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LASER APPLICATION NOTE
Filters and Mirrors for Laser Applications

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The use of lasers as the illumination source for fluorescence applications requires special consideration for the optical path. This special attention centers on the coherent nature of the light, the small beam diameter, the polarization, and the power. There are also circumstances where cone angle and scatter within the system play significantly into the design of the optics. While the entire beampath should be considered carefully, this brief note will emphasize the filters and dichroic mirrors used in the beam path.

In a typical epi-illumination configuration there are three optics that play the major role: excitation filter, dichromatic/dichroic beamsplitter, and emission filter. Usually, these optics are held in one mount called a cube which can be inserted and removed from the microscope at will.

In this standard epi-fluorescent paradigm, the excitation filter is designed to reject all light from deep ultraviolet (UV) to near infrared (NIR) other than the band needed for the excitation of the fluorochrome. Usually this is a bandpass filter design of 30-60nm full width, half-maximum transmission (FWHM). In standard microscopy, this optic has no surface flatness specification or transmitted wavefront requirements. The wedge can be huge (typically anything less than 8 arc minutes), and the scratch/dig specification is minimal at 80/50. There can be no measurable/observable pinholes. Typically there are no overarching concerns about the autofluorescence of the materials as this optic lies outside the imaging path. This optic is not typically anti-reflection (AR) coated and is made with float glass as the substrate. This excitation filter is designed for 0 degrees angle of incidence (AOI).

Compared to the epi platforms, the configuration for laser systems are extremely variable, even if most still use the same three types of optics listed. These filters and mirrors are not typically all together in one mount/cube and their locations in the beampath vary from system to system.

For laser applications, most of the specifications are significantly different. The clean-up filter (special name for the excitation filter used with lasers) must block only the unwanted output range from the laser. There are still many users that believe that lasers do not require clean-up filters, but this is rarely true. Virtually all plasma lasers produce multiple lines of varying power, and the newer generation of diodes have a much wider FWHM for their output emission. Furthermore, they may also have ‘noise’ well away from the primary line. The diode-pumped solid state lasers have emissions that are typically very narrow at their output but may be contaminated by the pump or primary lines. For these reasons, all laser systems should be tested for signal-to-noise with and without a clean-up filter. The transmitted wavefront must be better than 1 wave per inch, although some applications require ¼ wave or better. The wedge must be small, in the range of 1 arc minute, with no pinholes. Careful consideration of substrate materials that may auto-fluoresce may have to be done (depending on the application), and a scratch dig specification of 60/40 or better must be followed. This optic is typically 10-20nm FWHM, is ground and polished and has an AR-coating on any surface not used for the interference coating. While 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 from the beampath. The clean-up filters are designed for 3-5 deg AOI to insure that none of the reflected laser light goes back into the laser cavity. See figure 1.

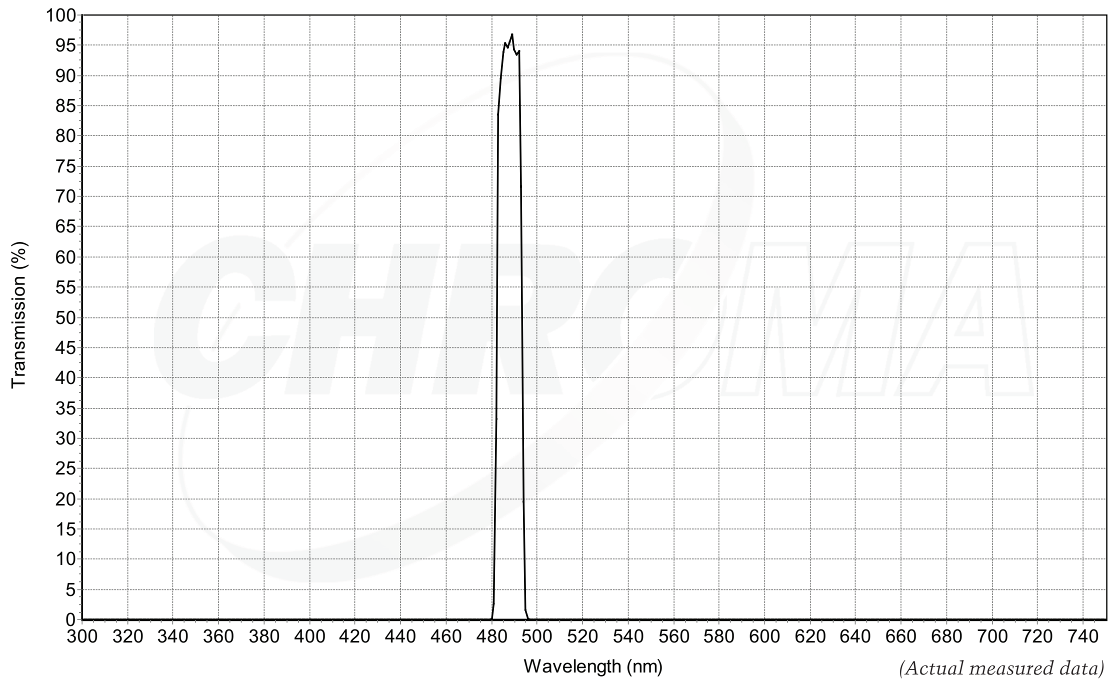


Fig. 1: ZET488/10x, common laser clean-up filter.

The second optic in the configuration is the dichromatic mirror also referred to as dichroic mirror or beamsplitter, which in widefield epi systems is typically specified as follows: less than 10 waves per inch of surface flatness, less than 1 wave per inch transmitted wavefront, 1 arc minute of wedge or less, 40/40 scratch/dig. The dichroic mirror should be made of fused silica as it is in both the excitation and emission paths in the epi systems. For standard applications, this optic is not AR-coated. These mirrors can be either longpass (reflect shorter wavelengths and transmit longer), or shortpass designs (transmit shorter wavelengths and reflect longer). Dichroic mirrors are designed for 45 deg AOI to change the excitation light by 90 degrees in standard microscope configurations.

In laser applications, the dichroic mirror must be made to much more exacting specifications: less than ½ wave per inch surface flatness before coating, less than ¼ wave transmitted wavefront distortion, less than 5 arc seconds of wedge, and usually 40/20 scratch/dig. All laser mirrors require AR-coating on any uncoated surface to reduce the laser second reflections and scatter, as well as increasing transmission. While most commercial microscopes call out 1mm thick dichroics, most of these specifications are easier with thicker fused silica. For that reason, systems built specifically for laser applications will often have thicker mirrors/dichroics. While most of the laser mirrors are designed for 45 deg AOI, some flow systems and newer versions of confocal microscopes use mirrors at 10-15 deg AOI to increase reflection efficiencies (and/or increase blocking) and to minimize polarization effects. All of the above specifications are considered a minimum, as some applications require that these mirrors have much higher standards.

