

# Angle-Tuned Thin-Film Interference Filters for Spectral Imaging

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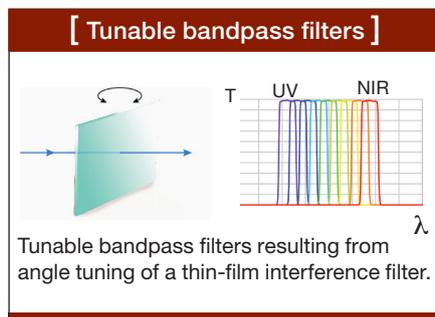
Semrock Inc.

By using angle-tuned thin-film interference filters, optical engineers can capture nonstandard wavelengths during spectral imaging without compromising performance.

Angle tuning an optical filter is an established trick that is used to select wavelength regions that are not covered by standard filters. It is especially helpful for optical engineers who wish to use a standard bandpass filter in order to avoid custom manufacturing. However, trying to capture as many precious photons as possible often results in a significant loss of filter performance, especially at larger tuning angles.

Angle-tuned thin-film interference filters—a recent innovation in thin-film filter design—overcome this problem. They allow for angle tuning over a wide spectral range without any degradation in performance.

It is well known that the spectral transmission of any thin-film filter shifts toward shorter wavelengths when the angle of incidence (AOI) increases from normal incidence to higher angles. However, the filtered spectrum becomes highly distorted at higher angles, and the shift can be significantly different for *s*- and *p*-polarized light, leading to a significant loss in performance and strong polarization dependence. When the AOI of light impinging on a filter is increased



beyond normal incidence ( $0^\circ$ ), the resulting spectral shift can be described by:

$$\lambda(\theta) = \lambda(0) \sqrt{1 - \frac{\sin^2(\theta)}{n_{eff}^2}} \quad (1)$$

where  $\theta$  is the angle of incidence and  $n_{eff}$  is called the effective index of refraction; this value is unique for each filter design and polarization state. This effect can be used to tune the filter spectrum, albeit over a limited range.

The optical filter design depends on the application. For applications that involve precise laser lines or telecommunication wavelengths, filters

with narrow passbands ( $<10$  nm) may be necessary. Wider passbands ( $\sim 20$  nm) are the norm for more common applications. Such filters are typically designed around multicavity Fabry-Perot structures. Optical filters with even wider ( $\sim 50$  nm) passbands are typically designed and manufactured to combine long-wave-pass (LWP) and short-wave-pass (SWP) edge filters.

Edge filters are formed by the edges of “stopband” regions that result from an approximately quarter-wave stack of high- and low-index thin-film layers formed from refractive oxides such as silicon dioxide ( $\text{SiO}_2$ ) and niobium pentoxide ( $\text{Nb}_2\text{O}_5$ ). These bandpass filters have found use in spectral imaging instrumentation, where the filter is mounted in a fixed geometry to capture light across a known spectral range.

Tweaking the filter angle to capture light from a different spectral range may seem like a good idea. However, as is evident from the spectral plot in the top figure on p. 13, angle tuning fixed filters has its limitations. Here, as the filter is angle-tuned from  $0^\circ$  to  $60^\circ$ , the spectrum becomes highly distorted: Transmission is reduced across the passband, and steep edges are lost. At a higher AOI, the passband ripple deteriorates substantially for *p*-polarized light, and the passband for *s*-polarized light disappears completely. The useful angle-tuning range is up to about  $15^\circ$  for this type of filter, resulting in a wavelength tuning range of 1 percent at best.

A new class of thin-film interference filters has been developed that can be angle tuned over large spectral ranges without sacrificing filter performance. Through a simple modification to both the SWP and LWP edges, one can realize wide bandpass edge filters. They can be angle tuned by as much as  $600^\circ$  with high out-of-band blocking, and no polarization splitting or edge steepness degradation. This novel design allows bandpass filters to be used in a variety of atypical geometries and in new instruments without a loss in filter performance.

## Tunable thin-film optical filters for spectral imaging

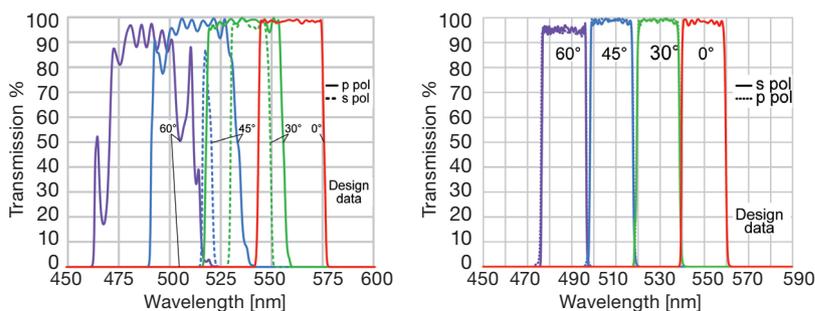
Invention drives innovation. An obvious new use of these widely tunable optical filters is in spectral imaging, which is used in myriad applications, including fluorescence microscopy, medical imaging, remote sensing, agricultural analysis and forensics. A good example is the use of tunable filters for fixed-cell imaging in fluorescence microscopy systems.

