Contents

ACKNOWLEDGEMENTS xi

GLOSSARY OF TERMS AND SYMBOLS xiii

1 **Introduction** 1

1.1 Motivation 1
1.2 A historical perspective 2
1.3 This book 3
1.4 Single molecule measurements 5
References 8

2 **Single molecule fluorescence techniques** 10

2.1 Introduction 10
2.2 Burst analysis 10
2.3 Photon counting histograms 12
2.4 Fluorescence correlation spectroscopy 24
2.5 Fluorescence resonance energy transfer 44
2.6 Measurements of immobilized single molecules 66
2.7 Other related techniques 80
References 89

3 **Single molecule fluorescence instrumentation** 97

3.1 Introduction 97
3.2 Optical arrangements for single molecule detection 102
3.3 Methods for discriminating signal from noise 119
3.4 Wavelength or polarization selection optics 122
3.5 Excitation sources 124
3.6 Microscope objectives for single molecule fluorescence detection 127
3.7 Detectors for single molecule fluorescence experiments 133
3.8 Acquisition cards and software 140
3.9 Realizing single molecule instrumentation 142
References 155

4 **Preparation of samples for single molecule fluorescence spectroscopy** 159
4.1 Introduction 159
4.2 Dye selection 160
4.3 Labelling of biomolecules 172
4.4 Doubly labelling single protein molecules for FRET studies 180
4.5 Optimizing biochemical systems for single molecule fluorescence studies 186
4.6 Immobilization methods 189
References 196

5 **Fluorescence spectroscopy of freely diffusing single molecules: examples** 201
5.1 Introduction 201
5.2 Single molecule studies of freely diffusing molecules 201
References 224

6 **Fluorescence spectroscopy of immobilized single molecules: examples** 225
6.1 Introduction 225
6.2 Single molecule studies of immobilized molecules 226
References 247

7 **The outlook for single molecule fluorescence measurements** 249
7.1 Outlook 249
References 252

INDEX 255