Workshop Training Series

The Application of iBOX Scientia and LI-COR CLx

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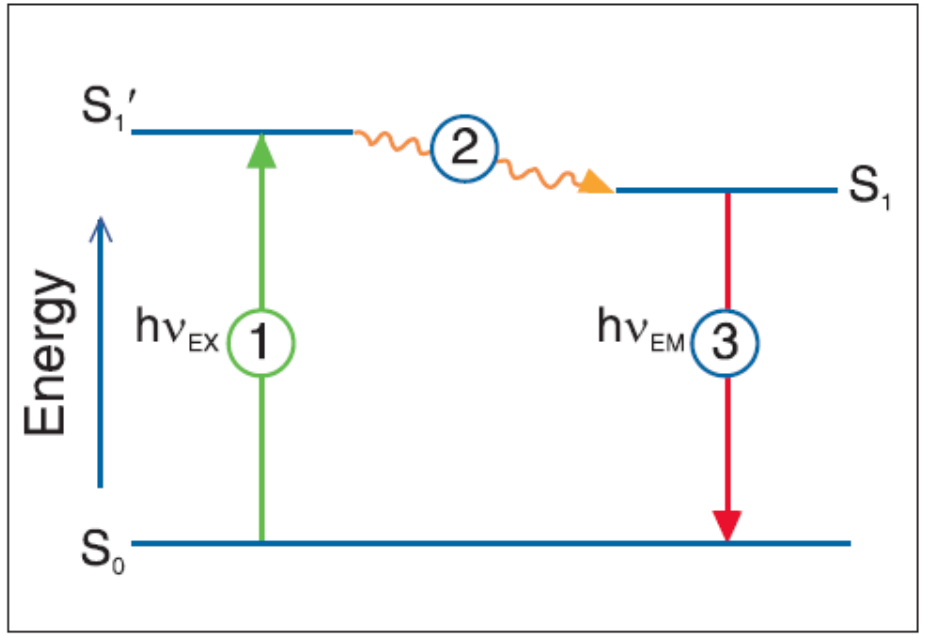
Basic Introduction to Fluorescence Techniques

- Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation.
- The fluorescence is widely used in lighting, analytical chemistry, forensics, mechanical engineering, and biomedical research.
- Fluorescent probes enable biomedical researchers to measure the products of biochemical reactions, detect particular components of complex biomolecular assemblies and visualize structures of interest in cells as well as whole bodies, with exquisite sensitivity and selectivity.

Electronic-State Diagram of Fluorescence (Jablonski Diagram)



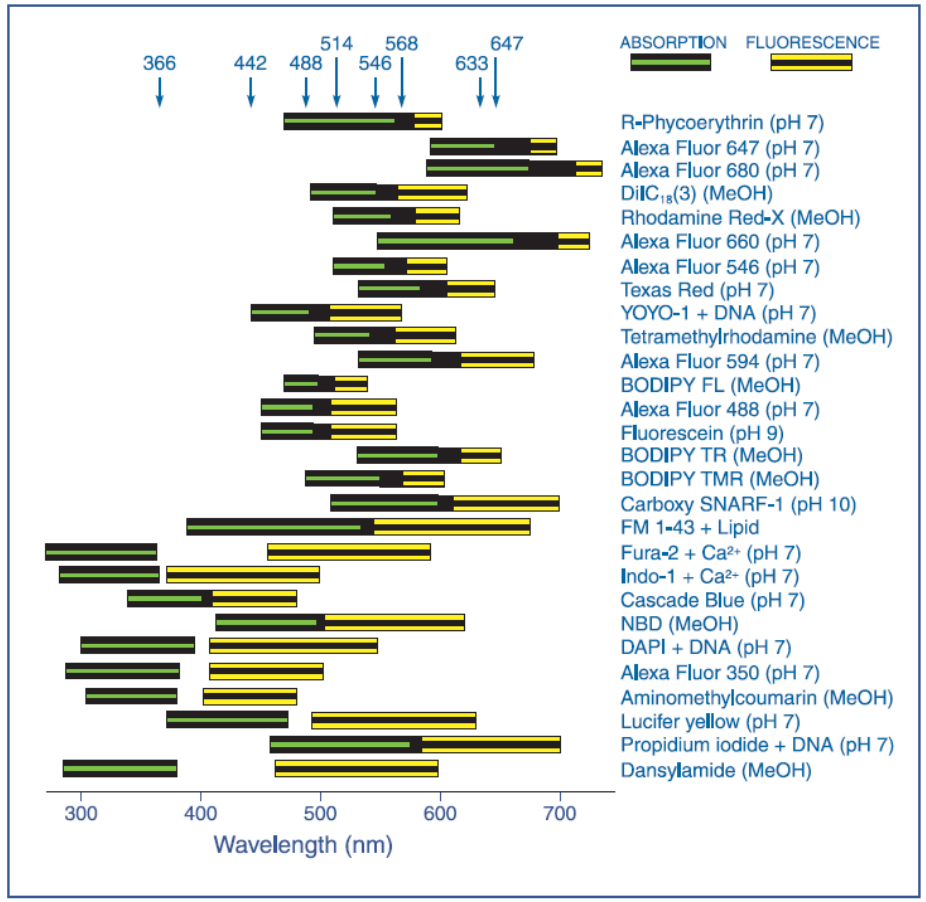
The photon energy is equal to the Planck constant (h) times a frequency of oscillation of an atomic oscillator (v, the Greek letter nu).

