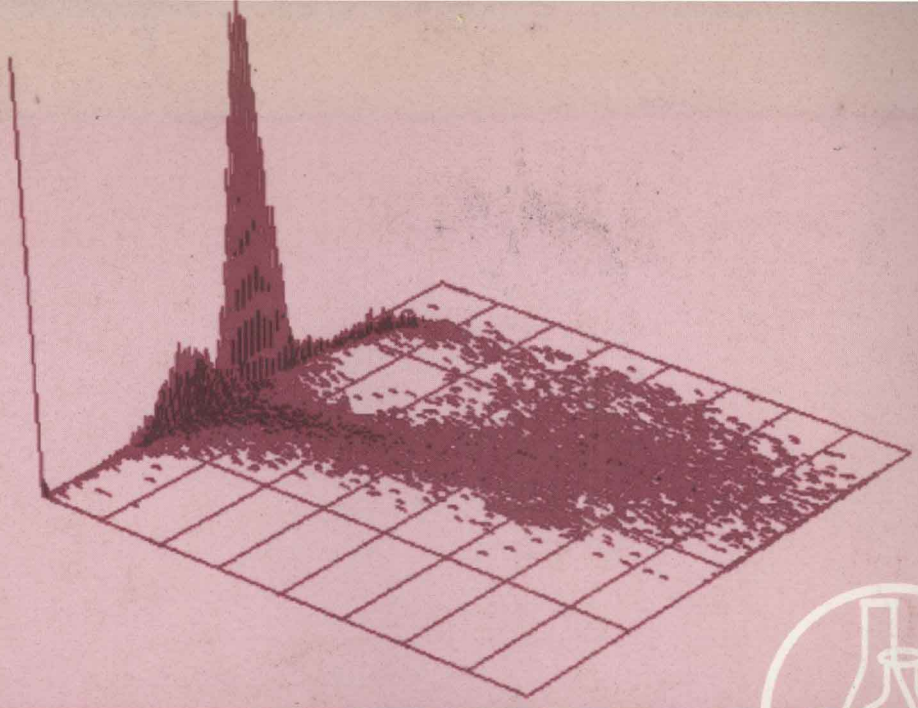

Flow Cytometry

Third Edition

Edited by

Michael G. Ormerod

PRACTICAL APPROACH

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A Practical Approach

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Preface

This book is intended as a handbook for every laboratory that has a bench-top flow cytometer or a fluorescence-activated cell sorter. It is an introduction and guide to those new to the field and a first point of reference for experienced practitioners who want to investigate a new technique.

The third edition has built on the ground covered in the first and second editions. Many of the chapters have been updated. Two chapters have been dropped and three new chapters added. The chapter on immunophenotyping—the most important clinical application of flow cytometry—has been retained and strengthened by the addition of a chapter on quality control in the clinical laboratory. The utility of the book in a clinical laboratory has been further enhanced by the addition of a chapter covering 10 other clinical applications (further clinical applications). Flow cytometry has found increasing application in the field of apoptosis research. A new chapter has been added to cover this important topic.

The size of the book (and hence its cost) has been kept within reasonable limits. Every flow cytometry laboratory can afford to have a copy on the shelf as a first point of reference. The book is not fully comprehensive, but it does aim to cover over 90% of the applications of flow cytometry in mammalian biology.

In an expanding field, new developments are continually appearing. It is recommended that everyone with a serious interest in flow cytometry should join the International Society for Analytical Cytology. The membership fee includes a subscription to the journal, *Cytometry*, which gives wide cover to new developments in this and related areas. The Society's Web site (www.isac-net.org) has links to the growing number of affiliated national organizations.

A computer is an essential element of all flow cytometers. The data generated is written to computer disc in a standard format so these files can be analysed off-line by a variety of computer programs. Correct analysis of the data is essential. Examples of data files generated by the applications described in this book are available on CD-ROM (1), which serves as a companion to this volume.

Reference

1. Ormerod, M. G. (1996). *Data analysis in flow cytometry—a dynamic approach.* Published by the author on CD-ROM.

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Abbreviations

7-AAD	7-aminoactinomycin D
ADB	1,4-diacetoxy-2,3-dicyanobenzene
ADC	analog-to-digital converter
ALG	anti-lymphocyte globulin
AO	Acridine Orange
APC	allophycocyanin
ATG	anti-thymocyte globulin
βAPP	beta-amyloid precursor protein
BCECF	2′,7′-bis-carboxyethyl-5(6)- carboxyfluorescein
BIODIPY	4,4-difluoro-4-bora-3α, 4α-diaza-*s*-indacene
bp	base pair
BrdUrd	5′-bromodeoxyuridine
BrdUTP	bromodeoxyuridine triphosphate
BSA	bovine serum albumin
$[Ca^{2+}]_i$	concentration of intracellular ionized calcium
CA3	chromomycin A3
CCCP	carbonyl cyanide *m*-chlorophenylhydrazone
CD	cluster of differentiation
CDC	Centers for Disease Control, USA
CFDA	carboxyfluorescein diacetate
CFDA SE	carboxyfluorescein diacetate, succinimidyl ester
CM-DiI	chloromethylbenzamido derivative of octadecylindocarbocyanine
CMFDA	5-chloromethylfluorescein diacetate
CMXRos	a chloromethyl derivative of X-rhosamine (MitoTracker Red)
CMTMR	5 (and 6)- ([(4-chloromethyl)benzoyl]amino) tetramethylrhodamine
CV	coefficient of variation
CVID	common variable immunodeficiency
CyA	cyclosporin A
Cy-chrome	phycoerythrin–cyanine5 conjugate
DAG	2-diacylglycerol

