

Contents

	List of Technical Terms and Symbols.....	XIII
1	Optical Signal Recording	1
2	Overview of Photon Counting Techniques.....	11
2.1	Steady-State Photon Counting	11
2.2	Gated Photon Counting	12
2.3	Multichannel Scalers	16
2.4	Time-Correlated Single Photon Counting (TCSPC).....	20
2.4.1	General Principle	20
2.4.2	The Classic TCSPC Setup	23
2.4.3	Reversed Start-Stop	24
3	Multidimensional TCSPC Techniques	27
3.1	Multidetector TCSPC	29
3.2	Multiplexed TCSPC	33
3.3	Sequential Recording Techniques.....	35
3.4	Scanning Techniques.....	37
3.5	Imaging by Position-Sensitive Detection	39
3.6	Time-Tag Recording	43
3.7	Multimodule Systems.....	45
4	Building Blocks of Advanced TCSPC Devices.....	47
4.1	Constant-Fraction Discriminators	47
4.2	Time Measurement Block	50
4.2.1	Time Measurement by Fast TAC / ADC Principle... ..	50
4.2.2	Digital TDCs	55
4.2.3	Sine-Wave Conversion	59
5	Application of Modern TCSPC Techniques.....	61
5.1	Classic Fluorescence Lifetime Experiments	61
5.1.1	Time-Resolved Fluorescence.....	61
5.1.2	Fluorescence Lifetime Spectrometers	65
5.1.3	Fluorescence Depolarisation Effects.....	69

5.1.4	Reabsorption and Reemission.....	72
5.1.5	High-Efficiency Detection Systems.....	72
5.1.6	Measurement of the Instrument Response Function	75
5.1.7	What is the Shortest Lifetime that can be Measured?	77
5.1.8	Fluorescence Anisotropy	79
5.1.9	Time Resolved Spectra	82
5.2	Multispectral Fluorescence Lifetime Experiments.....	84
5.3	Excitation-Wavelength Multiplexing.....	87
5.4	Transient Fluorescence Lifetime Phenomena	90
5.4.1	Chlorophyll Transients.....	90
5.4.2	Continuous-Flow Mixing Techniques	94
5.4.3	Stopped-Flow Techniques	95
5.5	Diffuse Optical Tomography (DOT) and Photon Migration..	97
5.5.1	Principle of Diffuse Optical Tomography	97
5.5.2	Scanning Mammography	102
5.5.3	Brain Imaging	106
5.5.4	Muscle and Bone Studies.....	112
5.5.5	Exogenous Absorbers	112
5.5.6	Fluorescence	113
5.5.7	Small-Animal Imaging.....	115
5.5.8	Technical Aspects of TCSPC-Based DOT	117
5.6	Autofluorescence of Biological Tissue	121
5.6.1	Autofluorescence Detection by Multispectral TCSPC.....	122
5.6.2	Two-Photon Autofluorescence	124
5.6.3	Ophthalmic Imaging	126
5.7	TCSPC Laser Scanning Microscopy.....	129
5.7.1	The Laser Scanning Microscope.....	131
5.7.2	Lifetime Imaging Techniques for Laser Scanning Microscopy.....	134
5.7.3	Implementation of Multidimensional TCSPC	137
5.7.4	Multispectral FLIM.....	143
5.7.5	High Count-Rate Systems.....	146
5.7.6	FRET Measurements by TCSPC FLIM.....	149
5.7.7	Technical Details of TCSPC Laser Scanning Microscopy	154
5.8	Other TCSPC Microscopy Techniques	163
5.8.1	TCSPC Lifetime Imaging by Scan Stages	163
5.8.2	Microfluorometry.....	166

