

## **Information and Specifications:** **CRI Inc. Maestro Multi-Spectral Fluorescence**

### **INFORMATION**

#### **Basic Concepts**

Generation of luminescence through ultraviolet or visible light that results in a molecular excited state is termed photoluminescence. Photoluminescence is divided into two subsets, fluorescence and phosphorescence. The electronic configuration of the excited states and the emission pathway will determine which of the two will prevail. Fluorescence is the property of some atoms and molecules to absorb light at a particular wavelength and to subsequently emit light of a longer wavelength (i.e., when blue light is shown onto a certain type of protein it will appear to glow green). The phosphorescence is similar to fluorescence, but it remains in the excited state longer. The process of fluorescence is regulated by three primary events. A molecule absorbs the incoming light of a photon and reaches an excited state. The absorption of light and the excited state is reached in femtoseconds ( $10^{-15}$  s). The second event is the loss of energy of this excited state; this occurs in picoseconds ( $10^{-12}$  s). Lastly, the emission of a lower energy/longer wavelength photon and return to the ground state occurs in nanoseconds ( $10^{-9}$  s). These steps are illustrated in Figure 1.

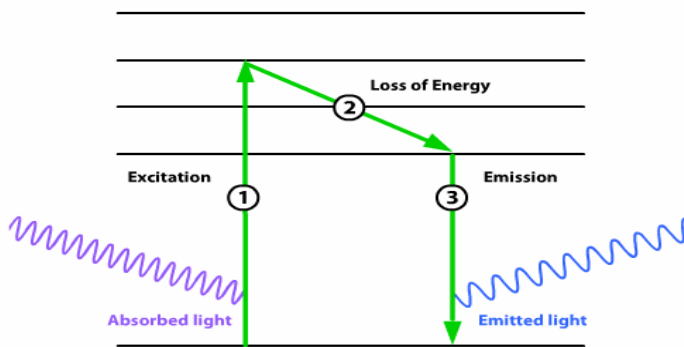


Figure 1: Three main steps in the fluorescence process  
([www.invitrogen.com/resources/education](http://www.invitrogen.com/resources/education)).

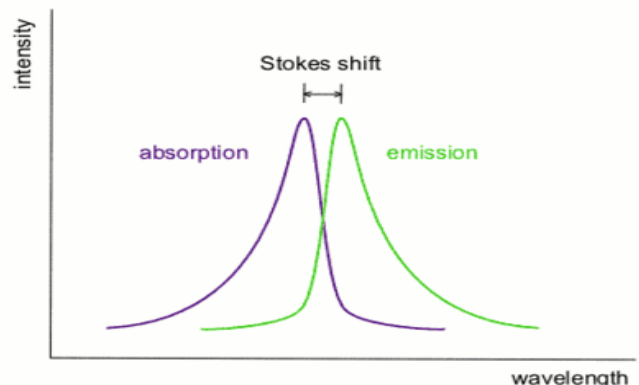


Figure 2: Stokes shift  
([http://en.wikipedia.org/wiki/Stokes\\_shift](http://en.wikipedia.org/wiki/Stokes_shift)).

Due to the loss of energy during the second step, there is always a shift in the energy from higher to lower. This phenomenon was first observed by the British scientist, Sir George G. Stokes in the late seventeenth century, and it was therefore termed Stokes shift. Stokes is also responsible for coining the name fluorescence, after the mineral fluorite (fluorspar). The electronic state of a molecule determines the distribution of negative charge and the overall molecular geometry. Several different electronic states exist for any particular molecule illustrated as S(0), S(1), and S(2) depending on the total electron energy and the symmetry of various electron spin states. Every electronic state is further divided into a number of vibrational and rotational energy levels associated with the atomic nuclei and bonding orbitals. The ground state for most organic molecules is an electronic singlet in which all electrons are spin-paired (have opposite spins).

