常见染料的多光子激发光谱

Probe	TPE Excitation Wavelength	Application
Alexa Fluor® 488 phalloidin	720 nm or 830 nm	Imaging F-actin organization in pancreatic acinar cells
Alexa Fluor® 594 hydrazide	810 nm	Ca^{2+}-insensitive, neuronal tracer *
Amplex® Red reagent	750 nm or 800 nm	Detection of reactive oxygen species (ROS) associated with amyloid plaques
CFSE, CMTMR	820 nm	Tracking T and B lymphocytes and dendritic cell motility patterns in intact mouse lymph nodes †
CM-H_2DCFDA	740 nm	Detection of localized reactive oxygen species release in cardiomyocytes ‡
DAPI, Hoechst 33342	740 nm	Imaging DNA in nuclei and isolated chromosomes
DiD	817 nm	Intravital imaging of mouse erythrocytes
FM® 1-43	840 nm	Monitoring synaptic vesicle recycling in rat brain slices
Fluo-5F §	810 nm	Imaging Ca^{2+} concentration dynamics in dendrites and dendritic spines
Fura-2	780 nm	Detection of GABA-mediated Ca^{2+} transients in rat cerebellar Purkinje neurons
Lucifer yellow CH	850 nm	Identification of gap junctions in rat brain slices
Laurdan	800 nm	Detection of ordered membrane lipid domains
Monochloro-bimane	780 nm	Imaging glutathione levels in rat brain slices and intact mouse brain
MQAE	750 nm	Fluorescence lifetime imaging (FLIM) of intracellular Cl^- concentrations in olfactory sensory neurons
Oregon Green® 488 BAPTA-1	880 nm	Imaging spatiotemporal relationships of Ca^{2+} signals among cell populations in rat brain cortex
Qdot® 525, Qdot® 585, Qdot® 655 nanocrystals	750 nm	Multiplexed immunohistochemical analysis of arterial walls **
SBFI	760 nm	Imaging of intracellular Na^+ gradients in rat cardiomyocytes
TMRE	740 nm	Mitochondrial membrane potential sensor ‡
X-rhod-1	900 nm	Simultaneous imaging of GFP-PHD translocation and Ca^{2+} dynamics in cerebellar purkinje cells

TABLE 1 Two-photon cross-sections of some common fluorophores[a]

Fluorophores[b]	Excitation wavelength (nm)	$\eta_2\delta$ (10^{-50} cm^4 s/photon)	δ (10^{-50} cm^4 s/photon)
Extrinsic fluorophores			
Rhodamine B	840		210 (±55)
Fluorescein (pH 11)	782		38 (±9.7)
Fura-2 (free)	700	11	
Fura-2 (with Ca^{2+})	700	12	
Indo-1 (free)	700	4.5 (±1.3)	12 (±4)
Indo-1 (high Ca)	700	1.2 (±0.4)	2.1 (±0.6)
Bis-MSB	691	6.0 (±1.8)	6.3 (±1.8)
Dansyl	700	1	
Dansyl hydrazine	700	0.72 (±0.2)	
$DiIC_{18}$	700	95 (±28)	
Coumarin-307	776	19 (±5.5)	
Cascade blue	750	2.1 (±0.6)	
Lucifer yellow	860	0.95 (±0.3)	
DAPI	700	0.16 (±0.05)	
BODIPY	920	17 (±4.9)	
Intrinsic fluorophores			
GFP wild type	~800		~6
GFP S65T	~960		~7
NADH	~700		~0.02
FMN	~700		~0.8
Phycoerythrin	1064		322 (±110)

[a] η_2, Fluorescence quantum efficiency under two-photon excitation (35, 42, 161).

[b] Abbreviations: Bis-MSB, p-bis(o-methylstyryl) benzene; $DiIC_{18}$, octadecyl indocarbo cyanine; DAPI, 4′,6-diamidino-2-phenylindole; BODIPY, 4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diazaindacene-2,6-disulfonic acid disodium salt; GFP, green fluorescent protein; NADH, reduced nicotinamide-adenine dinucleotide; FMN, flavin mononucleotide.

Blue/Cyan Dyes

Dye	Excitation
Alexa 350	780–800 nm
Hoechst	780–800 nm 900–1100 nm
DAPI	780–800 nm 900–1100 nm
CFP	800–900 nm

Green Dyes

Dye	Excitation
Oregon Green	800–860 nm
Alexa 488	800–830 nm
eGFP	920–990 nm
BODIPY	900–950 nm
FITC	750–800 nm
DiO	780–830 nm

Yellow/Orange Dyes

Dye	Excitation
YFP	890–950 nm
DiA	800–860 nm

Red Dyes

Dye	Excitation
DiI	830–920 nm
Rhodamine B	800–860 nm
Alexa 568	780–840 nm

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Table 1. Comparison of OP/TP excitation and emission data.

	OP maxima			TP maxima		Excitation max. shift	Emission max. shift	TP 850 nm excitation
	Abs. (nm)	Ex. (nm)	Em. (nm)	Ex. (nm)	Em. (nm)	OPE→TPE (nm)	OPE→TPE (nm)	Yield (Em_{850}/Em_{max})
ECFP$^{(ES)}$	444	436	475	850	477	−11	+2	
ECFP$^{(ref.)}$		434*‡	472*	860‡				
EGFP$^{(PS)}$	488	491	510	970	511	−4	±0	
EGFP$^{(ES)}$	489	492	510		511		±0	0.32
EGFP$^{(CS)}$		488	510	950	521	−14	+11	
EGFP$^{(ref.)}$		489*‡	508*†	920‡				
		471†						
EYFP$^{(ES)}$	514	515	530	970	530	−29	±0	
EYFP$^{(ref.)}$		514*‡	527*	960‡				0.33

CS, crude sample; ES, enriched sample; PS, pure sample; ref., reference.

*Patterson *et al.* (2001); †Labas *et al.* (2002); ‡Blab *et al.* (2001).