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Application note:

New Wavelength Filtering Technology for Light Microscopy

Introduction

Many light microscopy applications - particularly those involving fluorescence imaging - require wavelength discrimination, either to filter a broadband illumination source or to filter the images reaching the camera, or both. Traditional tools for wavelength filtering, such as monochromators and filter wheels, all have some type of limitation that often compromises their use in microscopy. However, a new type of wavelength filtering device called Flexible Wavelength Selector (FWS) now offers a combination of advantages for both illumination and image filtering. Specifically FWS devices combine the wavelength tunability and bandwidth control of a monochromator with the circular uniform aperture of a filter wheel. These devices are compact, simple, and cost-effective. In this article, we describe this newly patented technology and discuss its advantages for microscopy.

Image Filtering and Spectral Imaging

Wavelength selection. The mapping of fluorescent probes is based on wavelength-selective imaging. This may include a wavelength-selected illumination source, such as a laser, filtered lamp, LED, or filtered supercontinuum. The source wavelength is matched to the absorption peak of the target fluorophore for optimal excitation. Where multiple fluorophores are used in the same experiment, the use of two different wavelengths allows each to be selectively excited. This type of fluorescence microscopy also includes wavelength filtering in the imaging or detection part of the microscope, i.e., before the camera or eyepiece. In this way, the Stokes-shifted fluorescence from each fluorophore can be selectively observed with maximum signal-to-noise against a background that includes scatter from the excitation source and fluorescence at other wavelengths.

Wavelength selectivity and tunability is also useful in other types of light microscopy, for example phase contrast and oblique illumination methods used to image transparent samples absent endogenous fluorescence or added fluorophores. Here it is well-known that iteratively changing (tweaking) the illumination conditions, particularly the wavelength, can selectively optimize the contrast of different targets within the image field.

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Limitations of Legacy Methods

Methods for filtering light according to wavelength can be divided into three groups: filters (cut-off, dichroic, etc.), monochromators, and newer "niche" technologies. Each method has its advantages.

Filters. In the detection part of a microscope, filters and dichroic beamsplitters are the commonly used means of wavelength filtering just prior to the camera or eyepiece. Similarly, these filters can be used with a broadband light source for wavelength-selective illumination instead of an LED or laser. The filter is typically based on thin film dielectrics sometimes incorporating colored glass for extra wavelength blocking. For a single fluorophore, a bandpass or cut-off filter is typically used prior to the camera. For two or more fluorophores, the light may be split into two cameras according to wavelength, using a dichroic dielectric beamsplitter. Or the fluorophores may be imaged alternately using a filter wheel in front of a single camera.
The limitation of filters and filter wheels is their lack of flexibility since the bandpass and center wavelength are fixed for each filter. So images cannot be scanned as a function of wavelength nor can the wavelength and bandwidth be iteratively adjusted to find the optimum (e.g., high contrast) viewing conditions for a specific sample.

Monochromators. Conversely, the monochromator is very flexible. It relies on a either a diffraction grating or a dispersive prism in which incident light is deflected at a wavelength-dependent angle. In the typical setup, light is directed to an input slit before being deflected towards the output port. For filtering a light source, an output slit or fiber is used to select only a targeted wavelength window; the center of this band can be continuously tuned by rotating the grating (or prism) angle. And by adjusting the width of the input and output slit, the bandwidth of the transmitted light also can be smoothly varied. In a microscope or other application, the output port could be a slit with a single element detector such as a photomultiplier tube, or else a CCD or CMOS camera where different elements receive different wavelengths. However, monochromators are not commonly used in the image detection process. The reason is that only one axis of the camera array can be used for imaging since the other is used for wavelength dispersion. So an image must be built up line by line or alternatively pixel by pixel.

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Recent filtering technologies.

There are also a couple of other niche wavelength filtering technologies: the acousto-optic tunable filter (AOTF) and liquid crystal filters. The AOTF is a solid state component based on applying radio frequency (RF) input to an exotic crystal such as tellurium dioxide (TeO_2). The resultant acoustic vibrations act as a moving diffraction grating. The main advantage of the AOTF is that it has fast switching time and can control multiple ouput simultaneously. However, it has numerous well-documented drawbacks. First, it is a complex RF-powered system that is relatively costly. Plus, this cost increases non-linearly with aperture size. The AOTF also has poor out-of-band extinction, typically <102. In addition, there is a fixed relationship between bandwidth and center wavelength, and, in many devices, the output angle shifts with wavelength.

In liquid crystal based devices, the phase of linearly polarized light is manipulated in a liquid crystal cell sandwiched between wave plates. The use of polarizers means that only a certain wavelength band can pass through the device. These are useful niche devices, but they have some limitations: the bandwidth is fixed, the input must be linearly polarized, and for un-polarized light the total throughput efficiency is <25%. In addition, the out-of-band extinction is much lower than a monochromator or bandpass filter, and the transmission edge slope is poor. For some illumination applications the low damage threshold can be another limitation.