At room temperature, very few molecules have enough internal energy to exist in any state other than the lowest vibrational level of the ground state, and thus, excitation processes usually originate from this energy level. These particular energy relationships are illustrated below in the Jablonski diagram (Figure 3).

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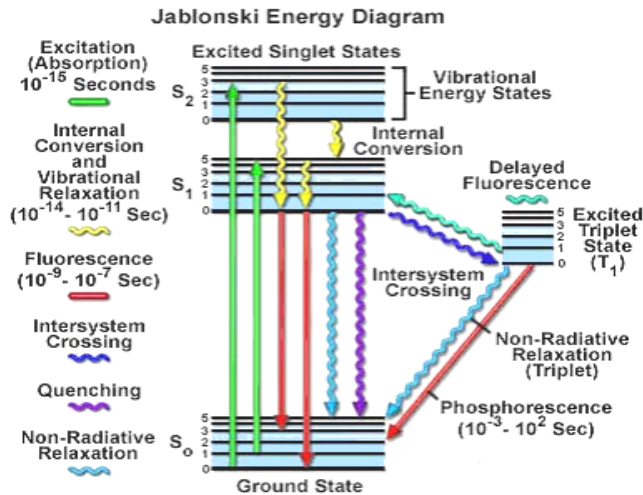


Figure 3: The Jablonski diagram outlines the photoluminescence processes of fluorescence and phosphorescence ([www.olympusmicro.com/primer/java/jablonski/jabintro/index.html](http://www.olympusmicro.com/primer/java/jablonski/jabintro/index.html)).

Often referred to as the Father of Fluorescent Spectroscopy, Alexander Jablonski received his doctorate in physics in 1930. His dissertation was titled, "On the Influence of the change of wavelengths of excited light on the fluorescence spectra." His work on the polarization of photoluminescence coupled with his desire to explain evidence found within the physics field prompted Jablonski to analyze the transition moments in absorption and emission of light.

The categories of molecules capable of undergoing electronic transitions that ultimately result in fluorescence are known as fluorochromes. Fluorochromes that are conjugated to a larger macromolecule (such as a nucleic acid, lipid, enzyme, or protein) through covalent bonds are termed fluorophores. Generally speaking, fluorophores are divided into two broad classes, intrinsic or extrinsic. Intrinsic fluorophores, such as

aromatic amino acids, neurotransmitters, and green fluorescent protein (GFP), are those that occur naturally. Extrinsic fluorophores are synthetic dyes or modified biochemicals that are added to a specimen to produce fluorescence with specific spectral properties. Each fluorophore has a specific spectral excitation range and will emit within a certain emission spectral range.

The discovery of GFP in the early 1960s enabled investigators to fuse this fluorophore to a wide variety of protein and enzyme targets, in order to monitor cellular processes in living systems. Since its discovery, many derivatives have been developed that emit light across the visible spectrum. However, because it requires an external light source to visualize the target, the target's depth must be very shallow so that enough light can reach the target and be emitted back without scatter.

Fluorescence light imaging is rapid, painless, and harmless to the animal. The typical experiment involves shining excitation light of the desired wavelength on the subject at a fluence rate of approximately  $4-20 \text{ mW/cm}^2$ . This fluence rate is about the same as a brightly lit room or outside on a sunny day. The emission light reflected from the subject is then filtered and imaged with a camera. Acquisition of an image takes a few seconds to a few minutes (see basic specifications), depending on the intensity of the light. The Maestro<sup>®</sup> system is unlike most fluorescent systems because it can perform spectral unmixing and multiplexing. This enables the user to 'remove' autofluorescence from an image and to perform imaging using multiple fluorescent signals simultaneously.

Using the maximum field of view, up to 3 whole mice can be imaged simultaneously, while another 3 can be anesthetized in an external induction chamber. Using this staggered system, large sets of animals can be processed in a relatively short period of time. Visualizing the growth of tumors in which fluorescent proteins such as GFP or red fluorescent protein (RFP) are stably expressed is a common use for the system.

