

# CONTENTS

|                                                                                                       |    |
|-------------------------------------------------------------------------------------------------------|----|
| Contributors                                                                                          | ix |
| Preface                                                                                               | xi |
| <b>1. Forces of a Single-Beam Gradient Laser Trap on a Dielectric Sphere in the Ray Optics Regime</b> |    |
| <i>A. Ashkin</i>                                                                                      |    |
| I. Introduction                                                                                       | 1  |
| II. Light Forces in the Ray Optics Regime                                                             | 4  |
| III. Force of the Gradient Trap on Spheres                                                            | 9  |
| IV. Effect of Mode Profiles and Index of Refraction on Trapping Forces                                | 16 |
| V. Concluding Remarks                                                                                 | 20 |
| Appendix I: Force of a Ray on a Dielectric Sphere                                                     | 21 |
| Appendix II: Force on a Sphere for Trap Focus along Y Axis                                            | 22 |
| Appendix III: Force on a Sphere for an Arbitrarily Located Trap Focus                                 | 23 |
| References                                                                                            | 25 |
| <b>2. Basic Laser Tweezers</b>                                                                        |    |
| <i>Ronald E. Sterba and Michael P. Sheetz</i>                                                         |    |
| I. General Description                                                                                | 29 |
| II. Microscope                                                                                        | 30 |
| III. Laser                                                                                            | 33 |
| IV. Optics and Layout                                                                                 | 35 |
| V. System Setup                                                                                       | 36 |
| VI. Alignment                                                                                         | 37 |
| VII. Translation                                                                                      | 38 |
| VIII. Video Recording and Analysis                                                                    | 38 |
| IX. Accessories                                                                                       | 39 |
| X. Summary                                                                                            | 40 |
| Appendix: Laser Tweezers Parts List                                                                   | 40 |
| References                                                                                            | 41 |
| <b>3. A Simple Assay for Local Heating by Optical Tweezers</b>                                        |    |
| <i>Scot C. Kuo</i>                                                                                    |    |
| I. Introduction                                                                                       | 43 |
| II. Methods                                                                                           | 44 |
| III. Results                                                                                          | 44 |
| References                                                                                            | 45 |

#### 4. Reflections of a Lucid Dreamer: Optical Trap Design Considerations

*Amit D. Mehta, Jeffrey T. Finer, and James A. Spudis*

|                                         |    |
|-----------------------------------------|----|
| I. Introduction                         | 47 |
| II. Choice of Trapping Laser            | 48 |
| III. Optical Layout                     | 50 |
| IV. Imaging                             | 53 |
| V. High-Resolution Position Measurement | 56 |
| VI. Noise Sources                       | 58 |
| VII. Feedback                           | 62 |
| VIII. Calibration                       | 65 |
| IX. Analysis                            | 67 |
| X. Conclusion                           | 68 |
| References                              | 68 |

#### 5. Laser Scissors and Tweezers

*Michael W. Berns, Yona Tadir, Hong Liang, and Bruce Tromberg*

|                               |    |
|-------------------------------|----|
| I. Introduction               | 71 |
| II. Mechanisms of Interaction | 74 |
| III. Biological Studies       | 81 |
| IV. Summary                   | 94 |
| References                    | 94 |

#### 6. Optical Force Microscopy

*Andrea L. Stout and Watt W. Webb*

|                           |     |
|---------------------------|-----|
| I. Introduction           | 99  |
| II. AFM-like Applications | 100 |
| III. Experimental Design  | 105 |
| IV. New Directions        | 113 |
| References                | 115 |

#### 7. Single Molecule Imaging and Nanomanipulation of Biomolecules

*Yoshie Harada, Takashi Funatsu, Makio Tokunaga, Kiyamu Saito, Hideo Higuchi, Yoshiharu Ishii, and Toshio Yanagida*

|                                                              |     |
|--------------------------------------------------------------|-----|
| I. Introduction                                              | 117 |
| II. Visualization of Single Fluorophores in Aqueous Solution | 118 |
| III. Application                                             | 123 |
| IV. Perspectives                                             | 127 |
| References                                                   | 128 |