- A photon of energy hu_{EX} is supplied by an external source such as an incandescent lamp or a laser and absorbed by the fluorophore, creating an excited electronic singlet state (S_1')
- The excited state exists for a finite time (typically 1–10 nanoseconds).
- A photon of energy hu_{EM} is emitted, returning the fluorophore to its ground state S_0. Due to energy dissipation during the excited-state lifetime, the energy of this photon is lower, and therefore of longer wavelength, than the excitation photon hu_{EX}.
- The entire fluorescence process is cyclical. Unless the fluorophore is irreversibly destroyed in the excited state (an important phenomenon known as photobleaching), the same fluorophore can be repeatedly excited and detected.
- The intensity of the emission light depends on the mount of fluorophore and can be qualified and quantified.

Fluorescence Detection Systems

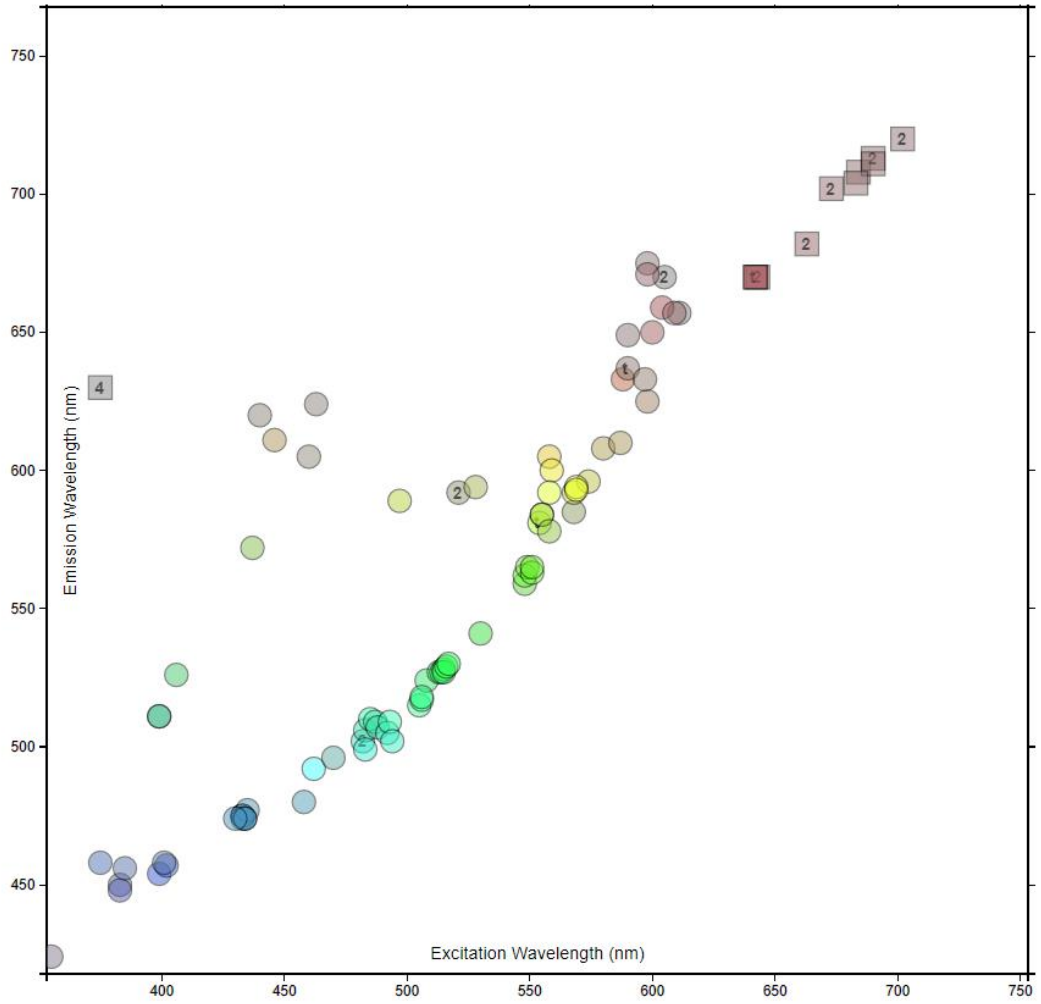
- An excitation source
- Fluorophores (dyes, Fluorescent proteins)
- Wavelength filters to isolate emission photons from excitation photons and
- A detector that registers emission photons and produces a recordable output, usually as an electrical signal or a photographic image.
- A software to analyze the data

Absorption and Fluorescence Spectral Ranges Of Fluorophores Most Used In Research



- **The range encompasses only those values of the absorbance or the fluorescence emission that are >25% of the maximum value.**