DAPI	4′,6-diamidino-2-phenylindole
DC	dendritic cells
DCH	2,3-dicyanohydroquinone
DCFH	2′,7′-dihydrodichlorofluorescein
DI	DNA index
$DiOC_6$	3,3′-dihexyloxacarbocyanine
$DiOC_{18}$	3,3′-dioctadecyloxacarbocyanine
DMSO	dimethylsulfoxide
DNTP	deoxynucleotide triphosphate
DOP-PCR	degenerate oligonucleotide-primed PCR
DPBS	Dulbecco's phosphate-buffered saline
dUTP	deoxyuridine triphosphate
EB	ethidium bromide
EBV	Epstein–Barr virus
ECD	phycoerythrin–Texas Red conjugate
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunoabsorbent assay
EMA	ethidium monazide
EQAS	external quality assurance survey
FALS	forward-angle light scatter
Fc	crystallizable fragment
FCM	flow cytometry
FBS	fetal bovine serum
FDA	fluorescein diacetate
FISH	fluorescent *in-situ* hybridization
FITC	fluorescein isothiocyanate
FL1, FL2, etc.	Fluorescence parameter 1, 2, etc., on the flow cytometer
GSH	glutathione
HE	dihydroethidium
HIV	human immunodeficiency virus
HLA	human leucocyte antigen
HPA	human platelet antigen
HPC	haematopoietic progenitor cells
IdUrd	iododeoxyuridine
IFN	interferon
IgG	immunoglobulin G
IL	interleukin
Indo-1	[1-[2 amino-5-[carboxylindol-2-yl]-phenoxy]-2–2′-amino-5′-methylphenoxy] ethane *N,N,N′N′*- tetraacetic acid
IP3	inositol 1,4,5-trisphosphate
ISEL	*in-situ* end-labelling
ISHAGE	International Society for Hematotherapy and Graft Engineering
IU	International units
JC-1	5,5′,6,6′,-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide
K_d	effective dissociation constant

laser	light amplification by stimulated emission of radiation
LGL	large granular lymphocytes
LI	labelling index
LWP	long wavelength pass
mAb	monoclonal antibody
mBrB	monobromobimane
mClB	monochlorobimane
MDR	multi-drug resistance
MESF	molecules of equivalent soluble fluorochrome
MLR	mixed lymphocyte reaction
MMP	mitochondrial membrane potential
MRD	minimal residual disease
MRP	MDR-associated protein
MTG	MitoTracker Green FM
NEQAS	National External Quality Assurance Scheme
NK	natural killer cell
PBL	peripheral blood lymphocytes
PBS	phosphate-buffered saline
PBSA	PBS with BSA
PC5	phycoerythrin–cyanine5 conjugate
PCD	programmed cell death
PCNA	proliferating cell nuclear antigen
PCR	polymerase chain reaction
PE	phycoerythrin
PerCP	peridinin chlorophyll-A protein
PE-Cy5	phycoerythrin–cyanine5 conjugate
PE-Cy7	phycoerythrin–cyanine7 conjugate
PFA	paraformaldehyde
PHA	phytohaemagglutinin
pH_i	intracellular pH
PI	propidium iodide
PIP2	phosphatidylinsitol 4,5-bisphosphate
p.l.m.	per cent labelled mitosis
PLP	periodate/lysine/formaldehyde mixture
PMA	phorbol myristate acetate
PMT	photomultiplier
PNH	paroxysmal nocturnal haemoglobinuria
PS	phosphatidyl serine
Py	pyronin Y
QC	quality control
RALS	right-angle light scatter
RM	relative movement
RPMI	Roswell Park Memorial Institute (medium)
SBIP	strand break induction by photolysis
SD	standard deviation

SNAFL	SemiNaphthoFluorescein
SNARF	SemiNaphthoRhodaFluor
SPF	S phase fraction
SV	simian virus
SWP	Short wavelength pass
T_C	cell-cycle time
Tdt	terminal deoxynucleotidyl transferase
T_{G2+M}	G_2/M transit time
TNF-α	tumour necrosis factor-α
T_{pot}	potential doubling time
T_S	S-phase transit time
TUNEL	Tdt-mediated dUTP nick end-labelling

Contents

Protocol list