#### 8. Signals and Noise in Micromechanical Measurements

*Frederick Gittes and Christoph F. Schmidt*

|                                                       |     |
|-------------------------------------------------------|-----|
| I. Introduction                                       | 129 |
| II. Spectral Data Analysis                            | 131 |
| III. Brownian Motion of a Harmonically Bound Particle | 136 |

|                                                                                                                                                                                |     |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| IV. Noise Limitations on Micromechanical Experiments                                                                                                                           | 140 |
| V. Sources of Instrumental Noise                                                                                                                                               | 147 |
| VI. Conclusions                                                                                                                                                                | 154 |
| References                                                                                                                                                                     | 154 |
| <br>9. Cell Membrane Mechanics                                                                                                                                                 |     |
| <i>Jianwu Dai and Michael P. Sheetz</i>                                                                                                                                        |     |
| I. Perspectives and Overview                                                                                                                                                   | 157 |
| II. Laser Optical Tweezers                                                                                                                                                     | 160 |
| III. Bead Coating                                                                                                                                                              | 160 |
| IV. Calibration of the Laser Tweezers                                                                                                                                          | 161 |
| V. Tracking the Bead Position                                                                                                                                                  | 165 |
| VI. Membrane Tether Formation and Tether Force Measurement                                                                                                                     | 166 |
| VII. Membrane Tether Force and Membrane Mechanical Properties                                                                                                                  | 167 |
| VIII. Membrane Tension and Its Significance                                                                                                                                    | 168 |
| References                                                                                                                                                                     | 170 |
| <br>10. Application of Laser Tweezers to Studies of the Fences and Tethers of the Membrane Skeleton that Regulate the Movements of Plasma Membrane Proteins                    |     |
| <i>Akihiro Kusumi, Yasushi Sako, Takahiro Fujiwara, and Michio Tomishige</i>                                                                                                   |     |
| I. Introduction                                                                                                                                                                | 174 |
| II. Studying Various Modes of Protein Motion in the Plasma Membrane Using Single-Particle Tracking                                                                             | 176 |
| III. Technical Details for SPT                                                                                                                                                 | 176 |
| IV. Instrumental Setup and Calibration of Laser Tweezers                                                                                                                       | 179 |
| V. Dragging Receptor Molecules along the Plasma Membrane and Finding Obstacles in the Path                                                                                     | 181 |
| VI. Laser Tweezers Together with SPT Can Reveal the Presence of Elastic Intercompartmental Barriers                                                                            | 185 |
| VII. Studying the Fence and Tether Effects of the Membrane Skeleton Using Laser Tweezers                                                                                       | 188 |
| VIII. SPT and Laser Tweezers Are Opening Up Possibilities for Scientists to Learn about the Molecular Mechanics in Cells by Directly Handling Single Molecules in Living Cells | 192 |
| References                                                                                                                                                                     | 192 |
| <br>11. <i>In Vivo</i> Manipulation of Internal Cell Organelles                                                                                                                |     |
| <i>Harald Felgner, Franz Grölig, Otto Müller, and Manfred Schliwa</i>                                                                                                          |     |
| I. Introduction                                                                                                                                                                | 195 |
| II. Optical Tweezers Setup                                                                                                                                                     | 196 |
| III. Reticulomyxa: Organelle Movement and Artificial Membrane Tubes                                                                                                            | 197 |
| IV. Spirogyra: Cytoplasmic Streaming                                                                                                                                           | 199 |
| References                                                                                                                                                                     | 202 |

## 12. Optical Chopsticks: Digital Synthesis of Multiple Optical Traps

*Justin E. Molloy*

|                                        |     |
|----------------------------------------|-----|
| I. Introduction                        | 205 |
| II. Trapping Configurations            | 206 |
| III. Mechanical Deflectors (Mirrors)   | 208 |
| IV. Acousto-optic Deflectors           | 208 |
| V. Position: Control and Noise         | 210 |
| VI. Computer Control of Trap Positions | 212 |
| VII. Other Developments                | 213 |
| References                             | 215 |

|       |     |
|-------|-----|
| Index | 217 |
|-------|-----|

|                   |     |
|-------------------|-----|
| Volumes in Series | 223 |
|-------------------|-----|