Cell Analysis

Volume 1

A Continuation Order Plan is available for this series. A continuation order will bring delivery of each new volume immediately upon publication. Volumes are billed only upon actual shipment. For further information please contact the publisher.

Cell Analysis

Volume 1

Edited by

NICHOLAS CATSIMPOOLAS

Boston University School of Medicine

Boston, Massachusetts

PLENUM PRESS • NEW YORK AND LONDON

Library of Congress Cataloging in Publication Data

Main entry under title:

Cell analysis.

Bibliography: p.

Includes index.

1. Cytology—Technique. 2. Cells—Analysis. I. Catsimpoolas, Nicholas. [DNLM: 1. Cells—Analysis—Periodical. W1 CE126H]

QH585.C44

574.87'028

82-5289

ISBN 978-1-4684-4099-7

ISBN 978-1-4684-4097-3 (eBook)

AACR2

DOI 10.1007/978-1-4684-4097-3

©1982 Plenum Press, New York

Softcover reprint of the hardcover 1st edition 1982

A Division of Plenum Publishing Corporation

233 Spring Street, New York, N.Y. 10013

All rights reserved

No part of this book may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise, without written permission from the Publisher

Contributors

- R. B. Allan**, *Department of Cell Biology, University of Glasgow, Glasgow G12 8QQ, U.K.*
- James W. Bacus**, *Medical Automation Research Unit, Rush–Presbyterian–St. Luke’s Medical Center, Chicago, Illinois 60612*
- S. A. Ben-Sasson**, *The Hubert H. Humphrey Centre for Experimental Medicine and Cancer Research, The Hebrew University–Hadassah Medical School, Jerusalem, Israel*
- Michael W. Berns**, *Department of Developmental and Cell Biology, University of California, Irvine, Irvine, California 92717*
- Francis Dumont**, *Unité de Cancérologie Expérimentale et de Radiobiologie, INSERM U95, 54500 Vandoeuvre-les-Nancy, France. Present address: Department of Immunology, Merck, Sharp and Dohme Research Laboratories, Rahway, New Jersey 07065*
- N. B. Grover**, *The Hubert H. Humphrey Centre for Experimental Medicine and Cancer Research, The Hebrew University–Hadassah Medical School, Jerusalem, Israel*
- Volker Kachel**, *Max-Planck-Institut für Biochemie, D-8033 Martinsried, West Germany*
- J. M. Lackie**, *Department of Cell Biology, University of Glasgow, Glasgow G12 8QQ, U.K.*
- J. Naaman**, *The Hubert H. Humphrey Centre for Experimental Medicine and Cancer Research, The Hebrew University–Hadassah Medical School, Jerusalem, Israel*
- Gary C. Salzman**, *Life Sciences Division, Los Alamos National Laboratory, University of California, Los Alamos, New Mexico 87545*
- Robert J. Walter**, *Department of Developmental and Cell Biology, University of California, Irvine, Irvine, California 92717*
- P. C. Wilkinson**, *Department of Bacteriology and Immunology, Western Infirmary, University of Glasgow, Glasgow G11 6NT, U.K.*

Preface

The selective combination of physical, biochemical, and immunological principles, along with new knowledge concerning the biology of cells and advancements in engineering and computer sciences, has made possible the emergence of highly sophisticated and powerful methods for the analysis of cells and their constituents. This series on *Cell Analysis* is, therefore, aiming at providing the theoretical and practical background on how these methods work and what kind of information can be obtained. *Cell Analysis* will cover techniques on cell separation, cell identification and classification, characterization of organized cellular components, functional properties of cells, and cell interactions. Applications in cell biology, immunology, genetics, toxicology, specific diseases, diagnostics and therapeutics, and other areas will be covered whenever relevant results exist.

Nicholas Catsimpoolas
Boston, Massachusetts

Contents

Chapter 1

Quantification of Red Blood Cell Morphology

James W. Bacus

I. History	1
II. Details of Red Cell Measurements	3
III. Cell Sample Population Distributions	11
IV. Discussion and Summary	25
References	30

Chapter 2

Laser Microirradiation and Computer Video Optical Microscopy in Cell Analysis

Michael W. Berns and Robert J. Walter

I. Introduction	33
II. Laser Microbeams	34
III. Computer-Enhanced Video Microscopy for Laser Microsurgery	35
A. Contrast Enhancement	37
B. Edge Enhancement	37
C. Pseudocolor Enhancement	40
IV. Chromosome Microsurgery	40
A. Mitotic Organelles	43
B. Cytoplasm	48
V. Plant Cell Development (Chloroplast Irradiation)	49
VI. Developmental Cellular Neurobiology	49
VII. Pattern Formation	50
VIII. Conclusions	51
References	51

*Chapter 3***Combination of Two Physical Parameters for the Identification and Separation of Lymphocyte Subsets***Francis Dumont*

I. Introduction	55
II. Methods	56
A. Preparation of Cell Suspensions	56
B. Fractionation of Cell Suspensions by Free-Flow Preparative Electrophoresis	57
C. Separation of Cells by Isopycnic Centrifugation in a Percoll Gradient	60
D. Cell Enumeration and Size Distribution Analysis	61
E. Construction of the Fingerprints	63
F. Interpretation of the Fingerprints	65
III. Applications	66
A. Fingerprint Analysis of Murine Thymocytes	66
B. Fingerprint Analysis of Murine Splenocytes	75
C. Fingerprint Analysis of Murine Lymph Node Cells	84
IV. Conclusion	87
References	87

