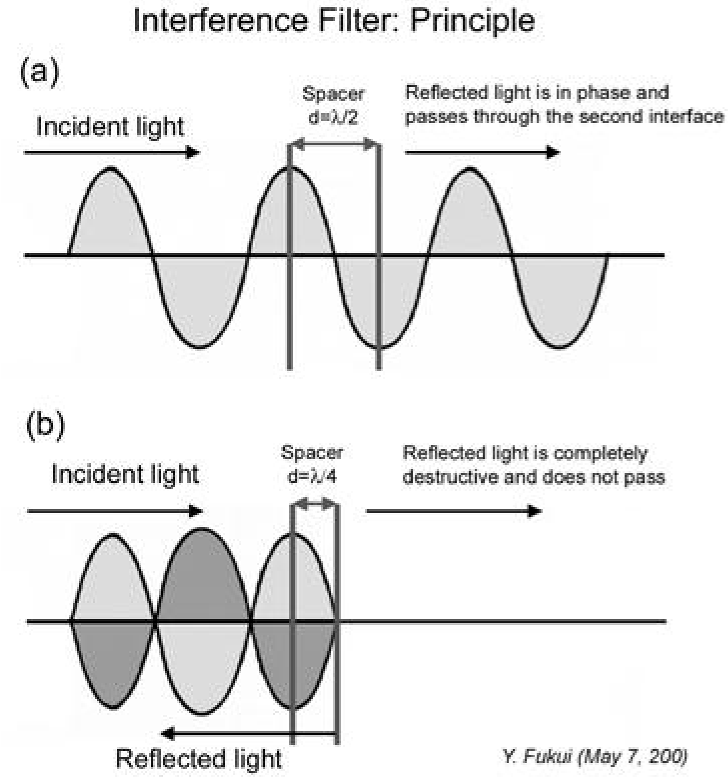
Interference Filter: Basics and Epifluorescence Application

(Principle)

When two or more waves vibrating in the same plane meet, they “interfere” and the resultant amplitude is the algebraic sum of individual components (“principle of superimposition”). For a short traveling distance, the waves are assumed maintain their phase constant, said to be “coherent”. Under this condition, waves are interfered constructively, destructively, or attenuated. Because light intensity is given as the square of amplitude, if the amplitude is doubled, the intensity is quadrupled. In contrast, if two waves are out-of-phase bringing in the superimposed amplitude is a half of original incident light, the resultant intensity is a quarter of the incident.

For a simplified example, when a wave passes an air-glass interface at a *normal incident*, travels a spacer of distance of a half wavelength (½ λ), then hits a semi-reflective glass-air interface, the reflected component has a phase difference of ½ λ and is *in phase*. In contrast, if a wave travels a distance of a quarter wavelength (¼ λ), the reflected light interferes destructively and no light passes the spacer. Given that the incident light has various wavelengths and the thickness of spacer is ½ λ, only light of wavelength of λ nm can pass the spacer. All other lights are in effect rejected or attenuated. A ¼ λ–thick film is in fact used for anti-reflective coating.

(Normal incident)



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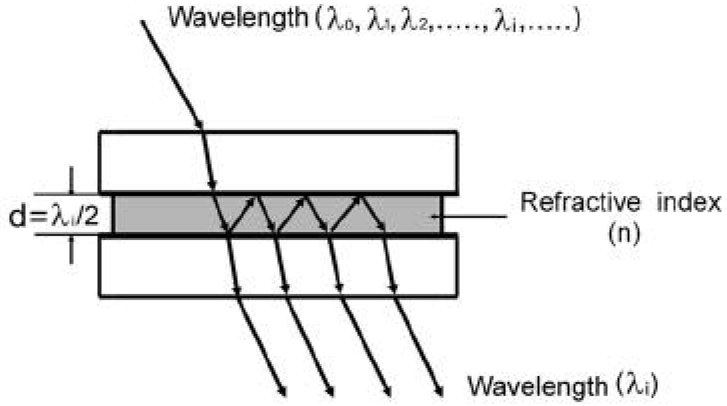
The figure below diagramatically illustrates the principle of interference filter. Note that incident light is drawn with angle in order to emphasize the behavior of light but in fact reflected light vibrates in same plane as shown in the previous figure, interfering with incident light. In this example, only electric field component having wavelength of λ_i can pass through the second interface of spacer of $\lambda_i/2$ in depth after enforced with reflected light vibrating completely *in phase*.

