

Principles of Fluorescence Spectroscopy

Third Edition

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 Springer

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*Dedicated to Mary,
for her continuous support and encouragement,
without whom this book would not have been written*

Preface

The first edition of *Principles* was published in 1983, and the second edition 16 years later in 1999. At that time I thought the third edition would not be written until 2010 or later. However, the technology of fluorescence has advanced at an accelerating pace. Single-molecule detection and fluorescence-correlation spectroscopy are becoming almost routine. New classes of probes have appeared, such as the semiconductor nanoparticles, or QDots, and genetically engineered green fluorescent probes. Additionally, it is now becoming possible to control the excited states of fluorophores, rather than relying only on spontaneous emission. These developments are changing the par-

adigm of fluorescence, from a reliance on organic fluorophores, to the use of genetic engineering, nanotechnology, and near-field optics.

I wish to express my appreciation and special thanks to the individuals who have assisted me in the preparation of the book. These include Ignacy Gryczynski for assistance with the figures, Krystyna Gryczynski for drawing the figures, Joanna Malicka for proofreading the chapters, Kazik Nowaczyk for the cover design and color digitizing of all figures, Tim Oliver for typesetting, and the NIH for their support of my laboratory. And finally, Mary, for her endless hours of typing, correspondence and support.

Joseph R. Lakowicz

Glossary of Acronyms

A	acceptor	C102	coumarin 102
AA	anthranilic acid	C152	coumarin 152
2-AA	2-acetylanthracene	C153	coumarin 153
Ac	acetonitrile	9-CA	9-cyanoanthracene
Ac	acetone or acridine	CaM	calmodulin
ACF	acriflavine	cAMP	cyclic AMP
AcH	acridinium cation	CFD	constant fraction discriminator
ACTH	adrenocorticotropin hormone	CG	calcium green
Alexa-Bz	Alexa-labeled benzodiazepine	CHO	Chinese hamster ovary
ADC	analog-to-digital converter	CC	closed circular
Adx	adrenodoxin	CCDs	charged-coupled devices
I-AEDANS	5-((((2-iodoacetyl)amino)ethyl)amino)- naphthalene-1-sulfonic acid	CH	cyclohexane
		Chol	cholesterol
AFA	aminofluoranthene	CLSM	confocal laser scanning microscopy
AN	anthracene	CNF	carboxynaphthofluorescein
2-AN	2-anilinonaphthalene	ConA	concanavalin A
2,6-ANS	6-(anilino)naphthalene-2-sulfonic acid	CRABPI	cellular retinoic acid binding protein I
AO	acridine orange or acoustooptic	CSR	continuous spectral relaxation
2-AP	2-aminopurine	CT	charge transfer
4-AP	4-aminophthalimide	CW	continuous wave
APC	allophycocyanin		
APDs	avalanche photodiodes	D	donor
9-AS	9-anthroyloxy stearic acid	Dansyl	5-dimethylaminonaphthalene-1-sulfonic acid
ASEs	asymptotic standard errors	DAPI	4',6-diamidino-2-phenylindole
AT	antithrombin	DAS	decay-associated spectra
		DBS	4-dimethylamino-4'-bromostilbene
B	benzene	DC	deoxycytosine
BABAPH	2-(sulfonatobutyl)-7-(dibutylamino)-2-aza- phenanthrene	DDQ	distance-dependent quenching
BABP	sulfonatobutyl)-4-[4'-(dibutylamino)- phenyl]pyridine	DEA	diethylaniline
BCECF	7'-bis(2-carboxyethyl)-5(6)-carboxyfluores- cein	DEE	diethyl ether
BSA	bovine serum albumin	DHE	dihydroequilenin
BODIPY	refers to a family of dyes based on 1,3,5,7,8- pentamethyl pyrromethene-BF ₂ , or 4,4- difluoro-4-bora-3a,4a-diaza-s-indacene; BODIPY is a trademark of Molecular Probes Inc.	DHP	dihexadecyl phosphate
β-PE	β-phycoerythrin	DiI or DiI ₁₂	1,1'-didodecyl-3,3,3',3'-tetramethyl lindi- carbocyanine
BPTI	bovine pancreatic trypsin inhibitor	DM	dodecylmaltoside
Bromo-PCs	brominated phosphatidylcholines	DMA	dimethylaniline
Bu	butanol	DMAS	N-dimethylaniline sulfonate
		DMF	dimethylformamide
		DMPC	dimyristoyl-L-α-phosphatidylcholine
		DMP	dimethyldiazaperopyrenium
		DMSO	dimethyl sulfoxide
		DMQ	2,2'-dimethyl-p-quaterphenyl
		10-DN	10-doxylnonadecane