Another consideration for the dichroic mirror is the polarization of the laser. Optics that are intended for use at 0 deg AOI have no effect on polarization states, but any optic at an angle works as a polarizer to varying degrees depending on specific design. Therefore, all laser dichroics/mirrors should be made with polarization properties of the laser in mind. See figure 2.

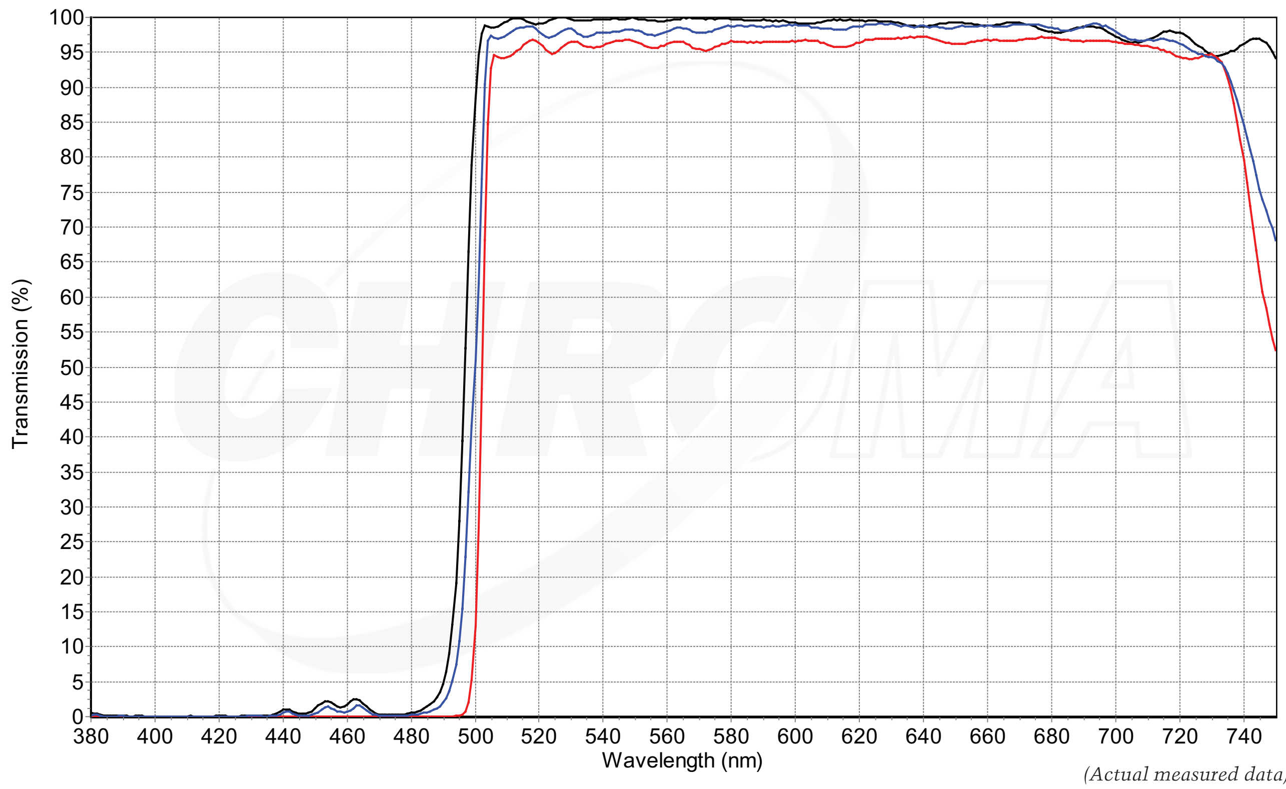


Fig. 2: ZT488rdc, typical laser dichroic/beamsplitter, shown with random (black), s-pol (blue), & p-pol (red). All dichroics are shown at 45 deg AOI. Some designs may vary more in polarization changes or differences.

Another consideration for all dichroic mirrors concerns the fact that any coating applied to a substrate such as fused silica will induce stress. This stress may be so minor that is not noticeable, but it may also be severe enough to exhibit extreme astigmatisms in the beam. Thicker substrates will almost always help with this, but may not be enough alone. Therefore, most manufacturers have some proprietary method for counteracting this stress, and reflection specifications for these mirrors must be carefully monitored. Note that transmitted wavefronts are dependent on the parallelism of the substrate outer surfaces and does not typically suffer from any coating effects.

One other possible cause of induced stress is the method of mounting this mirror into the system. The 45 degree surface that this mirror sits on should be polished to similar flatness as the substrate (i.e. ½ wave per inch), and whatever holds the mirror in place cannot exert any uneven pressure. This effect is most noticeable in TIRF (total internal reflection microscopy), and may require a fully "custom" cube/mount in place of the standard microscope cube.

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 from reaching the detector (whether the human eye or electronic 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 –log of transmission (T), and for widefield applications can vary from OD 3 to OD 6. This optic can be either a bandpass design or a longpass, with typical specifications of: less than 1 wave per inch transmitted wavefront, less than 1 arc-minute of wedge, 60/40 scratch/dig. AR-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 they should be AR-coated in all cases. These emission filters are also designed for 0-5 deg AOI, as are many of the modern widefield systems, to reduce internal reflections. See figure 3.

