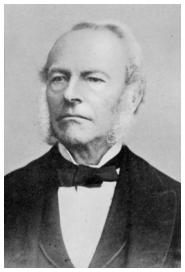


George Stokes
(1819 - 1903)

Max Born
(1882 - 1970)

E. Schroedinger
(1887 - 1961)

Historical Perspective

1853 Fluorescence described by Sir George G. Stokes

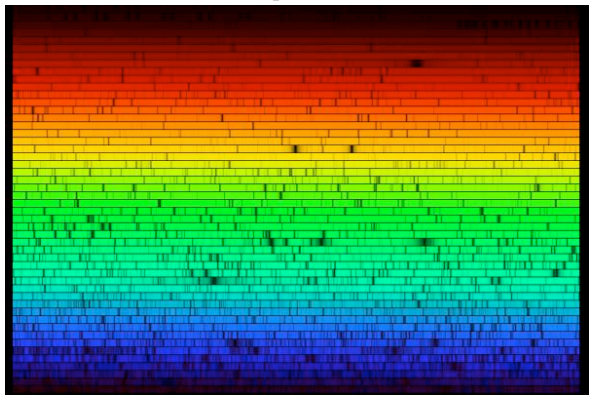
1913 Fluorescence microscope developed
Heinrich Lehmann
August Köhler & Carl Reichert (Zeiss)

1930s Secondary fluorescence technique
Max Haitinger

1945 Quinine based fluorophore
John Hershel

1950s Indirect Immunofluorescence
Albert Coons & Nathan Kaplan

Solar Spectrum

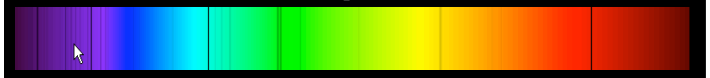


"Frauenhofer Lines"
(1814)

Kurucz et al (1984)

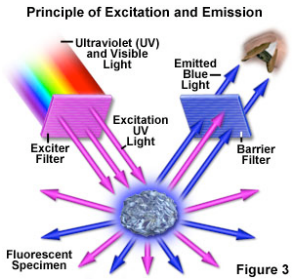
Visible Spectrum

Solar Spectrum

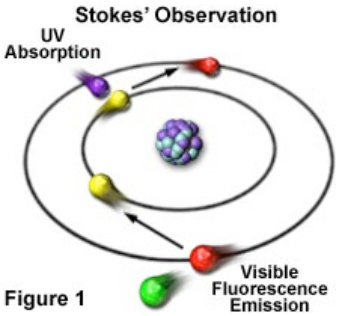


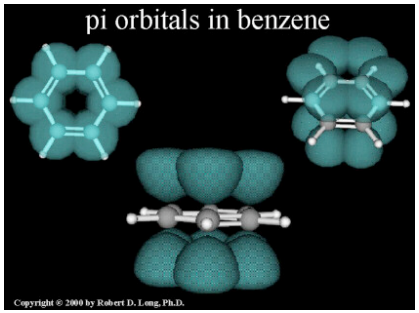
H_2
He_2
O_2
C
N_2
Si

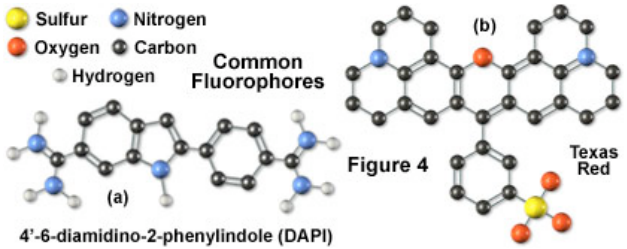
(Kirchhoff , Bunsen, others; 1860s)



