Image Processing and Analysis

A PRACTICAL APPROACH

Edited by
R. BALDOCK and J. GRAHAM

The Practical Approach Series
Series Editor: B. D. Hames

http://www.oup.co.uk/PAS

Image Processing and Analysis

A Practical Approach

Edited by

RICHARD BALDOCK
MRC Human Genetics Unit,
Western General Hospital,
Crewe Road, Edinburgh EM4 2XU

and

JIM GRAHAM
University of Manchester,
Imaging Science and Biomedical Engineering
Stopford Building, Oxford Road,
Manchester M13 9PT

OXFORD
UNIVERSITY PRESS

OXFORD
UNIVERSITY PRESS
Great Clarendon Street, Oxford OX2 6DP
Oxford University Press is a department of the University of Oxford
and furthers the University's aim of excellence in research, scholarship,
and education by publishing worldwide in
Oxford New York
Athens Auckland Bangkok Bogotá Buenos Aires Calcutta
Cape Town Chennai Dar es Salaam Delhi Florence Hong Kong Istanbul
Karachi Kuala Lumpur Madrid Melbourne Mexico City Mumbai
Nairobi Paris São Paulo Singapore Taipei Tokyo Toronto Warsaw
and associated companies in Berlin Ibadan
Oxford is a registered trade mark of Oxford University Press
Published in the United States
by Oxford University Press Inc., New York

A catalogue record for this book is available from the British Library
Library of Congress Cataloging in Publication Data
(Data available)
ISBN 0-19-963701-6 (Hbk)
0-19-963700-8 (Pbk)
Typeset by Footnote Graphics,
Warminster, Wilts
Printed in Great Britain by Information Press, Ltd,
Eynsham, Oxon.

Image Processing and Analysis

The Practical Approach Series

SERIES EDITOR

B. D. HAMES
Department of Biochemistry and Molecular Biology
University of Leeds, Leeds LS2 9JT, UK

See also the Practical Approach web site at **http://www.oup.co.uk/PAS**

★ indicates new and forthcoming titles

Affinity Chromatography
Affinity Separations
Anaerobic Microbiology
Animal Cell Culture (2nd edition)
Animal Virus Pathogenesis
Antibodies I and II
Antibody Engineering
Antisense Technology
★ Apoptosis
Applied Microbial Physiology
Basic Cell Culture
Behavioural Neuroscience
Bioenergetics
Biological Data Analysis
Biomechanics – Materials
Biomechanics – Structures and Systems
Biosensors
★ C Elegans
Carbohydrate Analysis (2nd edition)
Cell-Cell Interactions
The Cell Cycle
Cell Growth and Apoptosis
★ Cell Growth, Differentiation and Senescence
★ Cell Separation
Cellular Calcium
Cellular Interactions in Development
Cellular Neurobiology
Chromatin
★ Chromosome Structural Analysis
Clinical Immunology
Complement
★ Crystallization of Nucleic Acids and Proteins (2nd edition)
Cytokines (2nd edition)
The Cytoskeleton
Diagnostic Molecular Pathology I and II
DNA and Protein Sequence Analysis
DNA Cloning 1: Core Techniques (2nd edition)
DNA Cloning 2: Expression Systems (2nd edition)

DNA Cloning 3: Complex Genomes (2nd edition)

DNA Cloning 4: Mammalian Systems (2nd edition)

★ DNA Microarrays

★ DNA Viruses

Drosophila (2nd edition)

Electron Microscopy in Biology

Electron Microscopy in Molecular Biology

Electrophysiology

Enzyme Assays

Epithelial Cell Culture

Essential Developmental Biology

Essential Molecular Biology I and II

★ Eukaryotic DNA Replication

Experimental Neuroanatomy

Extracellular Matrix

Flow Cytometry (2nd edition)

Free Radicals

Gas Chromatography

Gel Electrophoresis of Nucleic Acids (2nd edition)

★ Gel Electrophoresis of Proteins (3rd edition)

Gene Probes 1 and 2

★ Gene Targeting (2nd edition)

Gene Transcription

Genome Mapping

Glycobiology

Growth Factors and Receptors

Haemopoiesis

★ High Resolution Chromotography

Histocompatibility Testing

HIV Volumes 1 and 2

★ HPLC of Macromolecules (2nd edition)

Human Cytogenetics I and II (2nd edition)

Human Genetic Disease Analysis

★ Immobilized Biomolecules in Analysis

Immunochemistry 1

Immunochemistry 2

Immunocytochemistry

★ *In Situ* Hybridization (2nd edition)

Iodinated Density Gradient Media

Ion Channels

★ Light Microscopy (2nd edition)

Lipid Modification of Proteins

Lipoprotein Analysis

Liposomes

Mammalian Cell Biotechnology

Medical Parasitology

Medical Virology

MHC Volumes 1 and 2

★ Molecular Genetic Analysis of Populations (2nd edition)