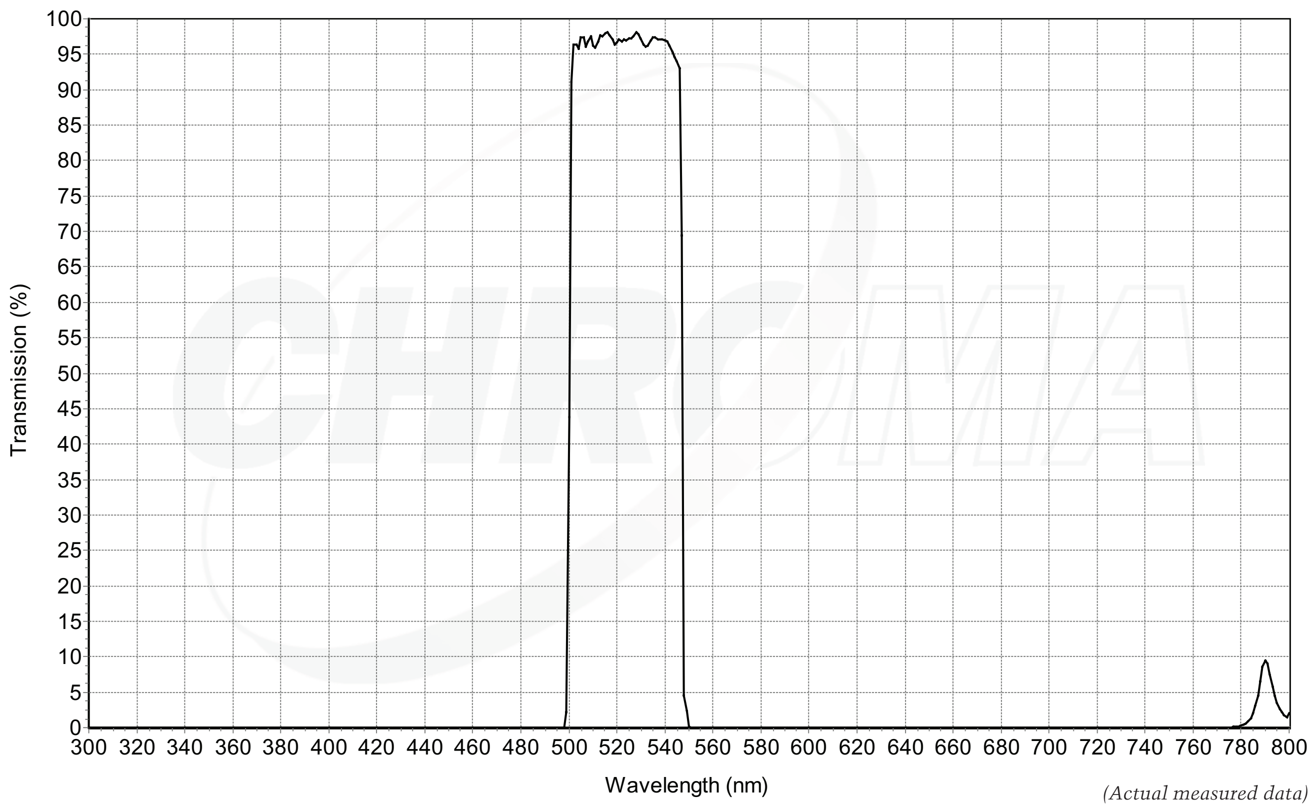


Fig 3: ET525/50m, typical emission filter for laser application (not shown OD6+ at 488 nm).

Furthermore, there are some specific applications that require further considerations.

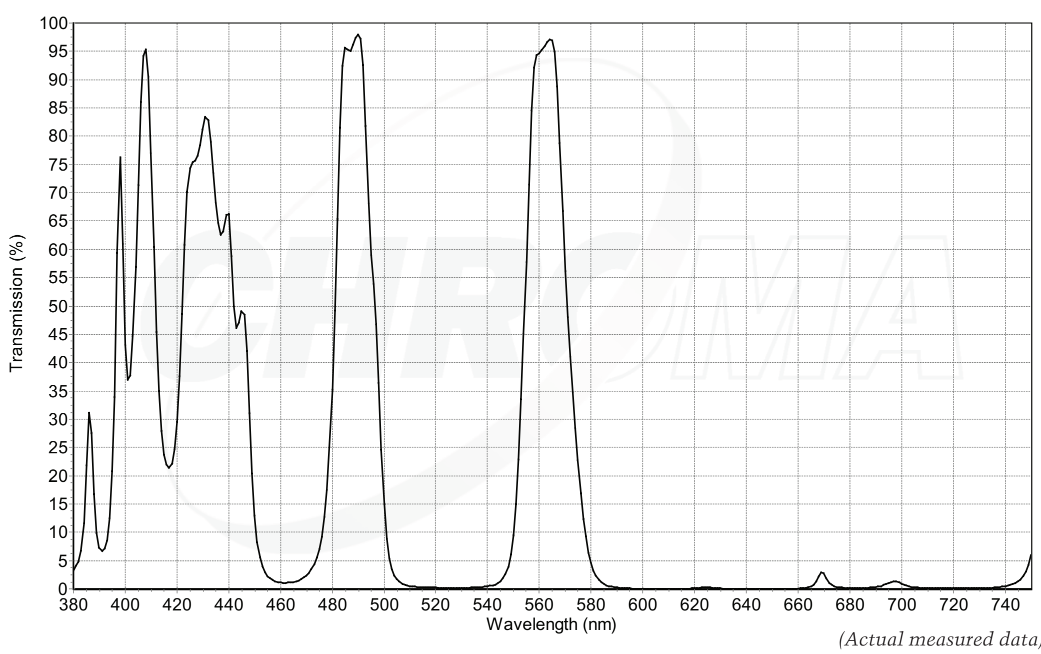
Standard Laser Scanning Confocals

Typical laser scanning units require the specifications listed above with the additional consideration for emission filters either in wheels or splitter units. If the confocal has only one detector (typically a PMT) and/or uses sequential imaging using emission filter wheels, then the emission filter used for each laser line must block only that laser line. However, if the confocal has multiple PMTs, with multiple emission filters, then each emission filter must block all laser lines being used. These multi-PMT systems would also use emission splitting dichroics to direct the light. These dichroics do not need to meet the more demanding specifications of the primary mirror, but may need to be image-quality with ar-coating in some applications.

As mentioned above, some of the newer laser scanning units use a primary dichroic that is not at 45 deg AOI, but may be 10-15 deg AOI, while the emission splitting dichroics are typically at 45 deg AOI.

Multi-Pinhole Confocals

The current commercial Nipkow Disk confocals can have two entirely different beam paths. One uses a primary dichroic which reflects the laser and therefore is very similar to the 'standard' confocal configuration. The other type uses a primary dichroic which transmits the laser/excitation light to the sample and then reflects the emission light to the detector (typically a CCD camera). This transmitting dichroic design is much more difficult for several reasons. The dichroic mirrors are somewhat more difficult to design and manufacture with a very narrow transmission band (for each laser in a multi-laser configuration) and broad reflection bands. This design also means that considerably more of the laser light will ultimately be reflected to the detector (also at differing angles), thereby putting more emphasis on the blocking ability of each emitter. And, since this transmitting mirror is in the focal path of this scope the thickness of the substrate is limited which complicates wavefront distortion control. See figure 4.