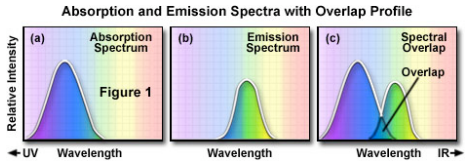
Fluorspar

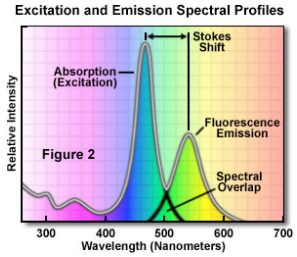


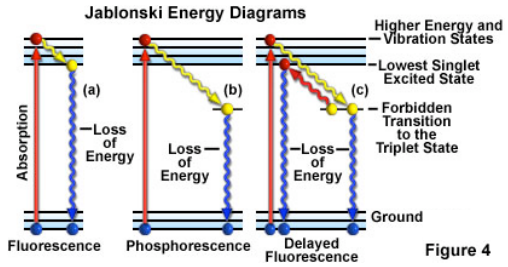


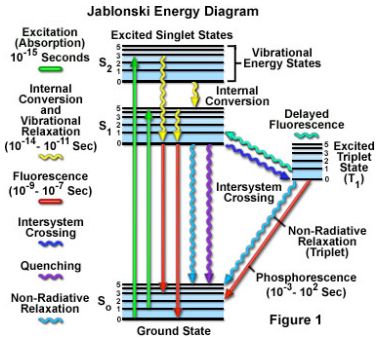


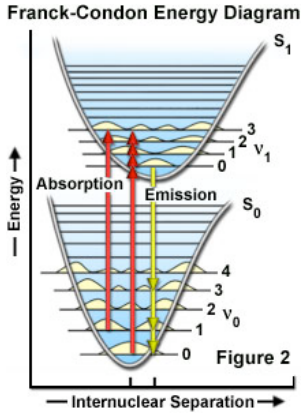
4'-6-diamidino-2-phenylindole (DAPI)

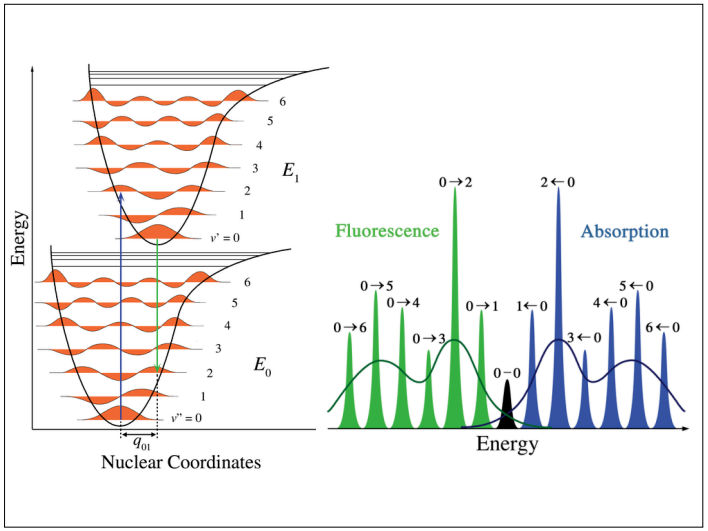

Absorption and Emission Spectra with Overlap Profile
(a)
Absorption Spectrum
(b)
Emission Spectrum
(c)
Spectral Overlap
Overlap
Relative Intensity
Figure 1
UV
Wavelength
Wavelength
Wavelength
IR

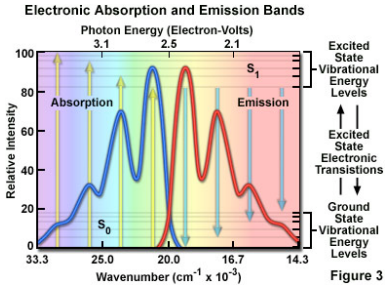

Excitation and Emission Spectral Profiles
Stokes Shift
Absorption (Excitation)
Fluorescence Emission
Spectral Overlap
Relative Intensity
Figure 2
300
400
500
600
700
Wavelength (Nanometers)