Molecular Genetics of Yeast

Molecular Imaging in Neuroscience

Molecular Neurobiology

Molecular Plant Pathology I and II

Molecular Virology

Monitoring Neuronal Activity

★ Mouse Genetics and Transgenics
Mutagenicity Testing
Mutation Detection
Neural Cell Culture
Neural Transplantation
Neurochemistry (2nd edition)
Neuronal Cell Lines
NMR of Biological Macromolecules
Non-isotopic Methods in Molecular Biology
Nucleic Acid Hybridisation
★ Nuclear Receptors
Oligonucleotides and Analogues
Oligonucleotide Synthesis
PCR 1
PCR 2
★ PCR 3: PCR In Situ Hybridization
Peptide Antigens
Photosynthesis: Energy Transduction
Plant Cell Biology
Plant Cell Culture (2nd edition)
Plant Molecular Biology
Plasmids (2nd edition)
Platelets
Postimplantation Mammalian Embryos
★ Post-translational Processing
Preparative Centrifugation
Protein Blotting
★ Protein Expression
Protein Engineering
Protein Function (2nd edition)
Protein Phosphorylation (2nd edition)
Protein Purification Applications
Protein Purification Methods
Protein Sequencing
Protein Structure (2nd edition)
Protein Structure Prediction
Protein Targeting
Proteolytic Enzymes
Pulsed Field Gel Electrophoresis
RNA Processing I and II
RNA-Protein Interactions
Signalling by Inositides
★ Signal Transduction (2nd edition)
Subcellular Fractionation
Signal Transduction
★ Transcription Factors (2nd edition)
Tumour Immunobiology
★ Virus Culture

Preface

Image processing, image analysis, and pattern recognition are techniques now widely used in bioscience and medicine. There is a plethora of packages and systems which one can buy, or download free, ranging from menu-driven systems to libraries for C-programming. There is also a large number of textbooks, with one or more of the above terms in their titles, which explain the computational basis of these techniques. Many of these textbooks are excellent in their mathematical and computational descriptions, but take as their audience engineers and computer scientists for whom the methods themselves present the scientific interest. This volume, in common with others in the *Practical Approach* series, takes a different perspective. We take our audience to be scientists whose interests are in other fields, and for whom these methods provide useful analytical tools. As is the case with all tools it is best for the user to have an idea how they work, so that their behaviour can be understood, without necessarily becoming immersed in the details. As this volume forms part of a series aimed at biologists, we have retained that emphasis. However, the methods we describe are equally applicable in many other fields—the earth sciences for example—and we hope it will be of interest to scientists in these areas also. Try to imagine your favourite images in the place of the chromosomes or fungal mycelia.

As in any field of engineering, there are carefully worked out computational and mathematical principles in image processing and analysis, from which practitioners have developed a collection of rules of thumb and established common procedures. It is the latter aspect, rather than the former, that we hope to emphasize here. Some of these procedures take the form of well-known algorithms, and these, together with rules of thumb and standard processes, are presented as Protocols. Some standard pieces of knowledge are more readily expressed in mathematical formulae. This is a mathematical topic, and it would be impossible to describe it without the use of mathematical language. We understand, however, that mathematics does not hold a deep fascination for at least some of our target audience, and we have tried to keep the mathematical descriptions at a level that would be acceptable to a final-year high-school or first-year university student. Many of the algorithms and formulae are already implemented in software packages and libraries. We hope that the descriptions here will give the user some understanding of the reasoning behind the functions given and the limitations of their operation. The algorithms should be presented in sufficient detail that a competent programmer, without much image analysis experience, should be able to implement them if required.

There is a wide range of material in the image processing and analysis literature. We have deliberately set out to avoid producing another all-encompassing

book. The material we have chosen has two aims. First, it is intended to cover the basic methods of which a user of the technology should be aware. Second, we present a selection of more advanced techniques that we hope will inspire the application of image analysis to a wider range of complex scientific problems. Each chapter describes in some detail a specific technique or collection of related techniques. To focus on the practicalities, each chapter relates its technical content to one (or maybe two) applications in bioscience or medicine. We hope that the reader should get an appreciation of not only how individual methods can solve real analytical problems, but also the range of applications which can be addressed.

The terms *image processing*, *image analysis*, and *pattern recognition* are often used interchangeably. In fact, they refer to different activities, but they overlap and in a given application, it is likely that all three will be used. Chapters 2, 3, and 4 address each of these topics. There is some overlap in their descriptions, where the individual topics merge. The chapters can be read individually, but together they should give a good grounding of the basic methods for dealing with digital images. These are preceded by a chapter on image acquisition. Many users of image analysis acquire their images using a television camera mounted on a microscope. Both of these components are becoming increasingly complex, and an understanding of their properties and limitations separately and in combination is important for achieving the highest quality data. Later chapters deal with more advanced computational methods, in the use of explicit mathematical models of image appearance, and the analysis of three-dimensional data. Not only in microscopy, but also in other research and clinical fields, analysis of structures in three dimensions is of increasing importance.