- **Fluorophores are arranged vertically in rank order of the maximum molar extinction coefficient (εmax), in either methanol or aqueous buffer as specified.**

Fluorescent Protein Properties



http://www.fpvis.org/FP.html

- Each fluorescent protein begins plotted with excitation wavelength on the x-axis and emission wavelength on the y-axis.
- The color is set based on its emission wavelength, and fades to gray as the brightness (product of exctinction coefficient and quantum yield) decreases.
- Intrinsically fluorescent proteins are shown as circles and proteins requiring an extrinisc cofactor are shown as squares.
- Dimeric proteins are indicated with a 2 inside the symbol; tandem dimeric proteins are shown with a t inside the symbol.

iBox Scientia Imaging Systems

- **High sensitivity cameras/optics**
  - Cooled and ultracooled CCDs
- **Increased resolution**
  - High megapixel CCDs
- **Capture and analytical software**
  - Integrates darkroom, camera, lens automation
- **Excitation and emission automation**
  - The BioLite Excitation light engine
  - 8 excitation filters (400-750nm)
  - Epi 365nm UV
  - 5 emission filters (to NIR)
- **Anesthesia system**
- **Temperature controlled imaging surface**

iBox® Scientia System

UVP

iBox Scientia Imaging Systems

- Non-Invasive, Visible and NIR In Vivo Imaging for Detection of Fluorescent Markers in Small Animals
- Unlimited selection of filters enables imaging in the fluorescent, visible and NIR ranges
- Optional anesthesia unit for small animals produces a state of immobilization that allows acquisition of repeatable, consistent images