Spectral imaging in fluorescence microscopy has been used to resolve microscopic structures within live and fixed cells that are labeled with fluorescent markers. There are two typical approaches for this type of spectral imaging—one based on optical filters and another on diffraction gratings. The latter technique suffers from light loss due to the intrinsic low efficiency of grating optics. Therefore, thin-film filters with high (more than 90 percent) transmission passbands are preferable. Although imaging with fixed filters has its advantages, imaging multiple fluorophores requires the mechanical interchange of several filters to cover the spectral region over which the fluorophores emit. This is cumbersome and time-consuming, especially in live-cell imaging.

With tunable filters, it is possible to move the narrow (say, 20-nm) filter passband over a large spectral range, thereby permitting imagery of multiple fluorophores with only one filter. Spectral imaging can also be used to untangle any fluorescence spectral overlap when combined with linear unmixing. The left part of the bottom figure to the right shows micrographs of a fixed cell stained with three spectrally distinct fluorophores. As the filter is angled tuned from 0° to 45°, it is possible to generate maps of spatially distinct regions within individual cells. At 0°, the entire outer cell body is evident; between 15° and 30°, the actin filaments can be observed, and with the filter tuned to 45°, one can view the cell nucleus. Tunable filters image multiple fluorophores from the same sample quickly; this experiment took less than two minutes.

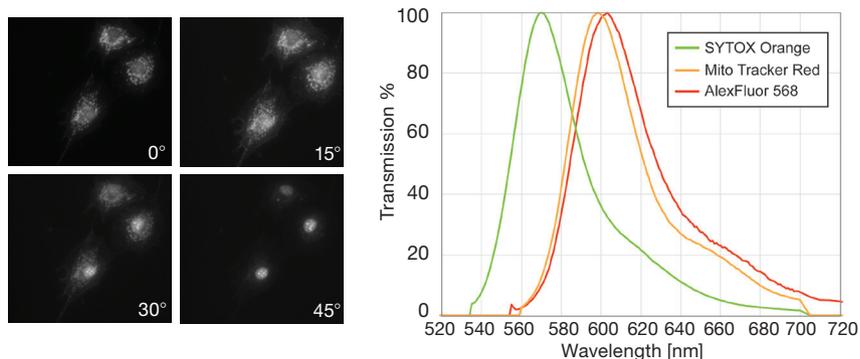
Another technology to benefit from tunable filters is hyperspectral imaging.

## [ Limitations of angle tuning fixed filters ]



(Left) Example of a fluorescence bandpass thin-film filter comprised of a combination of long-wave-pass and short-wave-pass filter coatings (FWHM ~35 nm). (Right) A similar filter that uses the novel thin film design. Five spectra are calculated for tuning angles from 0° to 60°.

## [ Fluorescence microscopy images and emission profiles ]



(Left) Fluorescence microscopy images acquired from a cell stained with MitoTracker Red (mitochondria), AlexaFluor 568 (actin filaments) and Sytox orange (nucleus) as a tunable thin-film filter is angle-tuned from 0° to 45°. (Right) Fluorescence emission profiles of the three fluorophores used to stain the cell.

This technique is used in airborne military surveillance to monitor ground activities; in remote sensing to map the spatial location of minerals; in agriculture to assess crop health; and in the pharmaceutical industry for rapid drug differentiation. Common hyperspectral imagers are based on diffraction gratings and CCDs that acquire spectral images (known as “datacubes”) via pushbroom scanning.

On each CCD image, one dimension encodes spatial information and the other encodes the spectrum. To generate a typical two-dimensional spatial image, the optical system scans the imaging field-of-view across the region to be imaged. Widely tunable optical filters allow an efficient alternative to push-broom scanning. The tunable filter would provide spectral scanning, permitting a capture mode where each

CCD frame captures a high-resolution two-dimensional spatial image.

Innovation in instrument design based around tunable filters could lead to increased adoption and widespread use of spectral imaging in industrial process control and environmental monitoring. In addition, their use in spectral imaging instruments would allow increased deployment of compact systems for field operations in agriculture, food inspection, security and counterfeiting. ▲

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## [ References and Resources ]

>> P. Yeh. *Optical Waves in Layered Media*, Wiley, N.Y., 1988.