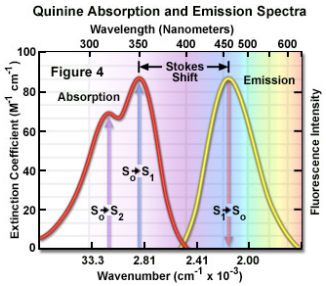

Jablonski Energy Diagrams
Higher Energy and Vibration States
Lowest Singlet Excited State
Forbidden Transition to the Triplet State
Ground
Absorption
(a)
(b)
(c)
Loss of Energy
Loss of Energy
Loss of Energy
Fluorescence
Phosphorescence
Delayed Fluorescence
Figure 4


Jablonski Energy Diagram
Excitation (Absorption) 10^{-15} Seconds
Excited Singlet States
Vibrational Energy States
Internal Conversion and Vibrational Relaxation (10^{-14}- 10^{-11} Sec)
Internal Conversion
Delayed Fluorescence
Excited Triplet State (T_1)
Fluorescence (10^{-9}- 10^{-7} Sec)
Intersystem Crossing
Intersystem Crossing
Quenching
Non-Radiative Relaxation (Triplet)
Non-Radiative Relaxation
Phosphorescence (10^{-3}- 10^{2} Sec)
S_2
S_1
S_0
Ground State
Figure 1


Franck-Condon Energy Diagram
S_1
S_0
Absorption
Emission
Energy
ν_1
ν_0
Figure 2
Internuclear Separation







Quantum Yield (photons emitted / photons absorbed)

Dye	Solvent	Exc	Em	QY
Acridine Orange	Ethanol	493	535	0.46
Benzene	Ethanol	248	300-350	0.04
Chlorophyll-A	Ethanol	440	685	0.23
Eosin	Water	521	544	0.16
Fluorescein	Water	437	515	0.92
Rhodamine-B	Ethanol	555	627	0.97

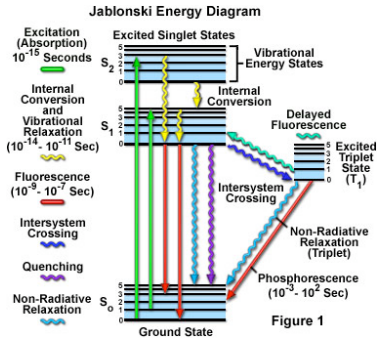
Solvent Effects

Triplet Blinking

Fluorescence Quenching

Photobleaching

Transition to the Dark Side

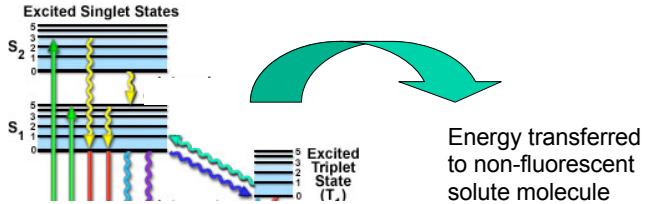


Triplet Blinking
- stable triplet state
- $S_1 \rightarrow T_1$ due to solvent
  - -halogens
  - -transition metals

Quenching

Collision with solutes

Resonance energy transfer



Energy transferred to non-fluorescent solute molecule

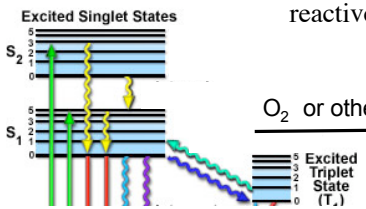
Death of a Fluor

Photobleaching

T_1 is highly reactive

O_2 likes T_1 electrons

Oxygen radicals are highly reactive & cytotoxic



O_2 or other solute → New compound (disrupted conjugation)