Filter Selection Chart

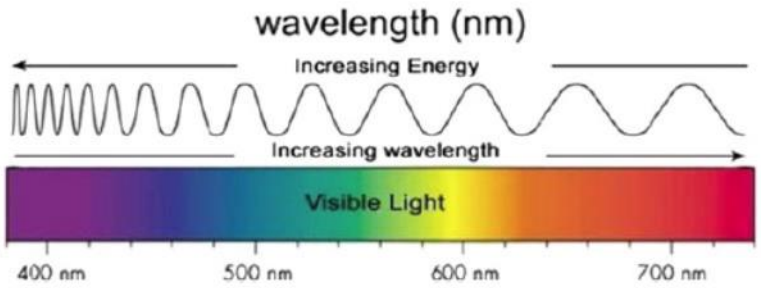
				UVP Darkroom Emission Filters											
			Part Number	38-0361-01	38-0352-01	38-0340-01	38-0344-01	38-0220-01	38-0220-03	38-0341-01	38-0339-01	38-0369-01	38-0362-01	38-0365-01	38-0368-01
			Wavelength	465-495	503-523	513-557	565-625	580-630	580-630	607-682	668-722	700-740	767-807	780lp**	800lp***
	Part Number (Auto BioLite)	Part Number (Manual BioLite)	Wavelength	Peak nm	480	513	535	595	605	605	645	695	720	787	NA	NA
UVP Excitation Filters for BioLite	38-0358-04	38-0358-03	450sp*	NA	CFP (mice)			SYPRO® Ruby								
	38-0340-04	38-0340-03	455-495	475		GFP (mice)	Alexa Fluor® 488, CF™488A, Cy2®, Fluo, FITC, FAM, GFP (gels, blots, plants), Oregon Green 488®, Rhodamine Green, 110, SYBR® Green, Gold, Safe, YFP, MultiFluor Blue, DyLight 488	SYPRO® Orange			SYPRO® Tangerine					
	38-0344-04	38-0344-03	502-547	525				Alexa Fluor® 546, 555, CF™543, 555, Cy3®, MultiFluor Green, DyLight 550	Ethidium Bromide ONLY (if others, see next column)	Ethidium Bromide, Deep Purple, RFP	Propidium Iodide					
	38-0341-04	38-0341-03	533-587	560							Alexa Fluor® 568, 594, CF™568, 594, 620R, Rhodamine Red, 6G, B, SYPRO® Red, Texas Red®	RedDot™1, RedDot™2				
	38-0359-04	38-0359-03	600-645	630								Alexa Fluor® 633, 647, CF™633, 640R, 647, 660C, 660R, Cy5® & 5.5, MultiFluor Red, DyLight 633, 650, 680/680B, Alexa Fluor 680, RedDot™1, RedDot™2	IRDye® 680, CF™ 680, RedDot™1, RedDot™2			
	38-0360-04	38-0360-03	687-748	715										Alexa Fluor® 750, CF™750, Cy7®, RedDot™1, RedDot™2	Alexa Fluor® 750, CF™750	
	38-0371-04	38-0371-03	700-740	720										CF™750	CF™750	Multiplexing IRDye® 680 & 800 or CF™ 680 & 770 (See Below)
	38-0370-04	38-0370-03	750-780	765												IRDye® 800, CF™ 770, 790
	Excited by UV Source (not BioLite)						Qdot™ 525				Qdot™ 655					

* 450 short pass filter. Can accommodate wavelengths 380-450nm based on BioLite energy emission

** 780 long pass filter. Can accommodate wavelengths above 780nm

FILTER SELECTION CHART

Consideration of Choosing Fluorophores/Fluorescent Proteins



- Generally, the distance travelled by emission light is determined by the wavelength of light and the tissue property. Scattering and absorption decrease significantly at wavelengths longer than 600 nm.
- Reduced signal-to-noise ratio of fluorescence imaging results from increased autofluorescence, which is relatively high in the visible range, but declines at longer wavelengths (>600nm).
- Many commercial fluorophores absorb and emit in or near the NIR (LI-COR's IRDye700 and IRDye800, for instance).
- Besides wavelength that affects the penetration and autofluorescence, the brightness and photostability are also need to be considered when choose fluorescent proteins used for your research.

