



Support Application Notes

Overview of Optical Filters

Introduction

Turner Designs fluorometers require an excitation and an emission filter; the Model 10-AU also uses a reference filter. Optical filters are chosen to be optimal for each application, cost effective, and durable. Filters are used to selectively pass a portion of the ultraviolet or visible spectrum.

In combination with a light source, the excitation filter allows only light which excites the molecule of interest to strike the sample. The emission filter allows the fluorescence from the sample to pass to the detector and blocks stray light from the light source or interfering components in the sample. The reference filter is used in the reference path of the 10-AU series and is a factor in determining the basic operating level of the instrument.

Filters can be used alone or in combination to select the desired spectral band. Optical filters obey the Bouguer-Lambert Law, which states that the spectral transmittance of two or more optical filters used simultaneously is equal to the product of the spectral transmittance of each filter.(1)

Filters with four types of spectral characteristics are used in Turner Designs fluorometers: broadband, narrowband, sharpcut, and neutral density.

- A **broadband** filter can pass a broad band of light. For instance, a broadband filter may transmit light from 300 - 400 nm, but block light with wavelengths shorter than 300 and longer than 400.
- **Narrowband** filters pass a narrow band of light (as little as 1 nm). For example, a 436 nm filter with a bandpass of 10 nm, will pass light from 431 - 441 nm (5 nm on either side of 436 nm).
- **Sharpcut** or edge filters can be used to block light that is longer or shorter than a nominal wavelength. A 450 nm long-wave filter will allow transmission of light that is longer than 450 nm, but it will block light that is shorter than 450 nm. A 450 nm short-wave filter will transmit light that is shorter than 450 nm and block light that is longer than 450 nm.
- A **neutral density** filter, primarily used as a reference filter, can be used to decrease the transmitted light across a very broad spectrum. For instance, a neutral density filter can be used to decrease the total light transmission by a factor of 10 or 100.

Three types of optical filters are used in Turner Designs Fluorometers: 1. Optical Glass, 2. Interference, and 3. Gel Wratten.

1. **Optical Glass Filters.** Optical glass filters are made from glass that absorbs specific wavelengths of the spectrum. They are relatively inexpensive and are very durable under most conditions. Both bandpass, sharpcut, and neutral density filters are available in optical glass. However, the choice of filter glasses is limited. The

amount of transmission and band width is dependent on the glass thickness. The following factors may affect optical glass:

- Thermal shock caused by a rapid temperature change;
- Solarization caused by prolonged exposure to ultraviolet light can cause an increase in absorption (decrease in transmission);
- Exposure to high humidity or corrosive environments can cause 'spotting' or 'staining', which changes the surface, resulting in increased light scattering off the surface and decreased transmission through the glass.(2)

However, we have found that glass filters can be used for years or decades under most conditions.

2. Interference Filters. In terms of spectral characteristics, interference filters can have broad or narrow bandpasses, or can be sharpcut filters. Interference filters used in Turner Designs fluorometers are primarily narrow bandpass. Interference filters are made by coating optical glass with two thin films of reflecting material separated by an even-order spacer layer. The central wavelength and bandwidth of the filter can be controlled by varying the thickness of the spacer layer and/or the number of reflecting layers. To ensure out-of-band blocking (blocking undesirable wavelengths of light) an additional blocking component is added. While the additional blocking eliminates out-of-band light transmission and decreases background noise, it also decreases the overall light transmission through the filter which decreases the fluorescent signal. Interference filters typically permit 10 to 70% light transmission. The minimum specified transmission depends on the transmitted wavelength and bandwidth.

Interference filters are affected by temperature. The center wavelength will shift linearly with, and in the direction of, changes in temperature. For example, the temperature coefficient for a 400 nm filter is about 0.015 nm/°C. The center wavelength and maximum transmission of interference filters can drift with age, especially under conditions of high humidity and variable temperatures.³ Good quality filters are hermetically-sealed to mitigate the affects of aging. Hermetically sealed filters are guaranteed for one year; we have found that under good ambient conditions, such as in a laboratory, the filters show minimum signs of aging after two years or more.

A new interference filter usually has a uniformly dark side and a uniformly reflective or mirrored side. To protect the filter from heat and light, the reflective side should always face the light source. A filter that is affected by age and humidity will show discoloration around the outside diameter, this discoloration will move toward the center of the filter with time and additional damage. A symptom of aging is a significantly decreased maximum transmission which results in less sensitivity for a fluorescent assay. The recommended operating conditions for interference filters is -40 degrees C to +70 degrees C, and a maximum temperature change of 5 degrees C/minute.(3)

3. Gel Wratten Filters. Gel Wratten filters can have broad or narrow bandpasses or can be sharpcut filters. Gelatin filters are made by dissolving specific organic dyes into liquid gelatin. The gelatin is coated onto prepared glass and when it is dry, it is stripped off the glass and coated with lacquer. Each filter is standardized for spectral transmittance and total transmittance. At Turner Designs, the gelatin filter is placed between two pieces of glass or in combination with other filters for use in the fluorometer. Like dyes in other applications, the spectral characteristics of the dyes used in filters may change depending on the dye used, age, and exposure to heat and light. Gelatin filters should be kept cool, dry and should not be subjected to temperatures higher than 50 degrees C.(1) Most of the gelatin filters used by Turner Designs have been found to be stable under test conditions, which include up to two

weeks of continuous exposure to several light sources.

References

1. Kodak Filters for Scientific and Technical Uses, Eastman Kodak Company, 3 ed. 1981.
2. Schott Color Filter Glass, Schott Optical Glass Inc., 1976
3. Andover Corporation Optical Filter Guide, Andover Corporation.

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