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### SMALL ANIMAL IMAGING APPLICATIONS

A useful application would be the simultaneous employment of various fluorophores, such as GFP, RFP, and mPlum on tumor cells in a rodent model as illustrated in Figure 4. Each individual spectral range was chosen. Their mixed signals were analyzed to pull out their individual signals as well as the background signals. The results display the pure RFP, GFP, and mPlum as well as their mixed signals and the background (autofluorescence from skin and food).

Another application is the use of a fluorophore that has one wavelength in an unbound state and another wavelength in a bound state. A fluorophore may red shift upon being bound and the Maestro would be able to accurately account for both bound and unbound. This could be used in drug pharmacokinetics studies to quantify the effectiveness of potential pharmaceutical products

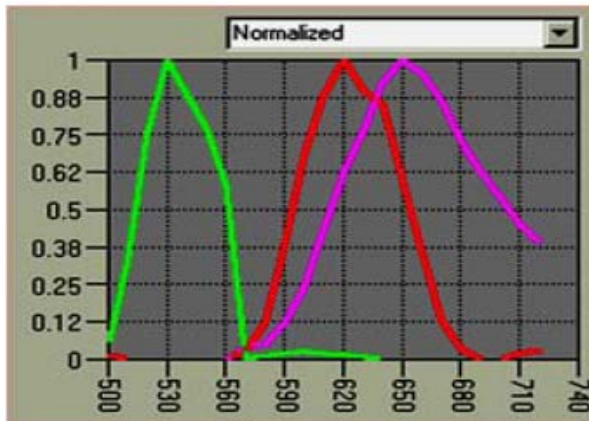
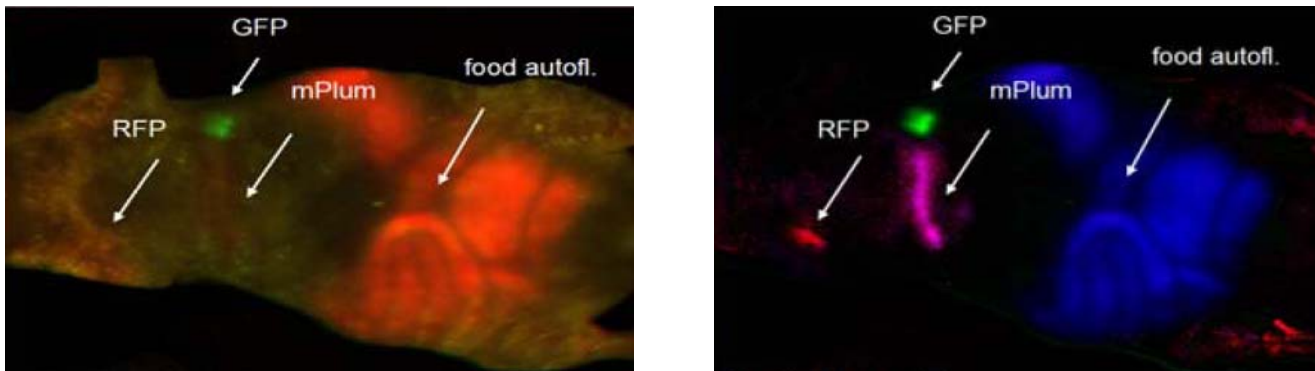


Figure 4: Results from a nude mouse with 3 subcutaneous tumors that are fluorescently labeled. Initial mixed image is illustrated on the left. An unmixed composite of RFP, GFP, mPlum, and food autofluorescence is shown on the right. The calculated spectra for RFP (red), GFP (green), and mPlum (magenta) are shown in the bottom figure ([www.crc-inc.org/](http://www.crc-inc.org/)).