Properties of the best FP variants

Class	Protein	Source laboratory (references)	Excitation[c] (nm)	Emission[d] (nm)	Brightness[e]	Photostability[f]	pKa	Oligomerization
Far-red	mPlum[g]	Tsien (5)	590	649	4.1	53	<4.5	Monomer
Red	mCherry[g]	Tsien (4)	587	610	16	96	<4.5	Monomer
	tdTomato[g]	Tsien (4)	554	581	95	98	4.7	Tandem dimer
	mStrawberry[g]	Tsien (4)	574	596	26	15	<4.5	Monomer
	J-Red[h]	Evrogen	584	610	8.8*	13	5.0	Dimer
	DsRed-monomer[h]	Clontech	556	586	3.5	16	4.5	Monomer
Orange	mOrange[g]	Tsien (4)	548	562	49	9.0	6.5	Monomer
	mKO	MBL Intl. (10)	548	559	31*	122	5.0	Monomer
Yellow-green	mCitrine[i]	Tsien (16,23)	516	529	59	49	5.7	Monomer
	Venus	Miyawaki (1)	515	528	53*	15	6.0	Weak dimer[j]
	YPet[g]	Daugherty (2)	517	530	80*	49	5.6	Weak dimer[j]
	EYFP	Invitrogen (18)	514	527	51	60	6.9	Weak dimer[j]
Green	Emerald[g]	Invitrogen (18)	487	509	39	0.69[k]	6.0	Weak dimer[j]
	EGFP	Clontech[l]	488	507	34	174	6.0	Weak dimer[j]
Cyan	CyPet	Daugherty (2)	435	477	18*	59	5.0	Weak dimer[j]
	mCFPm[m]	Tsien (23)	433	475	13	64	4.7	Monomer
	Cerulean[g]	Piston (3)	433	475	27*	36	4.7	Weak dimer[j]
UV-excitable green	T-Sapphire[g]	Griesbeck (6)	399	511	26*	25	4.9	Weak dimer[j]

Applications of the iBox in Biomedical Research

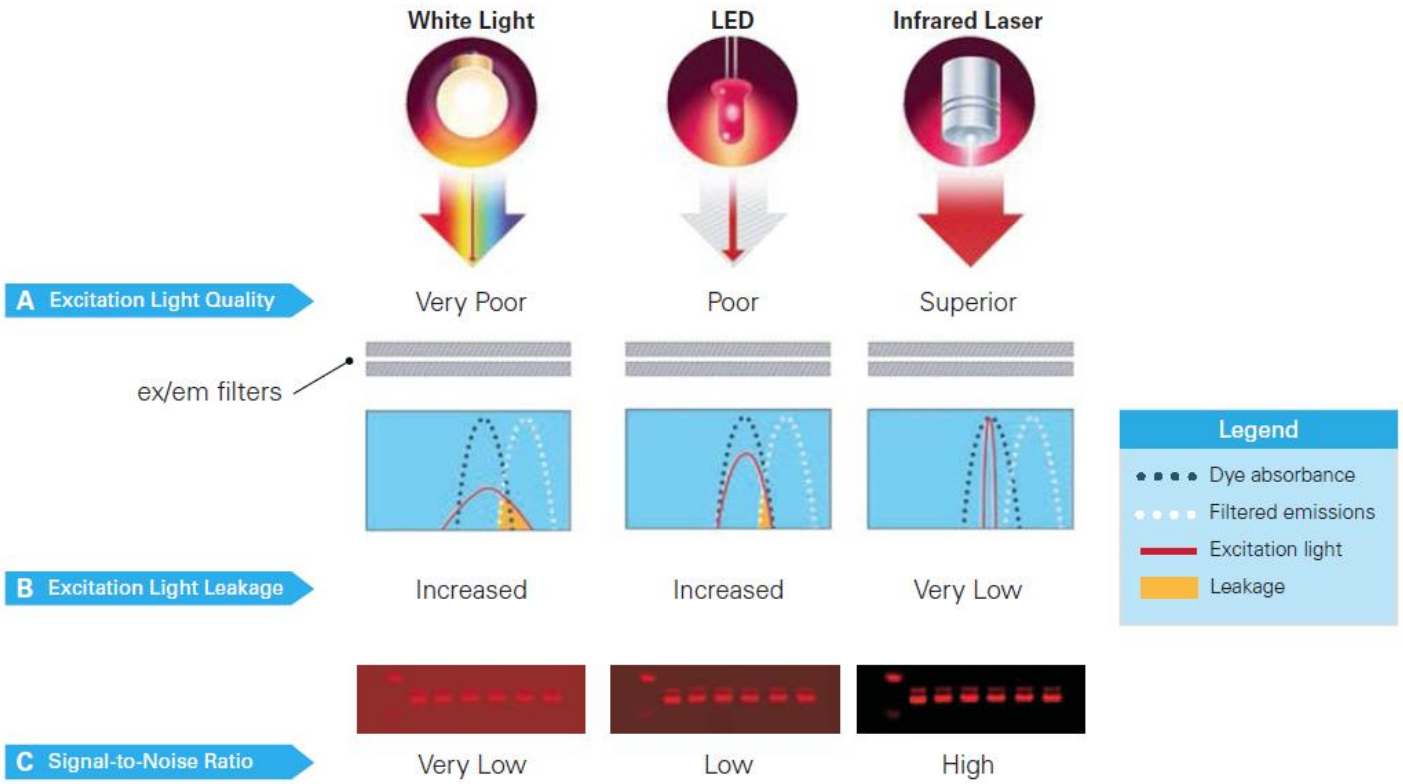
- Tumor studies
- Cancer research
- Heart disease
- Immunology
- Metastasis
- Gene expression

