



Flow Cytometry

Third Edition

Edited by

Michael G. Ormerod

**PRACTICAL
APPROACH**

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A Practical Approach

Edited by

Michael G. Ormerod

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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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M.G.O.

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 <i>m</i> -chlorophenylhydrazine |
| 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 |

ABBREVIATIONS

| | |
|--------------------|---|
| 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 <i>in-situ</i> 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 <i>N,N,N'N'</i> - tetraacetic acid |
| IP3 | inositol 1,4,5-trisphosphate |
| ISEL | <i>in-situ</i> 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 | phosphatidylinositol 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 |

ABBREVIATIONS

| | |
|---------------|---------------------------------------|
| 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 |

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