### INSTRUMENT SPECIFICATIONS

The Maestro in vivo fluorescence imaging system is a light-tight apparatus that uses a Cermex-type 300 Watt Xenon light source. This provides 5600°K that spans the electromagnetic spectrum from 500–950 nm. The CCD is a 16-bit, high-resolution, scientific grade-imaging sensor. Four fiber-optic adjustable illuminator arms yield an even distribution to the subject. The light radiating from the excitation source and filter passes through the sample to the long pass emission filter. The light then passes through the camera lens and through the solid-state liquid crystal tuning element and finally to the CCD. The excitation and emission filter

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sliders hold two 50 mm diameter longpass filters. The long pass filters removes the band light especially from the excitation source. These filters are color coded to indicate the wavelength they represent. Consult the Maestro Filter Selection Guide in the software section.

### **Hardware**

Field of View (length x width):	3.4 cm X 2.5 cm to 10.16 cm X 7.62 cm (variable zoom)
Resolution:	25 to 75 $\mu\text{m}$ (based on zoom lens position)
Fluence Rate:	4 to 20 $\text{mW}/\text{cm}^2$ (based upon light position)
Scan Time:	5 sec to 1 min
Reconstruction Time:	1 to 30 sec
Scans needed for 1 mouse (Nose to Rump):	1 scan (at farthest table position) to 3 scans (at nearest table position)
Maximum Whole Mice per Scan	3
Maximum Mice per Scan	4

Filter Types:	Excitation Range:	Emission Range:
Blue	445 to 490 nm	515 nm longpass
Green	503 to 555 nm	580 nm longpass
Yellow	575 to 605 nm	645 nm longpass
Red	615 to 665 nm	700 nm longpass
Deep Red	671 to 705 nm	750 nm longpass
Special NIR	725 to 775 nm	800 nm longpass
NIR	710 to 760 nm	800 nm longpass

### **Software**

Maestro<sup>®</sup> Software is the image acquisition and analysis product from CRI. The fundamental advantage of the Maestro fluorescence imager and software is its ability to reduce signal-to-noise ratio 300-fold by the process of separating individual fluorophores from each other and from background autofluorescence. This functional capacity is known as hyper-spectral fluorescence and is analogous to spectral unmixing. Its resolution is capable of unmixing a large range of multi-spectral fluorophores that may overlap and to extract their pure signals. Tissue, especially skin, absorbs light when excited in the 400–500 nm range. Because the Maestro scans images between 500 and 950 nm, this range reduces the problem correlated with tissue absorption. In addition, selecting for autofluorescence/background within any wavelength range has the potential to eliminate all background signals. Therefore, the function of selecting and filtering this obtrusive signal yields a pure form of the signal of interest.

It provides easy to use control panels that set and display all imaging parameters, from exposure time to wavelength ranges. Once acquisition is complete, the analysis section can quickly and accurately extract the desired fluorescent signal from the autofluorescent background.

Operating System:	Windows XP, 2000, NT
Supported File Format:	TIFF
Image Data Types:	32-bit floating-point and 32-bit RGB color
Average File Size:	5 Mb to 10 Mb
Possible Analysis Techniques:	Image one, two, three or four fluorophores simultaneously in the presence of autofluorescence in a single animal. Measuring the area, mean, standard deviation, minimum, and maximum of a selection or entire image.

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### **GLOSSARY OF TERMS**

**Adsorption** – The accumulation of gases, liquids or solutes on the surface of a solid or liquid.

**Autofluorescence** – Self-induced fluorescence.

**Covalent bond** – A bond formed between two or more atoms by sharing electrons.

**Fluence rate** – a constant flow of photons over a set angle.

**Fluorochrome** – Any of a group of fluorescent dyes used to stain tissues and cells for examination by fluorescence imaging and/or microscopy.

**Fluorophore** – The categories of molecules capable of undergoing electronic transitions that ultimately result in fluorescence

**Hyper-spectral** – The processing/unmixing of a multi-spectral image.

**Longpass filters** – An optical interference or colored glass filter that attenuates shorter wavelengths and transmits (passes) longer wavelengths over the active range of the target spectrum (ultraviolet, visible, or infrared).