Differential Photobleaching in Multiply-Stained Cell Cultures

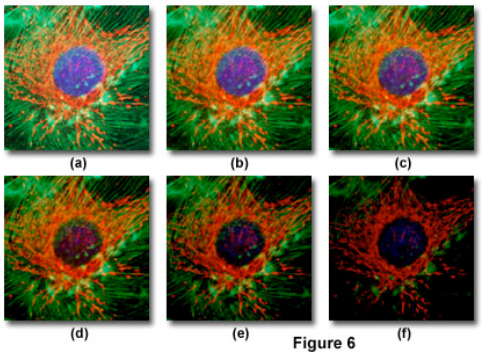


Figure 6

Differential Photobleaching in Multiply-Stained Tissues

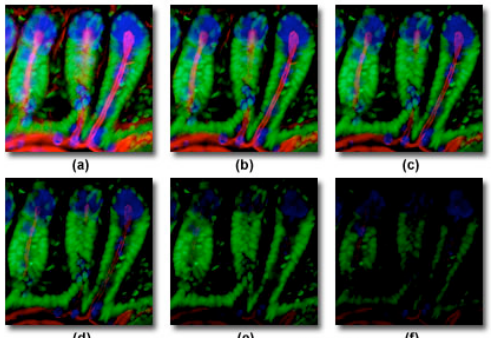


Figure 1

Reflected Light (Epifluorescence) Microscopy

Dichromatic Mirror Function in Reflected Light Fluorescence Illumination

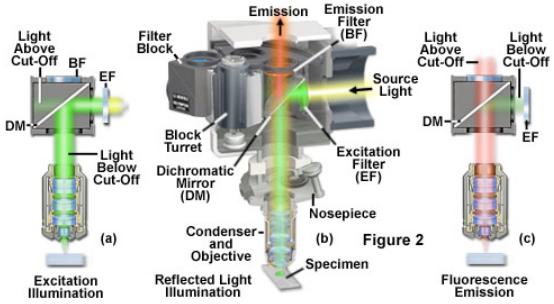


Figure 2

Fluorescence Filter Cube (Block) and Associated Spectra

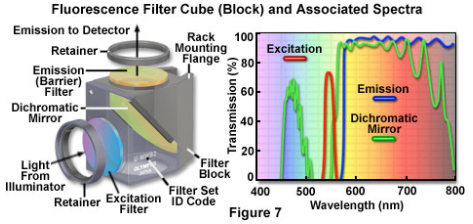


Figure 7

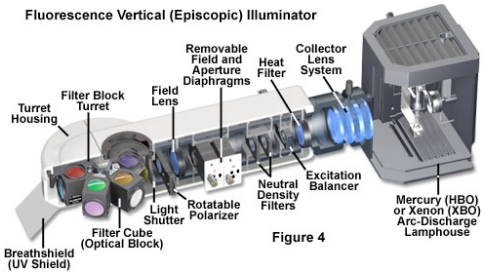


Figure 4