DNS	dansyl or 4-dimethylamino-4'-nitrostilbene	GPD	glyceraldehyde-3-phosphate dehydrogenase
DNS-Cl	dansyl chloride	GPI	glycosylphosphatidylinositol
DOS	trans-4-dimethylamino-4'-(1-oxobutyl) stilbene	GuHCl	guanidine hydrochloride
DPA	9,10-diphenylanthracene	GUVs	giant unilamellar vesicles
DPA	dipicolinic acid	H	n-hexane
DPE	dansyl-labeled phosphatidylethanolamine	HDL	high-density lipoprotein
DPH	1,6-diphenyl-1,3,5-hexatriene	HeCd	helium-cadmium
DPO	2,5-diphenyloxazole	HG	harmonic generator
DPPC	dipalmitoyl-L- α -phosphatidylcholine	HITCI	hexamethylindotricarbocyanine iodide
DPPC	dipalmitoylphosphatidylcholine	HLH	human luteinizing hormone
DP(M,O)PC(E)	dipalmitoyl(myristoyl, oleoyl)-L- α -phosphatidylcholine (ethanolamine)	HO	highest occupied
DTAC	dodecyltrimethylammonium chloride	HpRz	hairpin ribozyme
EA	ethyl acetate	HPTS	1-hydroxypyrene-3,6,8-trisulfonate
EA	ethanol	hrIFN- γ	human recombinant interferon γ
EAN	ethylaniline	HSA	human serum albumin
EB	ethidium bromide	17 β -HSD	17 β -hydroxysteroid dehydrogenase
EC	ethylcellulose	hw	half-width
ECFP	enhanced cyan fluorescent protein	IAEDANS	5-(((2-iodoacetyl)amino)ethyl)amino)-naphthalene-1-sulfonic acid
EDT	1,2-ethanedithiol	IAF	5-iodoacetamidofluorescein
EG	ethylene glycol	ICT	internal charge transfer
ELISA	enzyme-linked immunosorbent assays	IM	insertion mutant
eosin-PE	eosin-phosphatidylethanolamine	Indo-1-C ₁₈	indo-1 with a C ₁₈ chain
EP	1-ethylpyrene	IRF	instrument response function
EPE	eosin-labeled phosphatidylethanolamine	IXP	isoxanthopterin
ESIPT	excited-state intramolecular proton transfer	KF	Klenow fragment
ESR	excited-state reaction	KSI	3-ketosteroid isomerase
EO	electrooptic	LADH	liver alcohol dehydrogenase
EYFP	enhanced yellow fluorescent protein	LCAT	lecithin:cholesterol acyltransferase
F	single-letter code for phenylalanine	LDs	laser diodes
Fl	fluorescein	LE	locally excited
Fl-C	fluorescein-labeled catalytic subunit	LEDs	light-emitting diodes
FABPs	fatty acid binding proteins	LU	lowest unoccupied
FAD	flavin adenine dinucleotide	M	monomer
FC	fura-2 with calcium	MAI	N-methylquinolinium iodide
FCS	fluorescence correlation spectroscopy	MBP	maltose-binding protein
FD	frequency domain	MCA	multichannel analyzer
Fn	fibronectin	MCP	microchannel plate
Fs	femtosecond	Me	methanol
FITC	fluorescein-5-isothiocyanate	MEM	method-of-moments
FLIM	fluorescence-lifetime imaging microscopy	met RS	methionyl-tRNA synthetase
FMN	flavin mononucleotide	3-MI	3-methyl indole
FR	folate receptor	MLC	metal-ligand complex, usually of a transition metal, Ru, Rh or Os
FRET	fluorescence-resonance energy transfer	MLCK	myosin light chain kinase
FWHM	full width of half-maximum intensity	MLCT	metal-ligand charge transfer (state)
4FW	4-fluorotryptophan	MLE	maximum likelihood estimates
GADPH	glyceraldehyde-3-phosphate dehydrogenase	MPE	multiphoton excitation
GFP	green fluorescent protein	MPM	multiphoton microscopy
GGBP	glucose-galactose binding protein	MQAE	6-methoxy-quinolyl acetoethyl ester
GM	Goppert-Mayer	MRI	magnetic resonance imaging
GOI	gated optical image intensifier		
GP	generalized polarization		