Of course there are omissions. Some readers may feel that we might have included material on confocal microscopy or stereology, for example. These are both large topics which we felt are dealt with very well elsewhere for the practising biologist. We have, however, included the rather less well-known topic of projective stereology, which extends the established stereology literature.

We have had guiding principles in the choice of topics, but ultimately those selected, and the illustrative applications, reflect the interests of the editors and the social circles in which they move. We hope you like them.

September 1999

Jim Graham
Richard Baldock

Contributors

RICHARD A. BALDOCK
MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK. Richard.Baldock@hgu.mrc.ac.uk

ANDREW D. CAROTHERS
MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK. Andrew.Carothers@hgu.mrc.ac.uk

KENNETH R. CASTLEMAN
Perceptive Scientific Instruments, Inc., League City, Texas, USA. Castleman @persci.com

TIM COOTES
University of Manchester, Imaging Science and Biomedical Engineering, Stopford Building, Oxford Road, Manchester M13 9PT, UK. t.cootes@man. ac.uk

STEPHANIE L. ELLENBERGER
Image Processing, Information Systems Division, P.O. Box 155, 2600 AD Delft, The Netherlands. eberger@tpd.tno.nl

C. A. GLASBEY
Biomathematics and Statistics Scotland, The King's Buildings, Edinburgh EH9 3JZ, UK. c.glasbey@bioss.sari.ac.uk

JIM GRAHAM
University of Manchester, Imaging Science and Biomedical Engineering, Stopford Building, Oxford Road, Manchester M13 9PT, UK. Jim.Graham @man.ac.uk

DAVID J. HAWKES
UMDS Radiological Sciences, Guy's Hospital, London SE1 9RT, UK. D.Hawkes@umds.ac.uk

BILL HILL
MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK. Bill.Hill@hgu.mrc.ac.uk

G. W. HORGAN
Biomathematics and Statistics Scotland, Rowett Research Institute, Aberdeen AB21 9SB, UK. g.horgan@bioss.sari.ac.uk

C. A. REID
Biomathematics and Statistics Scotland, Rowett Research Institute, Aberdeen AB21 9SB, UK. c.reid@bioss.sari.ac.uk

DANIEL RUECKERT
UMDS Radiological Sciences, Guy's Hospital, London SE1 9RT, UK. D.Rueckert@umds.ac.uk

FONS J. VERBEEK
NIOB Hubrecht Laboratory, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands. verbeek@niob.knaw.nl

IAN T. YOUNG
Department of Applied Physics, Delft University of Technology, PO Box 5046, 2600 GA Delft, The Netherlands. young@ph.tu.tudelft.nl

Abbreviations

ADC	analogue-to-digital converter
ADU	analogue-to-digital converter unit
ASM	active shape model
AUC	area under the curve
CAR	computer-assisted reconstruction
CCD	charge-coupled device
CGH	comparative genomic hybridization
CLSM	confocal laser scanning microscope
CRT	cathode-ray tube
CT	computed tomography
CV	coefficient of variation
DAPI	4′,6-diamidino-2-phenylindole
DOF	depth of field
EM	expectation maximization
FEM	finite-element model
FFD	free form deformations
FFT	fast Fourier transform
FISH	fluorescent *in situ* hybridization
FITC	fluorescein isothiocyanate
FN	false negative
FOV	field of view
FP	false positive
FROC	free-response operating characteristic
GIS	geographic information system
ICP	iterative closest point
IR	infrared
ISCN	international system of human cytogenetic nomenclature
kbp	kilobase pairs
k-NN	k-nearest neighbour
LoG	Laplacian of Gaussian
LUT	look-up table
M-FISH	multicolour FISH
MLP	multilayer perceptron
MR	magnetic resonance
MRASM	multiresolution active shape model
NA	numerical aperture
NCC	normalized cross-correlation
NMR	nuclear magnetic resonance
PCA	principal components analysis

PET	positron emission tomography
PSF	point spread function
RBF	radial basis function
RGB	red green blue
ROC	receiver operating characteristic
ROI	region of interest
RMS	root mean square
SD	sampling density *also* standard deviation
SNR	signal-to-noise ratio
SPECT	single-photon emission computed tomography
SSD	squared sum of differences
SVD	singular value decomposition
TN	true negative
TP	true positive
TRITC	tetramethylrhodamine-5-isothiocyanate
TVP	total vertical projection
UV	ultraviolet
VIR	variance of intensity ratio
WDD	weight density distribution

Contents