(Actual measured data)

Fig. 4: ZT488/561trans-pc, typical dual transmitting polychroic.
(Inverse of % transmission is % reflection at least at visible wavelengths)

Total Internal Reflection Fluorescence, TIRF

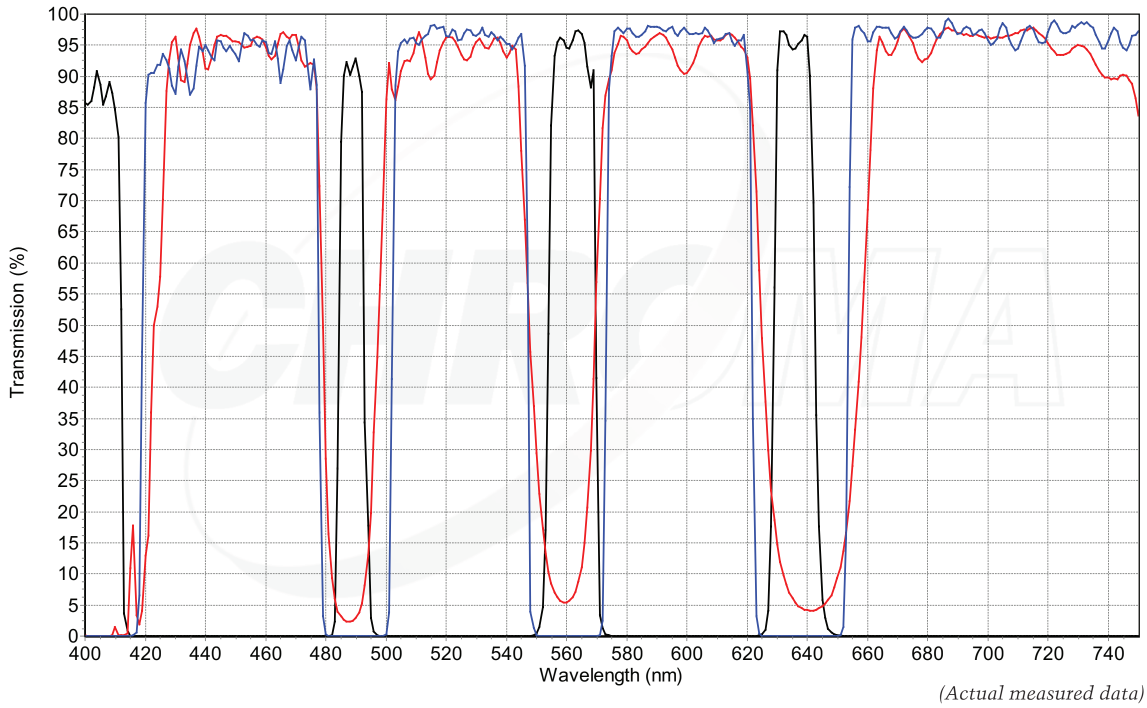
The original design of TIRF microscopes used prisms to bring the laser to the interface where the cells adhered. This presented constant alignment problems, but was fairly straight-forward from the filter standpoint since the reflected laser light was not collected by the objective. However, the later development of through-the-lens TIRF introduced the unusual circumstance where the laser excitation light is actually collected by the objective (serving here as both condenser and objective). The laser therefore travels back into the beampath at virtually full power. This reflected light requires that both the dichroic and the emitter in this TIRF application be made to deal with this huge amount of returning laser light/power. This laser light also poses a very real danger for anyone looking into the microscope and therefore is typically blocked with laser cut-out devices.

As mentioned earlier, the reflected wavefront distortion (RWD) for the dichroic mirror in a TIRF application must be of extremely exacting standards, usually starting with substrates at 1/10 wave surface flatness and 1/10 wave transmitted wavefront distortion (TWD). Great care must be taken to insure that the coatings applied do not stress the substrate beyond acceptable levels. As systems may vary, this requirement after coating can vary from ¼ to 1 wave per inch surface flatness/TWD.

The emission filters in TIRF rigs must block to a minimum of OD 6, and in many systems/applications, should block to OD 8. Only a few years ago, this was considered impossible, but is fairly routine now using advanced coating techniques and very special 'tricks of the trade'.

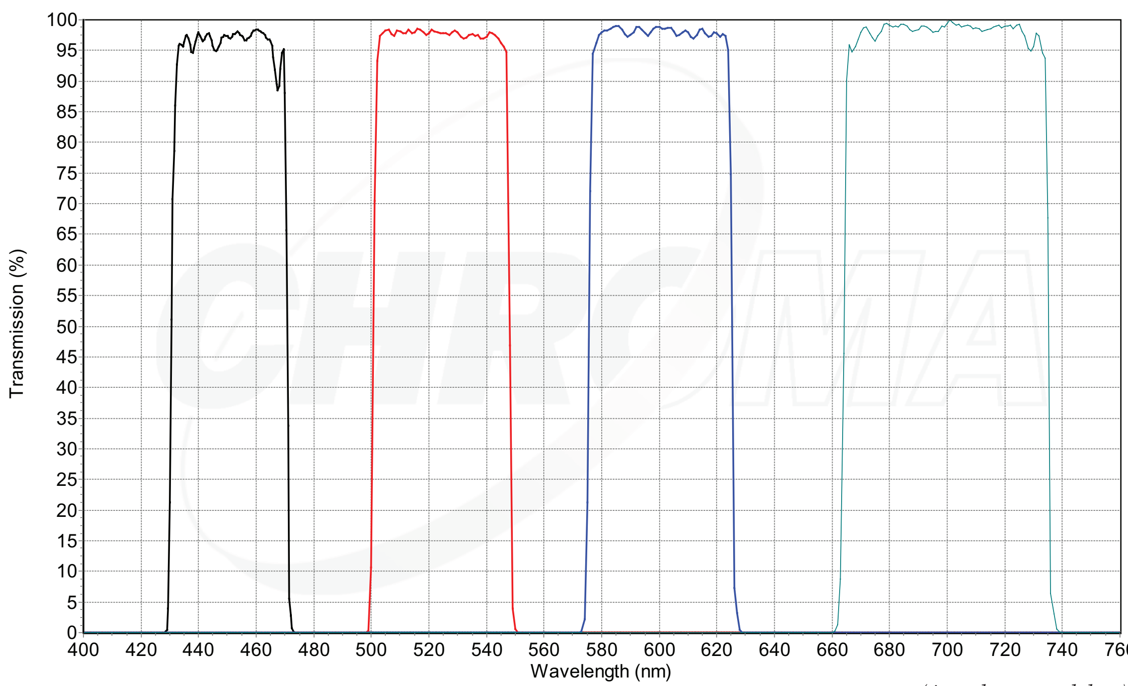
TIRF imaging is further complicated by the high angle reflections that occur by design in the system, since interference filters are designed to work at 0-5 deg AOI. Light beyond this cone angle has the effect of shifting the cut-on of the filter toward the blue/UV, which means toward the excitation source (the laser line in this case). Therefore, high angle excitation light in the system may require that the emission filter be slightly red-shifted in cut-on to allow for this shift to occur and still maintain the full blocking ability. Another option in this scenario is to use absorption glass, which is non-angle dependent, but which may greatly reduce the transmission of the filter.