NADH	reduced nicotinamide adenine dinucleotide	QDs	quantum dots
NATA	N-acetyl-L-tryptophanamide	QTH	quartz–tungsten halogen
NATyrA	N-acetyl-L-tyrosinamide		
NB	Nile blue	RBC	radiation boundary condition
NBD	N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)	RBL	rat basophilic leukemia
NBD-DG	1-oleoyl-2-hexanoyl-NBD-glycerol	R-PE	R-phycoerythrin
Nd:YAG	neodymium:YAG	REES	red-edge excitation shifts
NIR	near infrared	Re I	rhenium
NLLS	nonlinear least squares	RET	resonance energy transfer
NMA	N-methylantraniloyl amide	RF	radio frequency
NO	nitric oxide	RFP	red fluorescent protein
NPN	N-phenyl-1-naphthylamine	Rh	rhodamine
NR	neutral red	RhB	rhodamine B
NRP	neuronal receptor peptide	RhG	rhodamine green
5-NS	5-doxylstearate	R6G	rhodamine 6G
		RNase T ₁	ribonuclease T ₁
OG	Oregon green	RR	rhodamine red
OPO	optical parameter oscillator	Ru	ruthenium
ORB	octadecyl rhodamine B		
Os	osmium	SAS	species-associated spectra
		SBFI	sodium-binding benzofuran isophthalate
PBFI	potassium-binding benzofuran isophthalate	SBP	steroid-binding protein
PC	phosphatidylcholine	SBS	substrate-binding strand
PCSC	photon-counting streak camera	SC	subtilisin Carlsberg
PDA	pyrene dodecanoic acid	SDS	sodium dodecylsulfate
PDs	photodiodes	SEDA	dapoxyl sulfonyl ethylenediamine
PE	phycoerythrin	SMD	single-molecule detection
PE	phosphatidylethanolamine	SNAFLs	seminaphthofluoresceins
1PE	one-photon	SNARFs	seminaphthorhodafluors
2PE	two-photon	SP	short-pass
3PE	three-photon	SPQ	6-methoxy-N-[3-sulfopropyl]quinoline
PET	photoinduced electron transfer		
PcCN	3-cyanoperylene	T	tetramer
PG	propylene glycol	TAC	time-to-amplitude converter
PGK	phosphoglycerate kinase	TCE	trichloroethanol
Phe(F)	phenylalanine	t-COPA	16-octadecapentaenoic acid
PK	protein kinase	TCSPC	time-correlated single photon counting
PKI	protein kinase inhibitor	TD	time-domain
PMMA	poly(methylmethacrylate)	TEOS	tetraethylorthosilicate
PMT	photomultiplier tube	TFA	trifluoroacetamide
POPC	1-palmitoyl-2-oleoylphosphatidylcholine	TFE	trifluoroethanol
POPOP	1,4-bis(5-phenyloxazol-2-yl)benzene	THF	tetrahydrofuran
PP	pulse picker	TICT	twisted internal charge transfer
PPD	2,5-diphenyl-1,3,4-oxadiazole	TK	thymidine kinase
PPi	pyrophosphate	TL	tear lipocalin
PPO	2,5-diphenyloxazole	TMA	donor alone
PRODAN	6-propionyl-2-(dimethylamino)-naphthalene	TMR	tetramethylrhodamine
		TnC	troponin C
ps	picosecond	TNS	6-(p-toluidinyl)naphthalene-2-sulfonic acid
PSDF	phase-sensitive detection of fluorescence	TOAH	tetraoctylammonium hydroxide
PTP	phosphoryl-transfer protein	TOE	tryptophan octyl ester
Py2	pyridine 2	TPI	triosephosphate isomerase