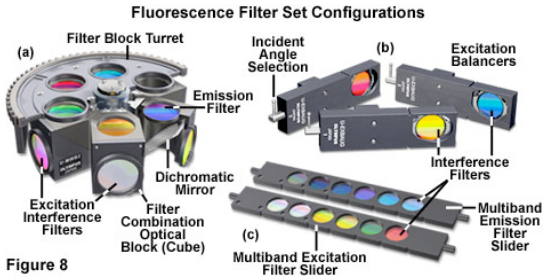


Figure 8

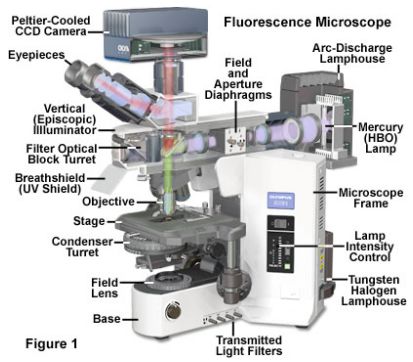


Figure 1

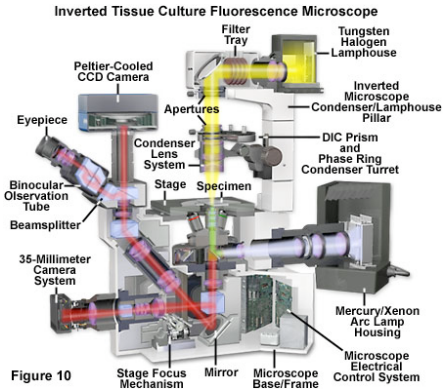


Figure 10

Transmission Fluorescence Microscopy

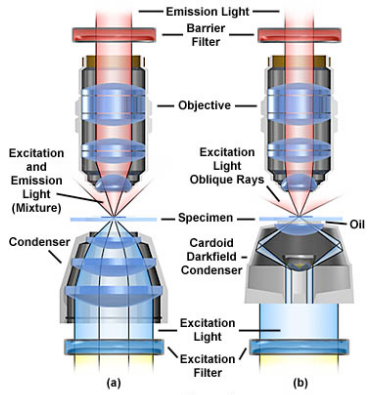


Figure 1

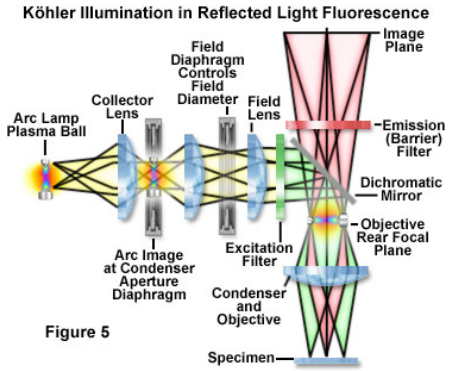
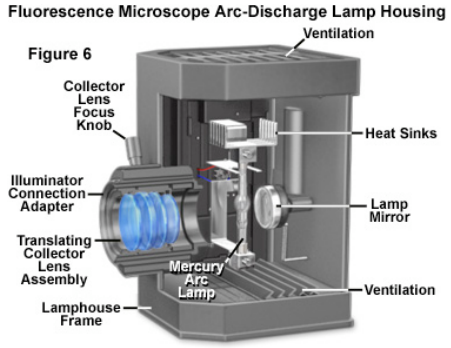


Figure 5



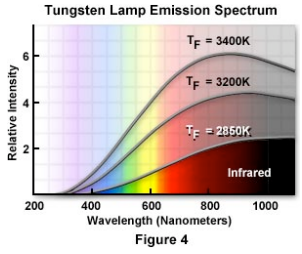


Figure 4

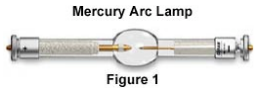


Figure 1

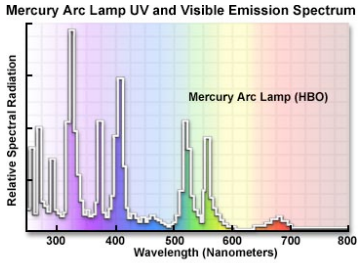
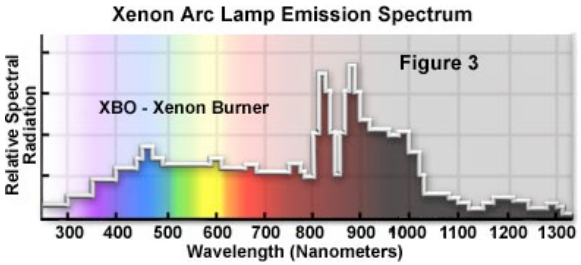
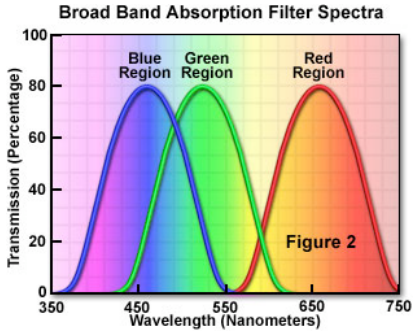