It is very common in the current through-the-lens TIRF systems to use an emission/barrier filter in the cube, or close to the mirror, with a second emission filter placed just before the detector or perhaps in an emission filter wheel. A well-blocked emitter in the cube also adds a significant safety factor for anyone accidentally looking into the scope without a laser lock. This 'doubled' emission design may be absolutely necessary in some systems but not in others. It should be noted here that standard very narrow "notch" filters (filters designed to have narrow rejection/blocking regions with transmissions at both shorter and longer wavelengths) do not work well in this application as this type of filter is very sensitive to the angle of the incoming light. See figures 5 & 6.



(Actual measured data)

Fig. 5: ZET405/488/561/640x, typical quad clean-up filter (black) ZT405/488/561/640rpc, quad polychroic (red). Makes alignment easier with no registration issues.ZET405/488/561/640m, quad emission filter (blue). Added blocking plus safety factor for users.

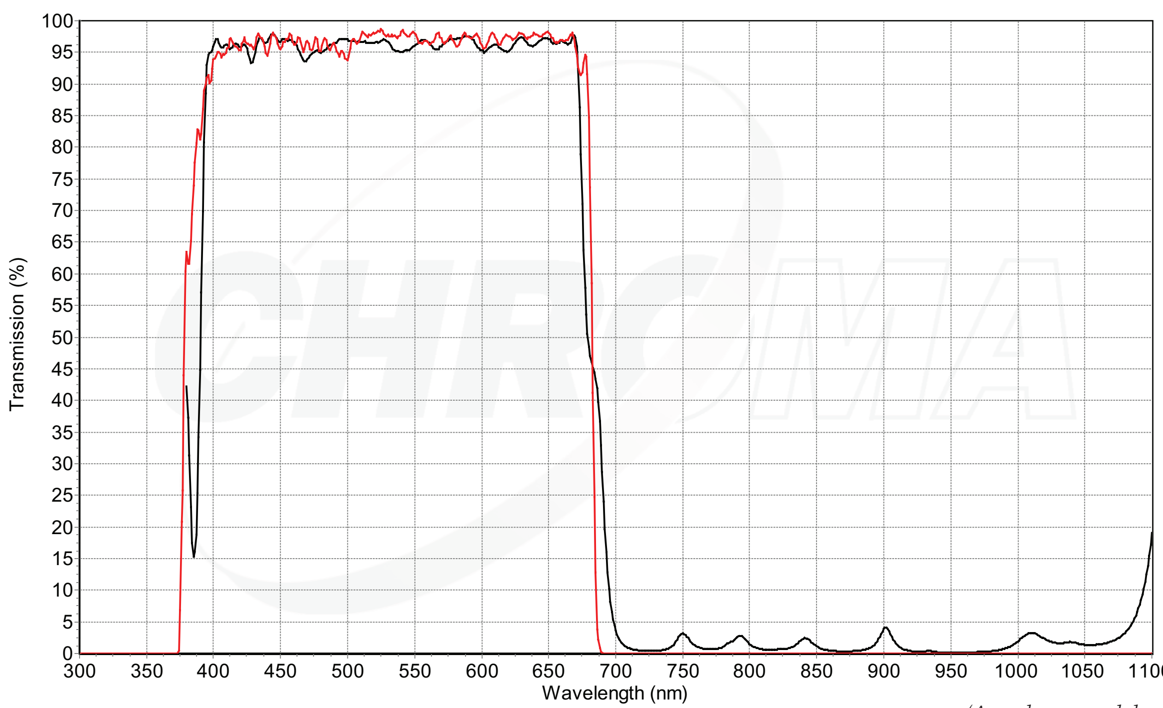


(Actual measured data)

Fig. 6: Single band emission filters designed to match the quad set in Fig. 5. ET450/40m (black); ET525/50m (red); ET600/50m (blue); ET700/75m (green)

Multi-photon Systems, 2/3-photon Laser Configurations

The development of 2-photon imaging systems that met earlier theoretical parameters (able to excite a fluorochrome at twice the wavelength, using very high powered pulsed lasers), proved to be very technically difficult for the filter manufacturers/industry. For these systems, in descanned mode, the primary mirror must be a shortpass design with reflection of the longer wavelengths and transmission of the shorter. This mirror must be designed to be extremely efficient in both reflection and transmission and also must not introduce any dispersion in the incident pulsed beam as this would decrease peak power considerably. This new technology also meant that the blocking/barrier filters for these systems must now have the maximum blocking effect on the longer wavelength side of the bandpass, as opposed to 1-photon blocking on the shorter side. Completely new filter designs had to be developed and refined over the years to the current standard of getting OD 8+ blocking across the full tuning range of these lasers (typically 680-1064nm). See figure 7.



(Actual measured data)

Fig. 7: One possible shortpass blocking optic/emitter, T680dcspxr (black), typical shortpass dichroic for 2p ET680sp-2p8 (red) (8 refers to average OD 8 across effective blocking range)

To further complicate the filter design, many wanted to image second harmonic generation (SHG) and be able to do 3-photon excitation, requiring the dichroic and emission filter to transmit into the UV range. These are perhaps among the most complex designs now available with interference technology. See figure 8.