TRES	time-resolved emission spectra	w	single-letter code for tryptophan
TrpNH ₂	tryptophanamide	W	water
TRITC	tetramethylrhodamine-5-(and-6)-isothio- cyanate	WT	wild type
tRNA ^{fMet}	methionine tRNA	WD	window discriminator
trp(w)	tryptophan	Xe	xenon
TTS	transit time spread	y	single-letter code for tryptophan
TU2D	donor-acceptor pair	YFP	yellow fluorescent protein
tyr(y)	tyrosine		
U	uridine		
7- UmP	7-umbelliferyl phosphate		

Glossary of Mathematical Terms

A	acceptor or absorption	pK_a	acid dissociation constant, negative logarithm
B_i	brightness of a fluorophore	q	efficiency for detection of emitted photons, typically for FCS
c	speed of light	Q	quantum yield
C_0	characteristic acceptor concentration in RET	r	anisotropy (sometimes distance in a distance distribution)
$C(t)$	correlation function for spectral relaxation	$\bar{r}$	average distance in a distance distribution
D	donor, or diffusion coefficient, or rotational diffusion coefficient	$r(0)$	time-zero anisotropy
$D_{ }$ or $D_{\perp}$	rate of rotation diffusion around or (displacing) the symmetry axis of an ellipsoid of revolution	$r(t)$	anisotropy decay
$D(\tau)$	part of the autocorrelation function for diffusion containing the diffusion-dependent terms	r_c	distance of closest approach between donors and acceptors in resonance energy transfer, or fluorophores and quenchers
E	efficiency of energy transfer	r_{0i} or r_{0g_i}	fractional amplitudes in a multi-exponential anisotropy decay
F	steady-state intensity or fluorescence	r_0	fundamental anisotropy in the absence of rotational diffusion
$F\chi$	ratio of χ_R^2 values, used to calculate parameter confidence intervals	r_{0i}	anisotropy amplitudes in a multi-exponential anisotropy decay
$F(\lambda)$	emission spectrum	r_{∞}	long-time anisotropy in an anisotropy decay
f_i	fractional steady-state intensities in a multi-exponential intensity decay	r_{ω}	modulated anisotropy
f_Q	efficiency of collisional quenching	R_0	Förster distance in resonance energy transfer
G	correction factor for anisotropy measurements	T	temperature
$G(\tau)$	autocorrelation function of fluorescence fluctuations	T_p	phase transition temperature for a membrane
hw	half-width in a distance or lifetime distribution	α_i	pre-exponential factors in a multi-exponential intensity decay
$I(t)$	intensity decay, typically the impulse response function	β	angle between absorption and emission transition moments
k_{nr}	non-radiative decay rate	γ	inverse of the decay time, $\gamma = 1/\tau$
k_s	solvent relaxation rate	Γ	radiative decay rate
k_T	transfer rate in resonance energy transfer	ε	dielectric constant or extinction coefficient
k_{st}	rate of singlet to triplet intersystem crossing	ε_A or ε	molar extinction coefficient for absorption
k_{ts}	rate of return to the singlet ground state from the triplet state	θ	rotational correlation time
m_{ω}	modulation at a light modulation frequency ω	θ_c	critical angle for total internal reflection
n	refractive index, when used in consideration of solvent effects	κ^2	orientation factor in resonance energy transfer
N	number of observed molecules in FCS	λ	wavelength
$N(t_k)$	number of counts per channel, in time-correlation single-photon counting	λ_{em}	emission wavelength
$P(r)$	probability function for a distance (r) distribution	λ_{em}^{max}	maximum emission wavelength
		λ_{ex}	excitation wavelength

$\lambda_{\text{ex}}^{\text{max}}$	maximum excitation or absorption wavelength for the lowest $S_0 \rightarrow S_1$ transition
λ_{max}	emission maxima
Λ_p	ratio of the modulated amplitudes of the polarized components of the emission
η	viscosity
μ_E	excited-state dipole moment
μ_G	ground-state dipole moment
μm	micron

GLOSSARY OF MATHEMATICAL TERMS

$\bar{\nu}$	specific gravity or wavelength in cm^{-1}
$\bar{\nu}_{\text{cg}}$	center of gravity of an emission spectrum in cm^{-1}
σ or σ_A	optical cross-section for absorption
σ_S	optical cross-section for scattering
τ	lifetime or time-delay in FCS
τ_D	diffusion time in FCS
τ_s	solvent or spectral relaxation time

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