

# Contents

|  |    |
|--|----|
| <b>Part I Flow Cytometry</b> .....                       | 1  |
| Chapter 1  |    |
| Operation of a Flow Cytometer                            |    |
| CHRISTOPH GÖTTLINGER, BIRGIT MECHTOLD                    |    |
| AND ANDREAS RADBRUCH .....                               | 3  |
| <b>Part II Immunofluorescence</b> .....                  | 27 |
| Chapter 2  |    |
| Conjugation of Fluorochromes, Haptens,                   |    |
| and Phycobiliproteins to Antibodies                      |    |
| WERNER MÜLLER .....                                      | 29 |
| Chapter 3  |    |
| Immunofluorescence: Basic Considerations                 |    |
| ANDREAS RADBRUCH .....                                   | 38 |
| Chapter 4  |    |
| Multicolor Immunofluorescence Analysis                   |    |
| CHIA-HUEI CHEN, KERSTIN WILLMANN, DIETER RECKTENWALD ... | 53 |
| Chapter 5  |    |
| Combined Intracellular and Surface Staining:             |    |
| Immunofluorescence of Cytokines in T Cells               |    |
| MARIO ASSENMACHER .....                                  | 63 |
| Chapter 6  |    |
| Cytometry and Sorting of Live Cells According            |    |
| to Their Secreted Products                               |    |
| RUDOLF A. MANZ .....                                     | 71 |

|   |            |
|---|------------|
| Chapter 7<br>Cytometry of Rare Surface Molecules<br>by Magnetofluorescent Liposomes<br>ALEXANDER SCHEFFOLD .....  | 77         |
| Chapter 8<br>Scatchard Analysis by Flow Cytometry<br>ROBERT F. MURPHY .....   | 82         |
| Chapter 9<br>Microbead Assay for Quantification of Neuronal Adhesion Molecule<br>Interaction by Flow Cytometry<br>ATTILA TÁRNOK, URSEL NÖHRENBERG, HANS-JÜRGEN VOLLMER<br>AND STEPHAN SCHUHMACHER ..... | 86         |
| <b>Part III DNA and Proliferation .....</b>   | <b>99</b>  |
| Chapter 10<br>Preparation and Staining of Fixed Cells<br>for High-Resolution DNA Analysis<br>FRIEDRICH J. OTTO .....  | 101        |
| Chapter 11<br>Simultaneous Flow Cytometric Detection of Bromodeoxyuridine<br>Incorporation and Cell Surface Marker Expression<br>WERNER MÜLLER .....  | 105        |
| Chapter 12<br>High-Resolution Cell Cycle Analysis: The Flow Cytometric<br>Bromodeoxyuridine-Hoechst Quenching Technique<br>MANFRED KUBBIES .....  | 112        |
| <b>Part IV Cellular Activation and Biochemistry .....</b>   | <b>125</b> |
| Chapter 13<br>Cell Activation: Indo-1 Ratiometric Analysis<br>of Intracellular Ionized Calcium<br>MANFRED KUBBIES .....   | 127        |

---

|  |            |
|--|------------|
| Chapter 14   |            |
| Detection and Purification of Rare Responders<br>by Fixed-Time Flow Cytometry        |            |
| ATTILA TÁRNOK AND HENNING ULRICH . . . . .   | 140        |
| <br>   |            |
| Chapter 15   |            |
| Biochemical Parameters of Cells Function   |            |
| GREGOR ROTHE AND GÜNTHER VALET . . . . .   | 159        |
| <br>   |            |
| Chapter 16   |            |
| The FACS-Gal Assay   |            |
| STEFFEN JUNG . . . . .   | 183        |
| <br>   |            |
| Chapter 17   |            |
| Ligand Acidification by Nonadherent Cells  |            |
| ROBERT F. MURPHY . . . . .   | 186        |
| <br>   |            |
| <b>Part V Cell Sorting . . . . .</b>   | <b>195</b> |
| <br>   |            |
| Chapter 18   |            |
| Powerful Preselection  |            |
| CHARLOTTE ESSER . . . . .  | 197        |
| <br>   |            |
| Chapter 19   |            |
| Magnetic Selection of Transfected Cells  |            |
| GREGOR SIEBENKOTTEN AND UTE BEHRENS-JUNG . . . . .                                   | 210        |
| <br>   |            |
| Chapter 20   |            |
| High Gradient Magnetic Cells Sorting   |            |
| STEFAN MILTENYI AND JÜRGEN SCHMITZ . . . . .   | 218        |
| <br>   |            |
| Chapter 21   |            |
| Setup of a Flow Sorter   |            |
| CHRISTOPH GÖTTLINGER, BIRGIT MECHTOLD,<br>KLAUS MEYER AND ANDREAS RADBRUCH . . . . . | 248        |
| <br>   |            |
| Chapter 22   |            |
| Sorting of Rare Cells  |            |
| WALTER WEICHEL, SIGRID IRLBUSCH, KIMITAKA KATO<br>AND ANDREAS RADBRUCH . . . . .     | 255        |

|  |     |
|--|-----|
| Chapter 23<br>Fluorescence-Activated Chromosome Sorting<br>JUDITH A. FANTES AND DARYLL K. GREEN .....  | 266 |
| Chapter 24<br>Analysis and Sorting of Plant Chromosomes by Flow Cytometry<br>JACKY VEUSKENS, DOMINIQUE MARIE, STEFAN HINNISDAELS<br>AND SPENCER C. BROWN ..... | 277 |
| Chapter 25<br>Large Particle Sorting<br>DAVID W. GALBRAITH AND SERGIO LUCRETTI .....   | 293 |
| <b>Part VI Safety</b> .....  | 319 |
| Chapter 26<br>Biological and Laser Safety<br>KLAUS L. MEYER .....  | 321 |
| <b>Appendices</b><br>BIRGIT MECHTOLD AND ANDREAS RADBRUCH .....  | 327 |
| Glossary .....   | 329 |
| Suppliers .....  | 331 |
| Cytometry on the Internet .....  | 347 |
| <b>Subject Index</b> .....   | 349 |