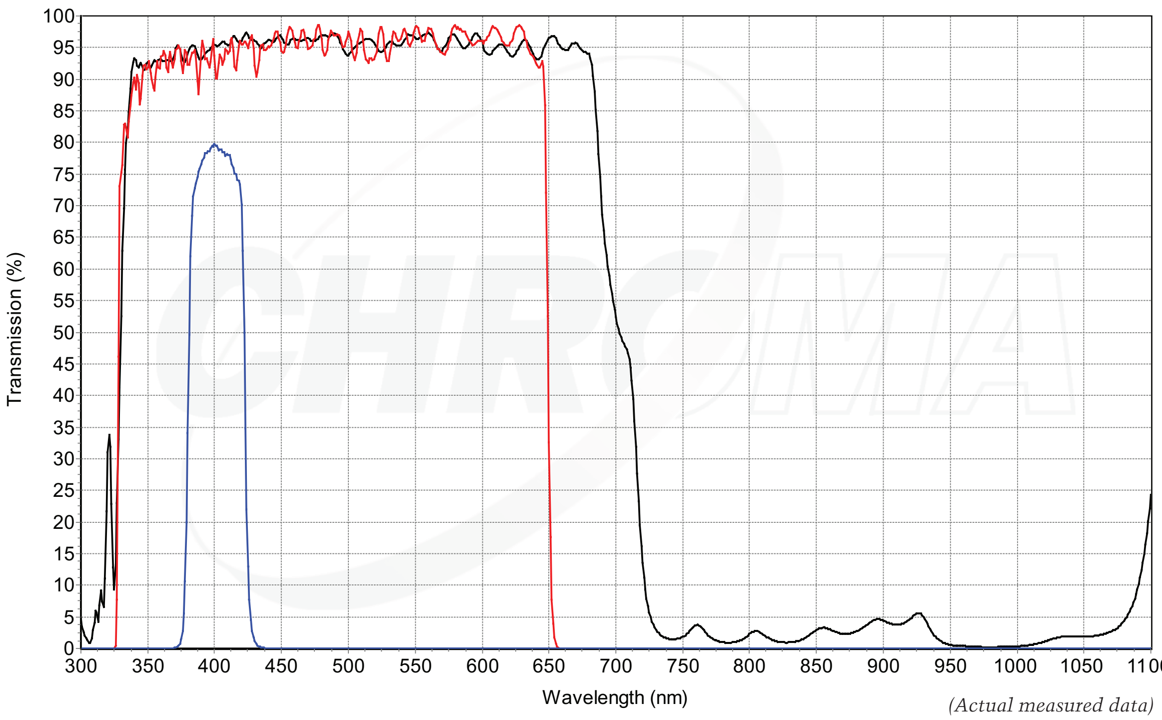


Fig. 8: Custom shortpass dichroic for 3-photon with transmission into the UV, T700dcspxru-3p (black); 3p shortpass blocking emitter, ET650sp-3p (red); and one option for the SHG bandpass at hq400/40m-2p (blue)

There are now many more users with non-descanned detector (NDD) systems available whereby the emission signal does not pass through the primary dichroic. These systems typically take the fluorescence directly from the sample and to the PMT. There may be a shortpass emission, to block the laser, at the beginning of this path (see figures 7 and 8) with bandpass emitters in front of the PMT or each PMT in a multi-detector system. These are typically arranged in cube mounts to simplify the beam path. See figure 9.

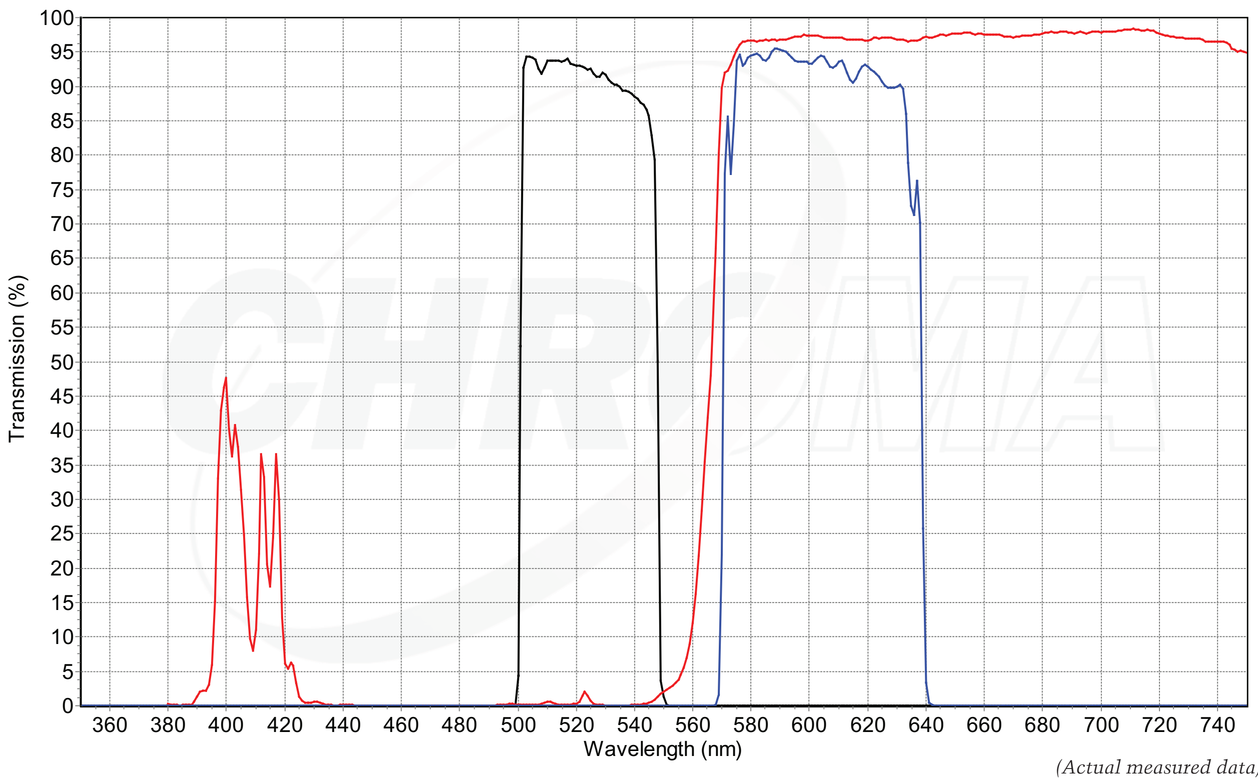
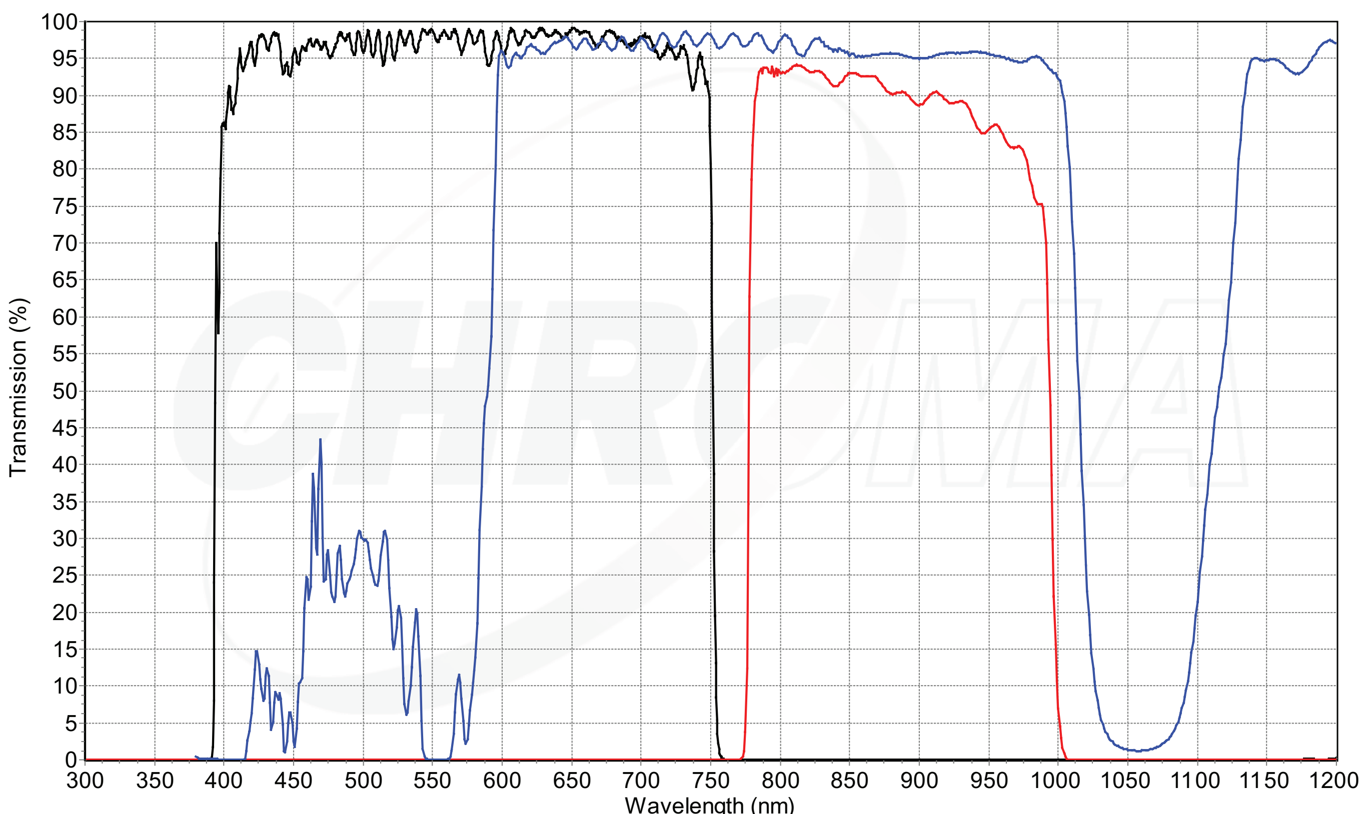