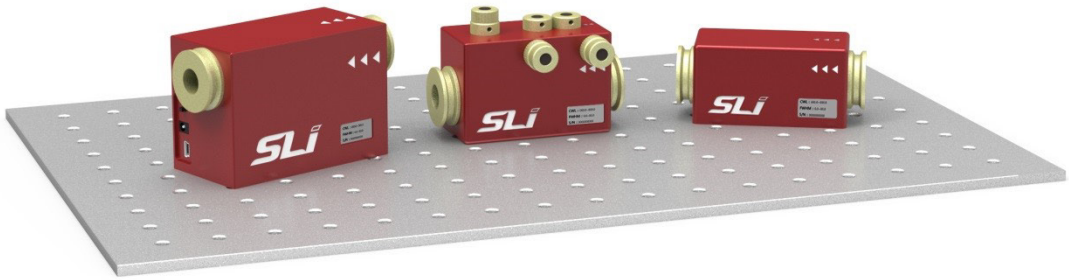

Fig. 1. Different models of Flexible Wavelength Selectors. From right: Auto, High Resolution and CenterLine.

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Flexible Wavelength Selectors

A new patented technology now combines the advantages of all these earlier methods without most of the drawbacks. Specifically, FWS provides the wavelength flexibility and precision of a monochromator with the large clear aperture of a filter. Moreover, it is simple, economical, robust, and it can be packaged as a compact device for microscopy.

Recent advances in ion beam sputtering (IBS) have enabled the fabrication of bandpass filters that combine a broad transmission band with very sharp edges, high (up to 10^{-6}) out-of-band extinction, and performance that is nearly immune to changes in temperature or humidity. FWS combine two of these broadband bandpass filters in a compact, light-tight housing.). This design enables independent rotation of the angle of incidence of each filter. These bandpass filters are all dielectric with transmission/reflectance characteristics determined solely by interference between the multiple coating layers.

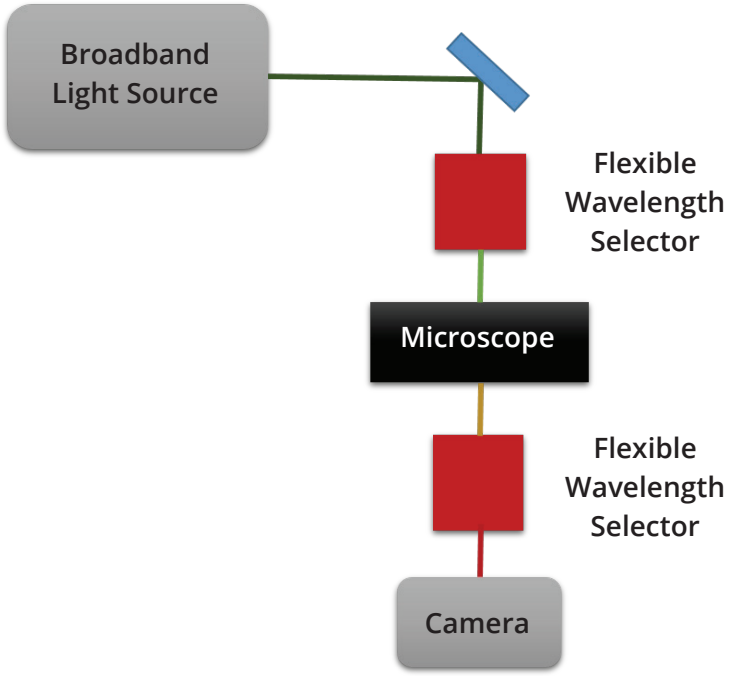

Fig. 2. A schematic diagram showing application of Flexible Wavelength Selector in fluorescence imaging setup.

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But, the center wavelength of each filter shifts as the angle of incidence changes so, for collimated input light, rotating the angle of incidence of one of these filters serves to smoothly tune its transmission spectrum. And, because there are two of these filters, they can be used to independently define the short and long wavelength edges of the overall transmission curve. In this way, both the center wavelength and bandwidth of transmitted light from any collimated source are fully adjustable. Just as important, a third glass plate is simultaneously rotated (manually or under automated control) to offset any slight lateral walk-off in the beam path as the angle of incidence is set or swept.

The bandwidth of FWS products can be user or factory adjusted from around 1.5 nm to 20 nm (nominal). And, the center wavelength can be chosen from most visible wavelengths (350 nm – 900 nm). Moreover, because they require collimated light, the infinity space in a microscope (i.e., prior to the camera) is an ideal location for compact devices based on this technology. In addition, the collimated input/output allows for simple fiber coupling, which is already offered in some commercial devices based on this technology.

This construction gives FWS devices several key advantages. Like a grating-based device, the out-of-band extinction is very high (10^{-6}). But, like a traditional filter (and unlike a grating monochromator with a narrow slit geometry), the spectral performance of a FWS device is uniform across 95% of the large (up to 10 mm diameter) clear aperture. These characteristics make it ideal for spectral filtering in the camera and the microscope light source.

As shown in Fig. 2, it is possible to use FWS for both excitation and emission. For excitation, it is necessary to select the optimal wavelength for maximal efficiency and to avoid cross-excitation in multi-fluorophore experiments. For emission, it is possible to scan through the wavelength range for spectral imaging.

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