**Luminescence** – The emission of light as a result of the excitation of atoms by energy other than heat

**Multi-spectral** – An array of spectral bands processed simultaneously.

**Neurotransmitter** – Chemical substances that transmit nerve impulses across a synapse to another nerve, muscle, or gland.

**Photoluminescence** – Luminescence induced by the absorption of infrared radiation, visible Light, or ultraviolet radiation.

**Phosphorescence** – Persistent emission of light following exposure to and removal of incident radiation.

**Redshift** – A shift in the spectra of very distant galaxies toward longer wavelengths (toward the red end of the spectrum).

### **REFERENCES:**

1. Lim, Yong Taik et al.(2003) Selection of Quantum Dot Wavelengths for Biomedical Assays and Imaging. Volume 2, Number 1, pp 50-64
2. Giepmans, Ben N.G. (2006) The Fluorescent Toolbox for Assessing Protein Location and Function.Science. Volume 312, pp 217-224
3. Levenson, Richard M. (2004) Spectral Imaging and Pathology: Seeing More. Laboratory Medicine Volume 35, Number 5, pp244-251
4. ansfield, JR et al. Autofluorescence removal, multiplexing and automated analysis methods for in-vivo fluorescence imaging. [http://www.cri-inc.com/files/MSI\\_BMO\\_Prep\\_2005.pdf](http://www.cri-inc.com/files/MSI_BMO_Prep_2005.pdf)
5. Invitrogen: Fluorescence Basics <http://www.probes.invitrogen.com/resources/education>
6. Fluorescence: <http://www.answers.com/fluorescence>
7. Jablonski biographical information <http://micro.magnet.fsu.edu/optics/timeline/people/jablonski.html>

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Or visit the company's site: <http://www.cri-inc.com/products/maestro.asp>

## Maestro Filter Selection Guide

Fluorophore			Filter Set						
Name	Excitation	Emission	Blue	Green	Yellow	Red	Deep Red	Special NIR	NIR
525 nm QDot®	<500	525	X						
545 nm QDot®	<520	545	X						
565 nm QDot®	<540	565	X						
605 nm QDot®	<580	605		X					
655 nm QDot®	<620	655			X				
705 nm QDot®	<650	708				X			
800 nm QDot®	<750	800							X
Alexa Fluor® 488	492	520	X						
Alexa Fluor® 500	500	530	X						
Alexa Fluor® 514	514	545	X						
Alexa Fluor® 532	532	554	X						
Alexa Fluor® 546	556	573	X						
Alexa Fluor® 555	555	580							
Alexa Fluor® 568	577	603		X					
Alexa Fluor® 594	590	618		X					
Alexa Fluor® 610	612	628		X					
Alexa Fluor® 633	632	650		X					
Alexa Fluor® 647	647	666		X					
Alexa Fluor® 660	668	698			X				
Alexa Fluor® 680	679	702			X				
Alexa Fluor® 700	700	719				X			
Alexa Fluor® 750	750	779					X		
Cy2™	498	506	X						
Cy3™	554	568	X						
Cy5™	649	666			X				
Cy5.5™	675	695			X				
Cy7™	743	805					X		
GFP	475	509	X						
FITC	490	525	X						
eGFP	498	515	X						
YFP	520	532	X						
Rhodamine	550	573	X						
RFP (dsRed)	558	583		X					
ICG	800	850						X	

**Color denotes usable filter set, X denotes recommended filter set**

QDot is a registered trademark of the Quantum Dot Corporation

Alexa Fluor is a registered trademark of Molecular Probes, Inc.

Cyanine dye (Cy2, Cy3, Cy5, Cy5.5 and Cy7) is a trademark of Amersham Biosciences.

Special NIR Excitation Filter available from Chroma Technology Corporation.