Fig. 9: Typical NDD emission splitting set

ET525/50m-2p (black); T565lpxr (red); ET605/70m-2p (blue) (not shown OD6+ to 1064nm)

Coherent Anti-Raman Stokes, CARS

This new technology, championed by Dr. Sunney Xie at Harvard, is a sophisticated multi-photon application that requires both a pump and a Stokes laser of varying wavelengths. Due to the huge powers of these lasers, extra blocking may be required, perhaps well beyond the OD 8+ level mentioned for standard 2-photon, and it is not unusual to use two blocking optics in series. See figure 10.

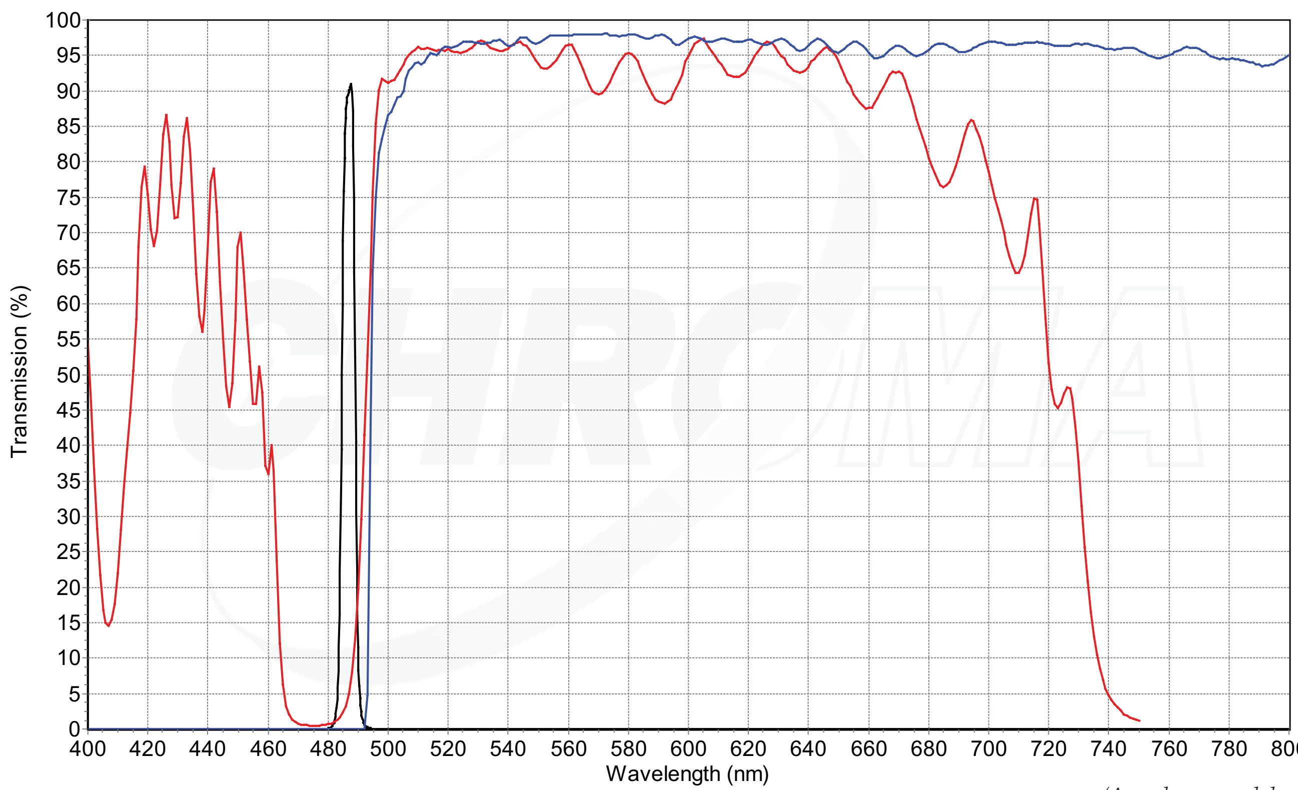


(Actual measured data)

Fig. 10: Possible shortpass blocker for CARS, ET750sp-2p8 (black), this design may be used in series; CARS-HQ890-220m as an example of a wide bandpass emitter (red); CARS-ZT1064dcrb (blue) is a 45 deg dichroic for combining the 1064nm laser with other lasers and/or the CARS emissions.