Figure 2





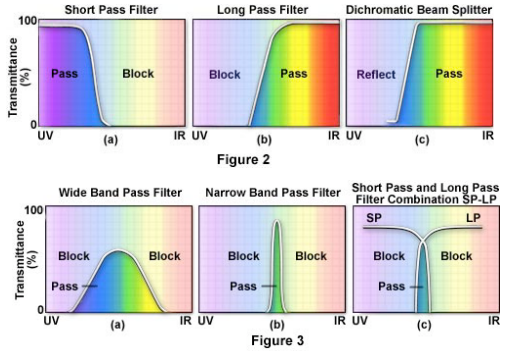


Figure 2

Figure 3

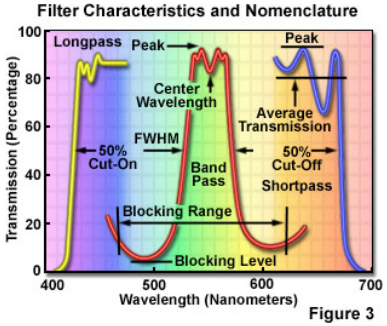


Figure 3

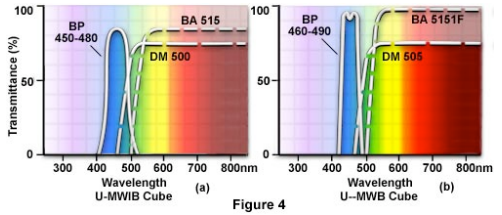


Figure 4

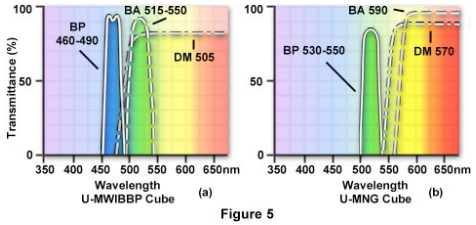


Figure 5

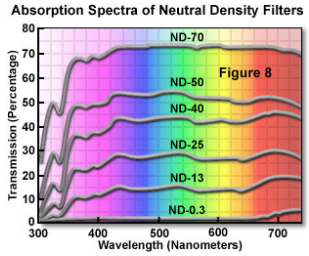
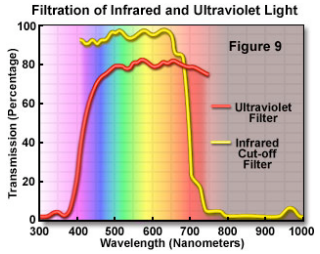
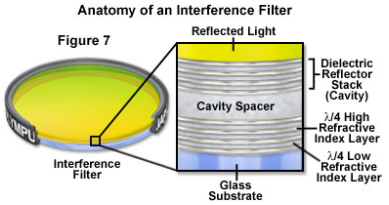
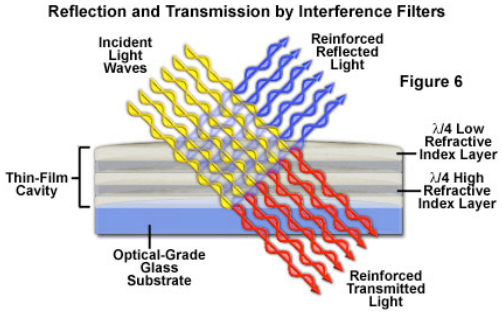
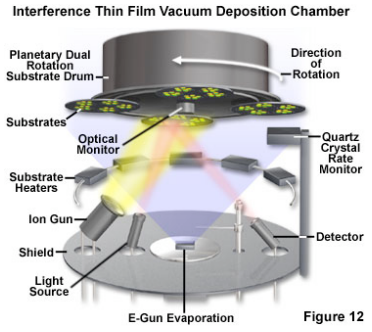


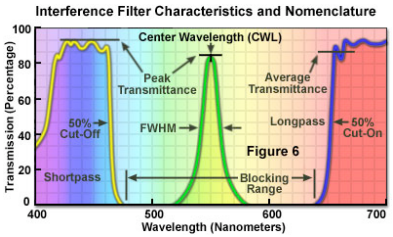
Figure 8


Filtration of Infrared and Ultraviolet Light
Figure 9
Transmission (Percentage)
100
80
60
40
20
0
300
400
500
600
700
800
900
1000
Wavelength (Nanometers)
Ultraviolet Filter
Infrared Cut-off Filter