*Chapter 4***Electrical Sizing of Cells in Suspension***N. B. Grover, S. A. Ben-Sasson, and J. Naaman*

I. Introduction	93
II. Theory	94
A. Electric Field	95
B. Hydrodynamic Field	96
C. Cell Volume	96
D. Shape and Orientation	97
E. Membrane Conductance	98
F. Electrical Size	99
III. Instrumentation	99
A. The Transducer	100
B. The Amplifiers	100
C. The Selector	100
D. Data Handling	102
E. Auxiliary Units	102

IV. Operation	102
A. Cell Suspensions	102
B. Orifice Dimensions	103
C. Amplification	103
D. Calibration	104
V. Applications	104
A. Fast Kinetics	104
B. High Fields	105
C. Membrane Permeability	105
VI. The Future	106
A. Sensitivity	106
B. Multiparameter Systems	107
References	107

Chapter 5

Light Scattering Analysis of Single Cells

Gary C. Salzman

I. Introduction	111
II. Theoretical Considerations	112
A. Scattering Theory	112
B. Coated Sphere Model Tests	113
C. Expected Detector Responses	113
III. Recent Applications of Forward and 90° Scatter	124
A. Human Peripheral Blood	124
B. Osmolarity Effects	125
C. Hematopoietic Stem Cells	126
D. T-Lymphocyte Subclasses	128
E. Non-Hodgkin's Lymphomas	129
F. Cervical Cell Analysis with 90° Scatter	129
G. Sputum Samples	129
IV. Multiangle and Multiwavelength Scattering	130
A. Multiangle Scattering	130
B. Two-Color Light Scattering	133
V. Fourier Transform Techniques	134
VI. Polarization Studies—New Directions	136
A. Polarization Transformations	136
B. Circular Intensity Differential Scattering	137
VII. Conclusion	138
References	138

Chapter 6

Methods for Measuring Leukocyte Locomotion

P. C. Wilkinson, J. M. Lackie, and R. B. Allan

I. Introduction	145
II. Visual Assays	151
A. Time-Lapse Filming	151
B. Indirect (Non-Time-Lapse) Methods	161
C. Analysis of Cell Movement	162
D. The Orientation Assay	165
E. The <i>Candida</i> Assay	168
III. The Micropore Filter Assay	171
A. Principle	171
B. Apparatus	171
C. Measurement of Leukocyte Locomotion in Filter Assays ..	174
D. The Checkerboard Assay	179
E. Automated Method	181
IV. The Under-Agarose Assay	182
V. Miscellaneous Techniques	184
A. The Capillary-Tube Assay for Macrophage Locomotion ..	184
B. The Agarose Microdroplet Assay	186
VI. Factors Influencing Locomotion	187
A. Cell-Substratum Interactions	187
B. Composition of the Fluid Phase	188
C. Cell-Cell Interactions	189
References	190

Chapter 7

Sizing of Cells by the Electrical Resistance Pulse Technique: Methodology and Application in Cytometric Systems

Volker Kachel

I. Introduction	195
A. Reasons for Measuring Size of Biological Cells	195
B. A Short Review on the History of Electrical Sizing	196
II. Basic Events and Problems in Electrically Sizing Transducers ..	198
A. The Basic Coulter Effect	198
B. Fundamental Relations of Particle Volume, Electrical Resistivity, Resistance Change, and Pulse Height	199

C. Flow Conditions and Flow Line Coordination in the Sensitive Zone	217
D. Behavior of Biological Particles in the Sizing Transducer	224
III. Instrumentation	248
A. General Conditions and Review	248
B. Transducer	249
C. Electronic Instrumentation	254
D. Electronic Pulse Handling Devices as Alternatives to Hydrodynamic Focusing	263
IV. Calibration Problems of Electrical Sizing Flow Cytometers	264
A. Calibration in Long Orifices with Homogeneous Field	264
B. Calibration with Short Orifices	274
C. Calibration with Reference Particles	276
V. Applications of the Metricell Cell Volume Analyzer	277
A. Erythrocyte Volume Distribution Curves of Young Rats	277
B. Red Blood Cell Aggregation in Humans	278
C. Thrombocyte Volume Distributions Measured from Whole Blood	279
D. Effect of Nerve Growth Factor on Pheochromocytoma Cells	281
VI. Combination of Electrical Sizing with Multiparameter Optical Flow Analysis	281
A. The Fluorescence-Volume (FLUVO) Transducer	281
B. Cell Volume-Cell Absorption Transducer	285
C. Electronic Evaluation	286
D. Examples of Combined Electrical-Optical Cell Analysis	290
VII. Sorting Activated by Electrical Sizing	297
VIII. Size-Triggered Imaging in Flow	302
A. Instrumentation for Size-Triggered Imaging	302
B. Problems of Visualization Cell Structures	306
C. Examples of Cell Imaging in Flow	307
Appendix	313
References	324
 Index	 333