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

R318

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





E200000322

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

© Oxford University Press, 2000

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, without the prior permission in writing of Oxford University Press.

Within the UK, exceptions are allowed in respect of any fair dealing for the purpose of research or private study, or criticism or review, as permitted under the Copyright, Designs and Patents Act, 1988, or in the case of reprographic reproduction in accordance with the terms of licences issued by the Copyright Licensing Agency. Enquiries concerning reproduction outside those terms and in other countries should be sent to the Rights Department, Oxford University Press, at the address above.

This book is sold subject to the condition that it shall not, by way of trade or otherwise, be lent, re-sold, hired out, or otherwise circulated without the publisher's prior consent in any form of binding or cover other than that in which it is published and without a similar condition including this condition being imposed on the subsequent purchaser

Users of books in the Practical Approach Series are advised that prudent laboratory safety procedures should be followed at all times. Oxford University Press makes no representation, express or implied, in respect of the accuracy of the material set forth in books in this series and cannot accept any legal responsibility or liability for any errors or omissions that may be made.

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
Apontosis

★ 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

 \bigstar In Situ Hybridization (2nd edition)

Iodinated Density Gradient Media

Ion Channels

 \bigstar 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
- Signalling by Inositides

 ★ Signal Transduction
 (2nd edition)
 Subcellular Fractionation
 Signal Transduction

RNA-Protein Interactions

- ★ 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 wellknown 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 finalyear 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

Preface

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

Contributors

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

xviii

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

Abbreviations

PET positron emission tomography

PSF point spread function RBF radial basis function

RGB red green blue

ROC receiver operating characteristic

ROI region of interest 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

Li	st of Contributors	xvii
Abbreviations		xix
1.	Microscope image acquisition	1
	Stephanie L. Ellenberger and Ian T. Young	
	1. Introduction Microscope systems Case study	1 1 2
	2. Basic optics Nature of light Illumination Optical system	3 3 7 9
	3. Microscope image acquisition system Microscope types and components Camera types and performance Images	12 12 19 28
	4. Application Objective Materials and methods Performance	29 29 30 34
	References	35
2.	Biological image processing and enhancement	37
	G. W. Horgan, C. A. Reid, and C. A. Glasbey	
	1. Introduction	37
	2. Contrast manipulation Functional transformation Histogram-based transformations Pseudocolour Thresholding Zooming	39 40 41 42 43 46
	3. Filtering Linear filters Non-linear filters	46 46 50

	4. Mathematical morphology Basic ideas Greylevel morphology Complex operations Top-hat transform and background subtraction	53 54 56 58 58
	5. Texture measures Statistical approaches Structural approaches Modelling approaches Texture transforms	60 61 64 64
	Acknowledgements	66
	References	66
3.	Image analysis: quantitative interpretation of chromosome images	69
	Kenneth R. Castleman	
	1. Introduction The karotyping problem Historical perspective Current practice Automatic karyotyping	69 70 70 70 70
	2. Image segmentation Thresholding The watershed algorithm Gradient image thresholding Laplacian edge detection Edge detection and linking Region growing	72 73 76 76 77 78 84
	3. Boundary refinement Active contours Binary image processing Morphological image processing Boundary curvature analysis Touch and overlap resolution	85 85 87 88 91 92
	4. Chromosome measurement Morphological features Banding pattern features	93 94 94
	5. Chromosome classification The Bayes classifier	96 96
	6. Karotype generation Chromosome straightening	99 99

	Chromosome enhancement Chromosome assignment	99 101
	7. Other cytogenetics applications Multiplex-FISH Metaphase finding	102 103 104
	8. Image processing software Academic packages Commercial packages	105 105 105
	References	106
4.	Pattern recognition: classification of	
	chromosomes	111
	Jim Graham	
	1. Introduction	111
	2. The chromosome classification problem	112
	3. Classification methods Defining classification in terms of probabilities Using Bayes' formula Non-parametric methods	114 114 115 121
	4. Features and feature selection Selecting features Combining features Clustering	124 126 128 129
	5. Neural networks Introduction Supervised training Unsupervised training	131 131 132 135
	6. Classifying chromosomes Features Data sets Classifiers	137 137 138 139
	7. Classifier validation The need for validation Cross-validation, the jackknife and the bootstrap The confusion matrix Validating issues for neural networks Training-set size and validation Validation issues for the two-class problem	141 141 141 142 143 143
	8. Available material Software Further reading	148 148

	Acknowledgements	150
	References	150
5.	Three-dimensional (3D) reconstruction from serial sections	153
	Fons J. Verbeek	133
	1. Introduction 3D reconstruction in microscopy Why serial sectioning is necessary	153 153 154
	2. Methodological aspects Modes of action Organizing the reconstruction data Methods and devices for the input of the data Spatial resolution Alignment and deformation correction	156 158 159 161 166 166
	23. Mathematical aspects Estimation of transformations Spatial transformation of image values Image correspondences Point-pattern matching methods Shape-based assessment methods Registration and congruencing using image moment Affine transform component estimation Evaluation using similarity measures Intensity-based methods	173 173 174 175 175 178 180 181 184
	4. Systems for routine application of 3D reconstruction Reconstruction under rigid transformation Reconstruction including deformation correction	186 187 187
	References	194
6.	3D analysis: registration of biomedical images	197
	Daniel Rueckert and David J. Hawkes	197
	1. Introduction	40=
	Intra-subject registration	197 198
	Inter-subject registration	198
	Serial registration	198
	Image to physical space registration Overview	198
	Overview	199