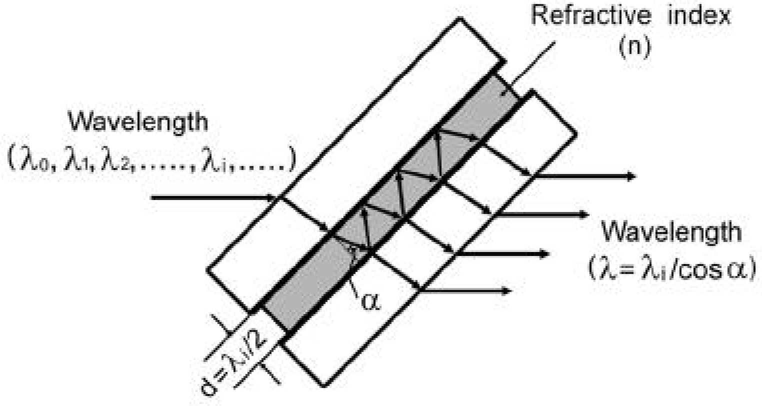
Interference Filter for Normal Incident



(Inclined incident)

If incident light is inclined relative to the plane of filter, transmitted light shifts in wavelength due to increased path length in the spacer. The diagram below shows mathematical relations between wavelength and incident angle.

Interference Filter for Inclined Incident



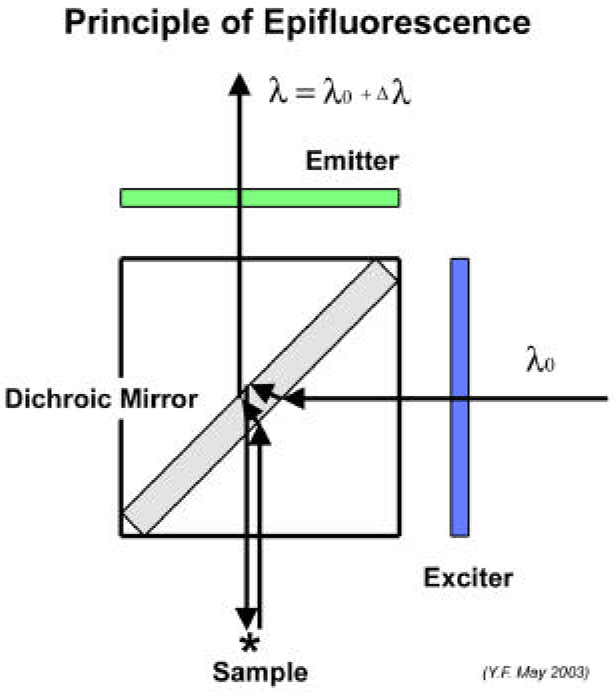
(Modern interference filter)

Modern interference filters are fabricated by a technology called "thin-film interference coating". The filter is made of multiple thin layers of semi-transparent, metal oxides, each of which has a unique refractive index. Thin layers are deposited with alternating high and low refractive indexes such that at each interface, light waves behave differently by virtue of wavelength. Usually, three or five layers are grouped into units called "cavities" and they are separated by a thicker "spacer" made of magnesium fluoride. The width of cavity spacer is even multiples of $\lambda/4$ or $\lambda/2$.

This technology makes it possible to construct many types of interference filters such as *"high-pass", "low-pass", "band-pass", or "band-rejection"*. Advantage of this technology is the capability to construct filters having extremely narrow band and sharp cut-off profiles. For example, a few nanometer bandwidth filter can be constructed by depositing over 75 layers. It is also possible to construct filters with a very unique and complex absorption/transmission profile as component of multi-band fluorescence filter sets (see **"Epifluorescence filters"** section below).

