

国外化学名著系列

(影印版)8

〔美〕 Joseph R. Lakowicz

Principles of Fluorescence Spectroscopy

(Third Edition)

荧光分析法原理

(第三版)



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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
	aminofluoranthene	Chol	cholesterol
AFA	anthracene	CLSM	confocal laser scanning microscopy
AN	2-anilinonaphthalene	CNF	carboxynaphthofluorescein
2-AN	6-(anilino)naphthalene-2-sulfonic acid	ConA	concanavalin A
2,6-ANS	acridine orange or acoustooptic	CRABPI	cellular retinoic acid binding protein I
AO	2-aminopurine	CSR	continuous spectral relaxation
2-AP	4-aminophthalimide	CT	charge transfer
4-AP	allophycocyanin	CW	continuous wave
APC	avalanche photodiodes		
APDs	9-anthroxly stearic acid	D	donor
9-AS	asymptotic standard errors	Dansyl	5-dimethylaminonaphthalene-1-sulfonic acid
ASEs	antithrombin	DAPI	4',6-diamidino-2-phenylindole
AT		DAS	decay-associated spectra
		DBS	4-dimethylamino-4'-bromostilbene
B	benzene	DC	deoxycytosine
BABAPH	2-(sulfonatobutyl)-7-(dibutylamino)-2-azaphenanthrene	DDQ	distance-dependent quenching
		DEA	diethylaniline
BABP	sulfonatobutyl)-4-[4'-(dibutylamino)-phenyl]pyridine	DEE	diethyl ether
		DHE	dihydroequeilenin
BCECF	7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein	DHP	dihexadecyl phosphate
		DiI or DiIC ₁₂	1,1'-didodecyl-3,3,3',3'-tetramethyl indo-carbocyanine
BSA	bovine serum albumin	DM	dodecylmaltoside
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.	DMA	dimethylaniline
		DMAS	N-dimethylaniline sulfonate
		DMF	dimethylformamide
		DMPC	dimyristoyl-L- α -phosphatidylcholine
		DMP	dimethyldiazaperopyrenium
β -PE	β -phycoerythrin	DMSO	dimethyl sulfoxide
BPTI	bovine pancreatic trypsin inhibitor	DMQ	2,2'-dimethyl-p-quaterphenyl
Bromo-PCs	brominated phosphatidylcholines	10-DN	10-doxylnonadecane
Bu	butanol		

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-doxylosteareate	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
PBFI	potassium-binding benzofuran isophthalate	SBFI	sodium-binding benzofuran isophthalate
PC	phosphatidylcholine	SBP	steroid-binding protein
PCSC	photon-counting streak camera	SBS	substrate-binding strand
PDA	pyrene dodecanoic acid	SC	subtilisin Carlsberg
PDs	photodiodes	SDS	sodium dodecylsulfate
PE	phycoerythrin	SEDA	dapoxyl sulfonyl ethylenediamine
PE	phosphatidylethanolamine	SMD	single-molecule detection
1PE	one-photon	SNAFLs	seminaphthofluoresceins
2PE	two-photon	SNARFs	seminaphthorhodafuors
3PE	three-photon	SP	short-pass
PET	photoinduced electron transfer	SPQ	6-methoxy-N-[3-sulfonylpropyl]quinoline
PeCN	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	tetraethoxysilicate
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	tropoin 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)-isothiocyanate	WT	wild type
tRNA ^{Met}	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 _⊥	rate of rotation diffusion around or (displacing) the symmetry axis of an ellipsoid of revolution	r(t)	anisotropy decay
D(τ)	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
Fχ	ratio of χ _R ² values, used to calculate parameter confidence intervals	r _{0i}	anisotropy amplitudes in a multi-exponential anisotropy decay
F(λ)	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(τ)	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, γ = 1/τ
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 _{is}	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_2	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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