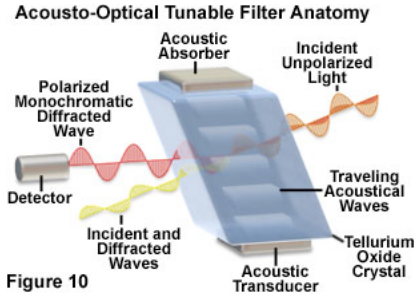

Anatomy of an Interference Filter
Figure 7
Reflected Light
Dielectric Reflector Stack (Cavity)
Cavity Spacer
λ/4 High Refractive Index Layer
λ/4 Low Refractive Index Layer
Interference Filter
Glass Substrate

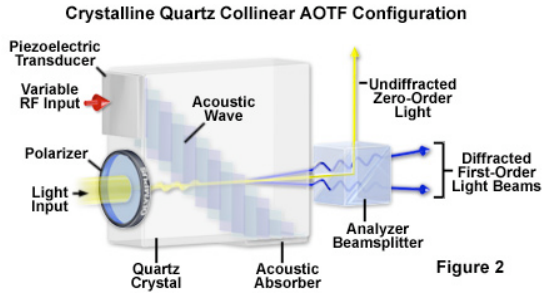

Reflection and Transmission by Interference Filters
Figure 6
Incident Light Waves
Reinforced Reflected Light
λ/4 Low Refractive Index Layer
λ/4 High Refractive Index Layer
Thin-Film Cavity
Optical-Grade Glass Substrate
Reinforced Transmitted Light

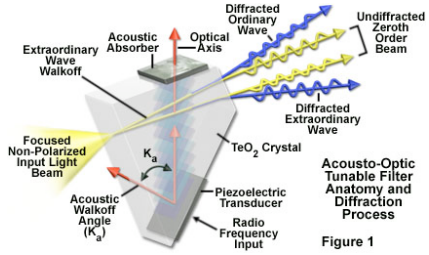

Interference Thin Film Vacuum Deposition Chamber
Planetary Dual Rotation Substrate Drum
Direction of Rotation
Substrates
Optical Monitor
Quartz Crystal Rate Monitor
Substrate Heaters
Ion Gun
Detector
Shield
Light Source
E-Gun Evaporation
Figure 12


Interference Filter Characteristics and Nomenclature
Transmission (Percentage)
100
80
60
40
20
0
400
500
600
700
Wavelength (Nanometers)
Center Wavelength (CWL)
Peak Transmittance
Average Transmittance
50% Cut-Off
FWHM
Longpass
50% Cut-On
Shortpass
Blocking Range
Figure 6

AOTF


Acousto-Optical Tunable Filter Anatomy
Acoustic Absorber
Incident Unpolarized Light
Polarized Monochromatic Diffracted Wave
Detector
Traveling Acoustical Waves
Incident and Diffracted Waves
Tellurium Oxide Crystal
Acoustic Transducer
Figure 10


Crystalline Quartz Collinear AOTF Configuration
Piezoelectric Transducer
Variable RF Input
Acoustic Wave
Undiffracted Zero-Order Light
Polarizer
Diffracted First-Order Light Beams
Light Input
Analyzer Beamsplitter
Quartz Crystal
Acoustic Absorber
Figure 2


Diffracted Ordinary Wave
Undiffracted Zeroth Order Beam
Extraordinary Wave Walkoff
Acoustic Absorber
Optical Axis
Diffracted Extraordinary Wave
Focused Non-Polarized Input Light Beam
TeO_2 Crystal
K_a
Acoustic Walkoff Angle (K_a)
Piezoelectric Transducer
Radio Frequency Input
Acousto-Optic Tunable Filter Anatomy and Diffraction Process
Figure 1

What to look for in a fluorophore

- Fluorescence Spectrum
- Quantum Yield
- Extinction Coefficient
- Stability (Photobleaching)
- Sensitivity to Environment
- Toxicity
- Reactivity
- Solubility