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Filters and mirrors for laser applications

Using lasers for fluorescence applications requires special consideration of the optical path. This special attention centers around the coherent nature of the light, the small beam diameter, the polarization and the power. Here I discuss the filters and dichroic mirrors used in the beam path, with an emphasis on Chroma's modified magnetron sputtering technology, MMS™: zet488/10x laser clean-up filter, zt488rdc laser dichroic mirror and et525/50m emission filter.

The products outlined here are part of Chroma's new sputtered offerings for laser applications. The spectral characteristics of the filters and mirrors described are shown in **Figure 1**. In a standard epifluorescence microscope, the excitation filter is designed to reject all light—from deep ultraviolet to 1,200 nm—other than the band needed for the excitation of the fluorochrome. Usually this is a band-pass design with 30–60 nm of full width, half-maximum transmission (FWHM). This optic in standard widefield microscopy has no surface flatness specification or transmitted wavefront requirements. It does not typically have an antireflective coating and is made with float glass as the substrate. This excitation filter is designed for a 0-degree angle of incidence (AOI).

For laser applications, most of the specifications are considerably different. The transmitted wavefront must be less than 1 wave per inch, although some applications require 1/4 wave per inch or better. The wedge must be small, in the range of 1 arc minute, with no pinholes. The clean-up filter (the exciter used with lasers) must block only the range of output from the laser that is not wanted. There are still many users who believe that lasers do not require clean-up filters, but this is rarely true. Virtually all lasers produce multiple lines and/or have 'noise' well away from the primary line. All laser systems should be tested for signal-to-noise with and without a clean-up filter. This optic is typically 10–20 nm FWHM, is ground and polished, and has an antireflective coating on any surface not used for bandpass coatings. Although standard laser applications work very well using float glass as the substrate, Raman applications and others may demand fused silica to reduce any chance of autofluorescence in the beam path. The clean-up filter is designed for 3–5 degrees AOI to insure that none of the reflected laser light goes back into the laser cavity. The Chroma zet488/10x meets all of the specifications listed above (**Fig. 1**).

The second optic in the configuration is the dichromatic mirror,

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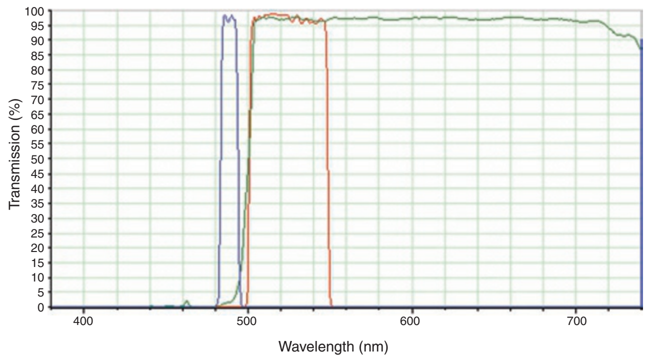

Figure 1 | A 488-nm laser set with sputtered optics. Typical laser set: zet488/10x (blue) to eliminate all light other than 488 nm; zt488rdc (green), dichroic mirror to reflect 488-nm laser to sample and transmit emission; et525/50m (red), bandpass emission filter to block 488 nm to OD ≥ 6 and transmit emission to detector.

which in widefield epifluorescence systems is typically specified with less than 10 waves per inch of surface flatness, less than 1 wave per inch transmitted wavefront, 1 arc minute of wedge or less. The dichroic mirror, also called the beam splitter, should be made of fused silica as it is in the excitation and emission path in most epifluorescence systems. For standard applications, this optic is not covered with an antireflective coating. These mirrors can have either long-pass (reflect shorter wavelengths and transmit longer) or short-pass designs. Dichroic mirrors are designed for 45 degrees AOI in standard microscope configurations.

In laser applications, the dichroic mirror must be made to much more exacting specifications: less than 1/2 wave per inch surface flatness before coating, less than 1/4 wave transmitted wavefront distortion and less than 5 arc seconds of wedge. All laser mirrors require antireflective coating on any uncoated surface to reduce the laser second reflection and scatter. Although most commercial microscope companies specify 1-millimeter-thick dichroics, all of these specifications are easier with thicker fused silica. Therefore, systems built specifically for laser applications will often have thicker dichroics.

Although most laser mirrors are designed for a 45-degree AOI, some flow systems and newer confocal microscopes use mirrors at 10–15 degrees AOI. Each of the above specifications is a minimum, as some applications require that these mirrors have much higher standards. The Chroma zt488rdc meets all of the above requirements (**Fig. 1**).

Another consideration for the dichroic mirror is the polarization of the laser. Optics that are used at 0 degrees AOI have no effect on polarization state, but any optic at an angle works as a polarizer. Therefore, all laser dichroics and mirrors should be made with the polarization properties of the laser in mind.

An important note regarding all dichroic mirrors is that any coating applied to a substrate, such as fused silica, will induce stress. This stress may be relatively minor and unnoticeable, but it may also be severe enough to produce extreme astigmatism in the beam. The use of thicker substrates will almost always decrease the effect of induced stress, but may not be enough alone. Therefore, Chroma, as well as most other manufacturers, have some proprietary method for counteracting this stress.

One more important possibility for inducing stress is the mounting of this mirror in the system. The cube or mount must not induce an uneven torque in the substrate.

The final optic in the configuration is the emission filter, also frequently called the barrier filter. The primary function of this optic is to block the excitation wavelengths of light from reaching the detector. The secondary function is to transmit the desired emission from the fluorochrome of interest. This means that the emission filter must block to high extinction any light transmitted through the excitation filter. This blocking is typically measured in optical density (OD), which is the negative log of transmission and for widefield applications can vary from OD 3 to OD 6. This optic can be either a band-pass design or a long-pass, and has the typical specifications of less than 1 wave per inch transmitted wavefront, less than 1 arc minute of wedge. Antireflective coating is an option specified by application.

For laser applications, the minimum requirements of the emission filter can be nearly identical to those above, except that the blocking at the laser line(s) should be a minimum of OD 6, and the filter should have an antireflective coating in all cases. These emission filters are also designed for 5 degrees AOI, as are many of the modern widefield systems, to reduce internal reflections. The Chroma et525/50m band-pass emitter meets all of the above requirements (**Fig. 1**).

In summary, laser optics must be designed and made differently from standard widefield filters and mirrors. Although it is true that most laser optics will work very well in widefield applications, the inverse is clearly not true, as shown by the differing specifications in each case. The differences in design and construction may not be apparent in every laser application, but they will be obvious in most applications, especially the more demanding ones, such as total internal reflection microscopy, Raman spectroscopy and multiphoton microscopy.