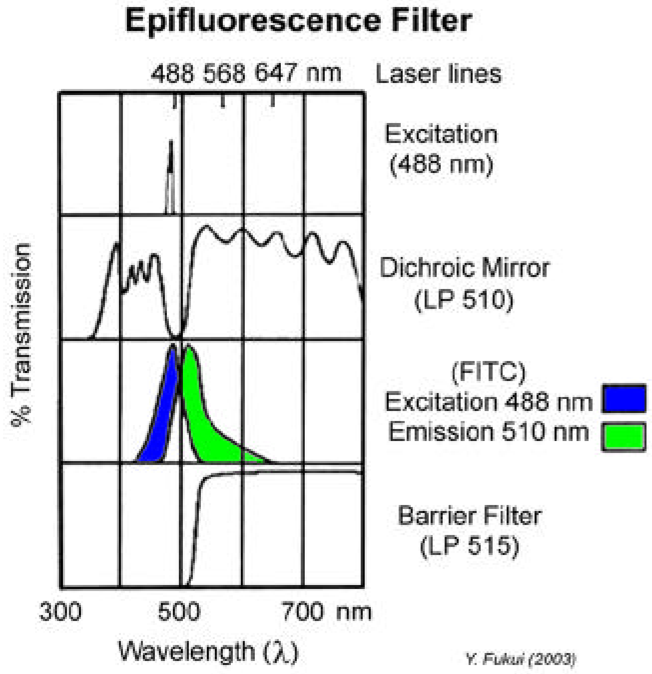
(Dichroic mirror)

If the back layer of interference filter is totally reflective to a specific wavelength, this filter is called a "dichroic mirror". When incident light is 45 ° reflecting a selected wavelength and reenters with its wavelength shifted, the light passes through the filter. This mechanism stands as the key element for epifluorescence where fluorescence light emitted from a specimen always has longer wavelength than the excitation wavelength (Stokes' Law). The diagram below illustrates the principle of epifluorescence.

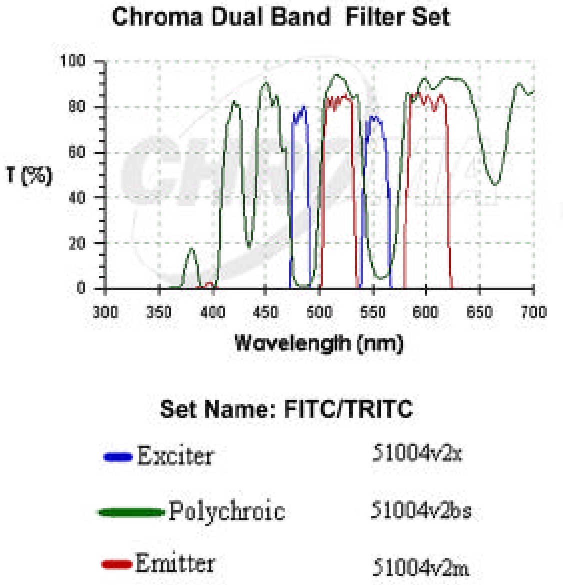


(Epifluorescence filters)

The excitation filter, dichroic mirror and emission (also called “barrier”) filters are assembled into a single unit called a fluorescence “cube” or “cassette” in basic configurations. In this configuration, when a different wavelength is selected for dual or multiple fluorescence observations, the cube can be manually changed to another. In a more sophisticated system, the excitation filter is installed separately in an electric shutter, allowing precisely controllable, millisecond order excitation. In highest-end configuration, the excitation and emission filters are separated and installed into excitation and emission filter wheels. The diagram below shows optical properties of interference filters, dichroic mirror and the absorption/emission property of a fluorochrome “fluorescence isothiocyanate (FITC)” as for an example of epifluorescence microscopy.



As for another configuration, there are “dual-” and “triple-band” fluorescence filter systems commercially available, for example from Chroma Company (http://www.chroma.com). These systems are made possible by virtue of wealth of sophisticated thin-film interference filter technology and capable of observing emissions of multiple fluorescence dyes simultaneously or separately (with separated excitation filter configuration) without changing the cube. This eliminates possible focus and geometric shift in image position due to unique behavior of lights with different wavelength. An example of “dual band” filter systems is shown below).


Chroma Dual Band Filter Set
T (%)
100
80
60
40
20
0
300
350
400
450
500
550
600
650
700
Wavelength (nm)
Set Name: FITC/TRITC
Exciter
51004v2x
Polychroic
51004v2bs
Emitter
51004v2m