Raman Spectroscopy

Raman techniques have been around for many, many years. The most common applications currently used look at only positive-Stokes Raman signals on the longer wavelength side of the laser (longpass dichroic mirrors and longpass emission filters). Occasionally, researchers prefer to look at negative Stokes Raman reactions (using shortpass dichroics and bandpass or shortpass emission optics). Each application must have specialized mirrors and filters, but the major requirement is maximum blocking of the laser due to the very low Raman signal. The Raman signal is so low, typically, that the optics for both clean-up filters and emission filters must also be made using fused silica, instead of float glass, to reduce any autofluorescence in the system. Otherwise, the dichroic and emission filter designs must also be as close as possible to the laser line. Fortunately, many of the positive Stokes experiments can use a longpass emission filter, which is actually easier to design only a few nanometers from the laser, and still achieve optimal blocking of the laser. See figure 11.

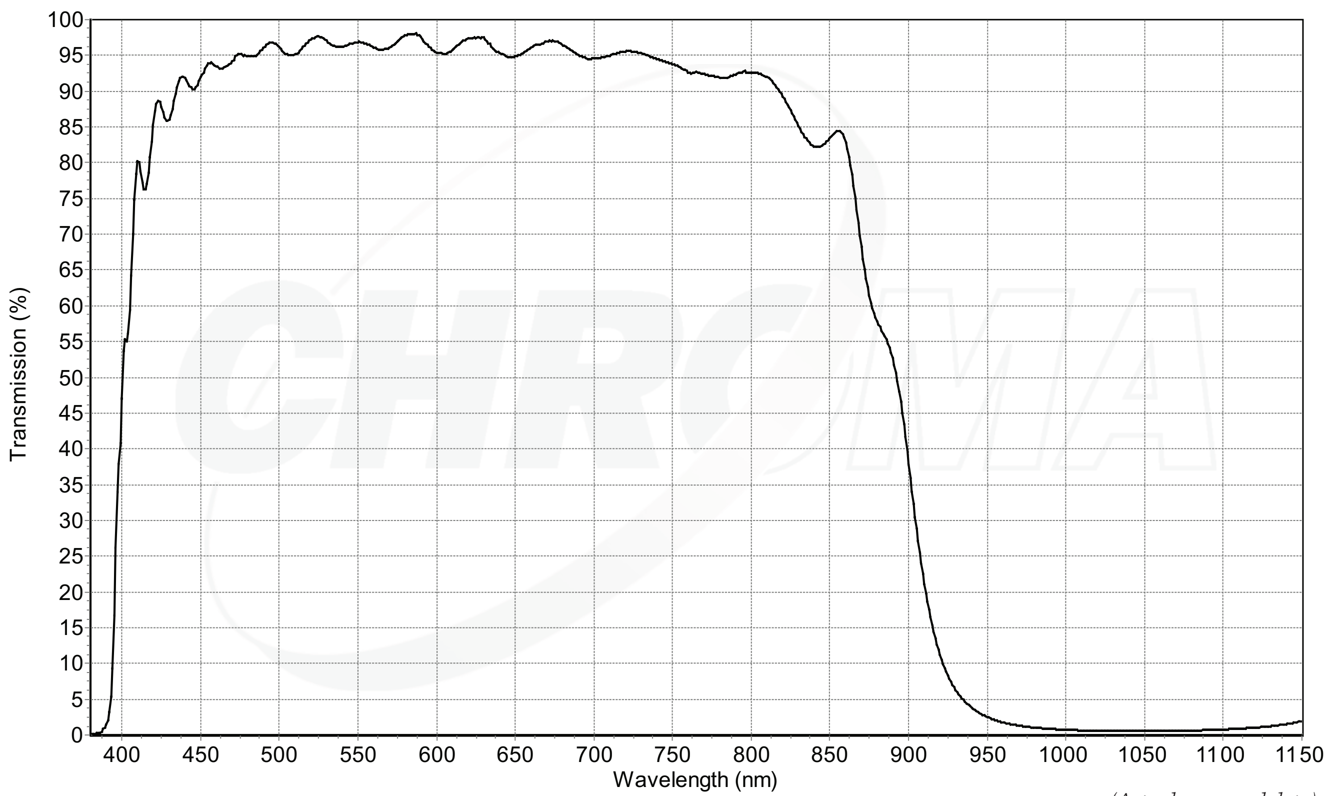


(Actual measured data)

Fig. 11: Typical Raman laser set. R488/5x, clean-up filter (black); Z488rdc, dichroic mirror (red); R488lp, longpass emission filter (blue)

Laser Traps and Tweezers

Frequently, these systems use a 1064nm laser or similar as a focused beam at the specimen plane. To achieve this, most systems use a shortpass dichroic with reflection in the near infrared (NIR) and transmission in the visible. Additionally, all emission filters must block the trap laser line, similar to 2-photon blocking, as described above. See figure 12.



(Actual measured data)

Fig. 12: Typical shortpass dichroic for traps & tweezers, Z1064rdc-sp. 45 deg AOI with 96+% reflection at 1064nm.

Flow Cytometry

Since flow systems use lasers, they also require special consideration even if some of the specifications can be relaxed due to the inherent ability of these machines to compensate for noise by using both scatter laser light and emission light in their calculations. These systems are typically non-imaging which does allow for the use of non-imaging components. One of the greatest challenges with flow cytometry optics is the drive to collect signal from more and more fluorochromes simultaneously. This means that both dichroics (which may be longpass or shortpass depending on the design of the instrument) and the emission filters may be designed for 4-6 different fluorochromes all in the same emission path. This necessitates very steep dichroic designs and very steep, and narrow, emission designs that must be custom designed for the different flow systems on the market. A full cytometry application note will be developed and available at www.chroma.com.

Summary

In summary, laser optics must be designed and made differently from standard widefield filters and mirrors. While it is true that most laser optics will work fine in widefield applications, the inverse is clearly not true, as shown by the different specifications in each case. The differences in design and construction may not be apparent to the end-user in every laser application. However, these differences will be painfully obvious in most experiments if these specifications are not met, especially with more demanding applications such as TIRF, Raman, and multi-photon microscopy.

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