5.8.3	Time-Resolved Scanning Near-Field Optical Microscopy	167
5.8.4	TCSPC Wide-Field Microscopy	168
5.9	Picosecond Photon Correlation	169
5.9.1	AntiBunching Experiments.....	170
5.9.2	Practical Details	173
5.10	Fluorescence Correlation Spectroscopy	176
5.10.1	Combined FCS/Lifetime Experiments by TCSPC	177
5.10.2	FCS in Laser Scanning Microscopes	182
5.10.3	Practical Tips	184
5.11	Combinations of Correlation Techniques.....	187
5.11.1	Combining Picosecond Correlation and FCS	188
5.11.2	Correlation of Delayed Detector Signals	188
5.11.3	Synchronisation of TCSPC Modules.....	189
5.12	The Photon Counting Histogram.....	191
5.13	Time-Resolved Single Molecule Spectroscopy.....	193
5.13.1	Burst-Integrated Fluorescence Lifetime (BIFL) Experiments	194
5.13.2	Identification of Single Molecules.....	196
5.13.3	Multiparameter Spectroscopy of Single Molecules.....	198
5.14	Miscellaneous TCSPC Applications	201
5.14.1	Two-photon Fluorescence with Diode Laser Excitation	201
5.14.2	Remote Sensing	204
5.14.3	Laser Ranging	206
5.14.4	Positron Lifetime Experiments	206
5.14.5	Diagnostics of Barrier Discharges	208
5.14.6	Sonoluminescence.....	210
5.14.7	The TCSPC Oscilloscope	211
6	Detectors for Photon Counting.....	213
6.1	Detector Principles	213
6.1.1	Conventional Photomultiplier Tubes	213
6.1.2	Channel and Microchannel PMTs.....	214
6.1.3	Position-Sensitive PMTs.....	215
6.1.4	Single-Photon Avalanche Photodiodes.....	217
6.1.5	Hybrid PMTs	220
6.1.6	Other Detector Principles.....	221

6.2	Characterisation of Detectors	222
6.2.1	Gain.....	222
6.2.2	Single-Electron Response	222
6.2.3	Signal Transit Time	224
6.2.4	Transit Time Spread.....	224
6.2.5	Pulse Amplitude Jitter.....	226
6.2.6	Cathode Efficiency.....	229
6.2.7	Dark Count Rate	231
6.2.8	Afterpulsing Probability.....	233
6.2.9	Prepulses	234
6.3	Measurement of PMT Parameters.....	234
6.3.1	Single Electron Response	234
6.3.2	Transit Time Spread.....	236
6.3.3	Pulse Amplitude Distribution	237
6.3.4	Afterpulsing Probability.....	239
6.3.5	Luminous Sensitivity and Quantum Efficiency	241
6.4	Photon Counting Performance of Selected Detectors	242
6.4.1	MCP-PMTs.....	243
6.4.2	Cooled PMT Modules With GaAs Based Cathodes.....	245
6.4.3	PMT Modules with Internal Discriminators	247
6.4.4	Miniature PMTs in TO-8 Housing.....	248
6.4.5	Photosensor Modules	249
6.4.6	Multianode PMTs	252
6.4.7	Linear Focused PMTs	254
6.4.8	Side-Window PMTs.....	256
6.4.9	Channel Photomultipliers.....	258
6.4.10	Single Photon Avalanche Photodiodes	258
7	Practice of TCSPC Experiments	263
7.1	Excitation Sources.....	263
7.2	Optical Systems.....	268
7.2.1	Lenses	269
7.2.2	Filters	273
7.2.3	Beam Splitters.....	278
7.2.4	Monochromators and Polychromators	279
7.2.5	Optical Fibres.....	282
7.2.6	Reflections in Optical Systems	285
7.2.7	Baffles, Aperture Stops and Field Stops	288
7.2.8	Detectors	289
7.2.9	Choosing the Detector.....	289
7.2.10	Quick Test of PMTs.....	292

7.2.11	Signal-Dependent Background	294
7.2.12	Instability in PMTs	295
7.2.13	Timing Stability	296
7.2.14	PMT Voltage Dividers	299
7.2.15	Preamplifiers	300
7.3	Detector Control and Overload Protection	302
7.4	Generating the Synchronisation Signal	304
7.5	System Connections	307
7.5.1	Connector Systems	307
7.5.2	Cables	308
7.5.3	Attenuators and Power Splitters	310
7.5.4	Shielding and Grounding	311
7.6	Safety Considerations	315
7.7	Setting the TCSPC System Parameters	317
7.7.1	Optimisation of the CFD in the Detector Channel	317
7.7.2	Reference CFD	322
7.7.3	Adjusting the Delay in the Detector and Reference Channel	323
7.7.4	Choosing the TAC Parameters	326
7.8	Differential Nonlinearity	330
7.9	Counting Loss in TCSPC Systems	332
7.9.1	Classic Pile-Up Effect	332
7.9.2	Counting Loss	338
7.9.3	Dead-Time-Related Signal Distortion	343
7.10	Calibration of the Time Scale	345
8	Final Remarks	347
9	References	351
	Index	389