ODYSSEY® CLx Infrared Imaging System

Front Panel and Scanning Surface

The Odyssey CLx is the next generation multifunctional imaging platform that can provide a wide range of applications. You can use it to scan DNA/RNA and protein gel, detect western blots, perform in-cell and on-cell western analysis, and perform ELISA/FLISA. Odyssey CLX can also be used to perform small animal imaging: in vivo, whole organ and tissue section.

Infrared Laser Excitation Out-performs LED And Visible White Light Systems



The Features of ODYSSEY® CLx Infrared Imaging System

- Quantitative analysis and a wide linear dynamic range are available with ODYSSEY® CLx Infrared Imaging System
- ODYSSEY® CLx Infrared Imaging System results in the highest signal-to-noise ratios, and the best detection sensitivity.
- Detect strong and weak bands on the same blot, without blowouts or hidden bands
- Detect two targets simultaneously on the same membrane to increase quantification accuracy
- At the 700 nm and 800 nm infrared wavelengths, both autofluorescence and light scatter produced by biological materials are dramatically reduced.
- Infrared dyes offer advanced signal stability that allows for convenient and reproducible data that are not time-sensitive – data are not contingent on the lifespan of an enzymatic reaction

BrightSite™ IRDye® Small Animal Imaging Agents

Application	IRDye EGF	IRDye RGD	IRDye 2-DG	IRDye PEG	IRDye HA	IRDye YC-27	IRDye BoneTag	CellVue	PSVue
Tumor Imaging	✓	✓	✓	✓	✓	✓			
Metabolic Imaging			✓						
Inflammation/Arthritis		✓	✓						
Vasculature (Contrast)				✓	✓				
Lymphatic Imaging					✓	✓			
Lymph Node Imaging					✓	✓			
Structural Imaging							✓		
Cell Trafficking								✓	
Apoptosis									✓

IRDye *YC-27 and IRDye *HA can be provided by LI-COR Custom Services group.

BrightSite™ IRDye® Small Animal Imaging Agents

- Near-infrared fluorescent probes for targeting of tumors, bone, lymphatics, and more.
- Validated with cell-based assays, microscopy, small animal imaging, and histology to ensure high affinity and specificity
- Simply administer the agent, then image the animal with a small animal imager equipped with an appropriate 680 nm and 800 nm filter set
- Compatible with most small animal imaging systems, including instruments from LI-COR Biosciences (Pearl® Imager and Odyssey®Imager), PerkinElmer (Xenogen, Caliper, CRi, and VisEn), and Bruker (Carestream and Kodak)

The Application of ODYSSEY® CLx Infrared Imaging System