system, *Exp. Cell Res.*, 165, 380, 1986.
7. **Wampler, J. E. and Kutz, K.,** Quantitative fluorescence microscopy using photomultiplier tubes and imaging detectors, in *Methods in Cell Biology*, Wang, Y. L. and Taylor, D. L., Eds., Academic Press, San Diego, 1989, 239.
8. **Sasov, Y.,** An integrated PC-based image analysis system for microtomography and quantitative analysis of inner micro-object structure, *J. Microsc.*, 156, 91, 1989.
9. **Frank, J.,** Image analysis of single macromolecules, *Electron Microsc. Rev.*, 2, 53, 1989.
10. **Dhawan, A. P.,** A review on biomedical image processing and future trends, *Comput. Methods Prog. Biomed.*, 31, 141, 1990.
11. **Kenny, P. A., Dowsett, D. J., Vernon, D., and Ennis, J. T.,** A technique for digital image registration used prior to subtraction of lung images in nuclear medicine, *Phys. Med. Biol.*, 35, 679, 1990.
12. **Holmquist, J., Antonsson, D., Bengtsson, E., Danielsson, P. E., Eriksson, O., Hedblom, T., Martensson, A., Nordin, B., Olsson, T., and Stenkvist, B.,** TULIPS — The Uppsala-Linkoping image processing system, *Anal. Quant. Cytol.*, 3, 182, 1981.
13. **Smith, K. C. A.,** On-line digital computer techniques in electron microscopy: general introduction, *J. Microsc.*, 127, 3, 1982.
14. **Skarnulis, A. J.,** A computer system for on-line image capture and analysis, *J. Microsc.*, 127, 39, 1982.
15. **Spring, K. R.,** Application of video to light microscopy, in *Membrane Biophysics II, Physical Methods in the Study of Epithelia*, Alan R. Liss, New York, 1983, 15.
16. **Caldwell, D. E.,** New developments in computer-enhanced microscopy (CEM), *J. Microbiol. Methods*, 4, 117, 1985.
17. **Wollmer, W.,** Application of a small microcomputer to cell image analysis, *Anal. Quant. Cytol. Histol.*, 9, 535, 1987.
18. **Gronsky, R.,** Spectroscopic information from high resolution images, *Ultramicroscopy*, 24, 155, 1988.
19. **Stewart, M.,** Computer image processing of electron micrographs of biological structures with helical symmetry, *J. Electron Microsc. Tech.*, 9, 325, 1988.
20. **Lin, J. A. and Cowley, J. M.,** Reconstruction from in-line electron holograms by digital processing, *Ultramicroscopy*, 19, 179, 1986.
21. **De Paz, P., Barrio, J. P., and Renau-Piqueras, J.,** A basic program for determination of numerical density of cytoplasmic compartments. II. Analysis of ellipsoids and cylindrical particles, *Comput. Biol. Med.*, 16, 273, 1986.
22. **Koss, L. G., Sherman, A. B., and Adams, S. E.,** The use of hierarchic classification in the image analysis of a complex cell population. Experience with the sediment of voided urine, *Anal. Quant. Cytol.*, 5, 159, 1983.
23. **Preston, K., Jr.,** High-resolution image analysis, *J. Histochem. Cytochem.*, 34, 67, 1986.
24. **Preston, K., Jr., Fagan, L. M., Huang, H. K., and Pryor, T. A.,** Computing in medicine, *IEEE Trans. Biomed. Eng.*, 784, 294, 1984.
25. **Kaufman, A. G., Nathwani, B. N., and Preston, K., Jr.,** Subclassification of follicular lymphomas by computerized microscopy, *Hum. Pathol.*, 18, 226, 1987.
26. **Wittekind, C. and Schulte, E.,** Computerized morphometric image analysis of cytologic nuclear parameters in breast cancer, *Anal. Quant. Cytol. Histol.*, 9, 480, 1987.
27. **Gunzer, U., Aus, H. M., and Harms, H.,** Letter to the editor, *J. Histochem. Cytochem.*, 35, 705, 1987.
28. **Pradère, P. and Thomas, E. L.,** Image processing of partially periodic lattice images of polymers: the study of crystal defects, *Ultramicroscopy*, 32, 149, 1990.
29. **Nierzwicki-Bauer, S. A., Balkwill, D. L., and Stevens, S. E., Jr.,** Three-dimensional ultrastructure of a unicellular cyanobacterium, *J. Cell Biol.*, 97, 713, 1983.
30. **Gras, H. A.,** "Hidden line" algorithm for 3D-reconstruction from serial sections — An extension of the NEUREC program package for a microcomputer, *Comput. Programs Biomed.*, 18, 217, 1984.

31. **Jimenez, J., Santisteban, A., Carazo, J. M., and Carrascosa, J. L.,** Computer graphic display method for visualizing three-dimensional biological structures, *Science*, 232, 1113, 1986.
32. **Harauz, G. and Flannigan, D.,** Structure of ribosomes from *Thermomyces lanuginosus* by electron microscopy and image processing, *Biochim. Biophys. Acta*, 1038, 260, 1990.

Methods of Image Analysis