# Advances in Filter Technology for Multiphoton Microscopy

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# Advances in Filter Technology for Multiphoton Microscopy

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## ABSTRACT

A new optical interference filter deposition technology is demonstrated that provides the deep blocking and extended transmission regions required for multiphoton fluorescence applications. This technology allows for the deposition of high phase thickness coatings with many more layers than was previously possible. Theoretical blocking at a level greater than optical density 9 is achieved. We present examples of shortpass edge filters and bandpass filters with high transmission and deep blocking. A dichroic mirror with high reflection in the near infrared and an extended region of high transmission throughout the visible is also presented.

Keywords: Optical filter, interference filter, dielectric thin film, multiphoton fluorescence, shortpass filter, bandpass filter

## 1. INTRODUCTION

Multiphoton excited fluorescence microscopy is a relatively new technique requiring an optical filter solution unique from that of traditional single photon excitation[1-5]. Typically it requires a dichroic filter to separate the incoming laser light from the fluorescence signal, and a second filter to further attenuate the scattered laser light. A high near-infrared rejection ratio is typically required, as most applications utilize femtosecond pulsed Ti:Sapphire lasers. Since there may be as much as 400 nm separation between the excitation and emission wavelengths, transmission edge steepness is not as important as it is with single photon excitation. The most important filter properties are deep blocking in the near infrared, and high transmission in the visible region, with minimal autofluorescence from the filter assembly.

In recent years there have been improvements in optical interference filter deposition technology allowing for the deposition of high phase thickness coatings with high spectral contrast. Omega has developed a new deposition technology, hereafter referred to as Alpha technology, that allows for the construction of high phase thickness shortpass and longpass edge filters with deep blocking and extended regions of high transmission. Traditionally, optical filters are based on a quarter wave stack design, in which alternating layers of high and low refractive index materials are deposited on a glass substrate in quarter-wave optical thicknesses[6]. As more layers are deposited, higher blocking and steeper edge slopes are achieved. The number of layers that can be deposited using optical monitoring is limited since the growth of the filter is typically monitored in the reflection band. Alpha technology avoids monitoring in the reflection band, so the number of layers that can be deposited is limited in practice only by the stress characteristics of the film. Zinc sulfide and cryolite ($Na_5Al_3F_{14}$) are an ideal pair of materials for coatings in the visible region, with a good net balance between tensile and compressive stress[7]. With Alpha technology employed in the deposition of ZnS/ cryolite films, coatings in excess of 100 layers have been achieved. Another ideal combination of materials for multilayer coatings is $TiO_2$ and $SiO_2$, which have the added advantage of durability and abrasion resistance.

In this paper we demonstrate the application of Alpha technology to shortpass edge and bandpass filters for multiphoton fluorescence applications. These filters are deeply blocking in the near infrared region of the spectrum, and highly transmitting in the visible. We present theoretical results showing that blocking as high as optical density 9 is achieved. A dichroic mirror with high reflection in the near infrared and high transmission throughout the visible is presented.


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## 2. SHORTPASS EDGE FILTERS

Alpha technology was used to fabricate shortpass edge filters composed of alternating layers of ZnS and cryolite. These materials are evaporated onto a glass substrate in an evacuated bell jar using filament heated crucible sources. These are soft and hygroscopic materials, so they must be protected from the ambient environment with an epoxy-cemented cover slip. The shortpass edge filters are constructed on float glass with no absorption glass components. Figure 1 illustrates an example of a shortpass edge filter that is blocking (reflecting) from 750-975 nm and transmitting down to 475 nm. This filter is composed of 41 layers, and the maximum blocking is greater than optical density 5. Measurements of blocking are limited to approximately optical density 5 (0.001% transmission) due to spectrophotometer signal-to-noise limitations.

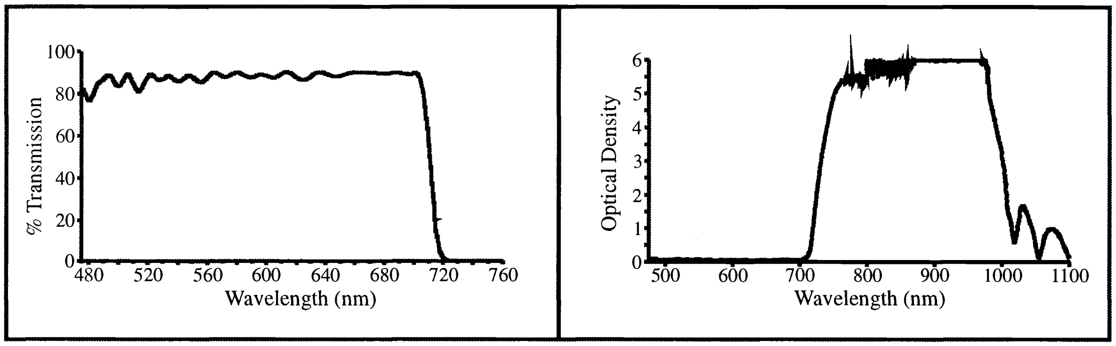


Figure 1: Transmission and blocking curves of shortpass edge filter designed for laser in 750-975 nm region.

We can calculate the level of blocking with commercially available thin film software. TFCalc3.4 (Software Spectra, Inc.) was used to model the transmission and blocking curves of the filter shown in Figure 1. The resulting theoretical curves are shown in Figure 2. We find that the maximum optical density achieved is 9.3, between the wavelengths of approximately 820 nm and 860 nm. Blocking is greater than optical density 5 from 736 nm to 970 nm. The difference in edge slope between the measured and theoretical curves is attributed to the experimental measuring conditions. All spectra presented in this work were measured by a Cary 5 UV-Vis spectrophotometer (Varian) using an f/8 beam. The theoretical curves in Figure 2 were calculated using a perfectly collimated f/∞ beam. The effect of an increase in the incident angle on a dielectric interference filter coating is a shift in the transmitted spectrum to shorter wavelengths. When a slightly divergent beam is used to measure a transmission spectrum, the resulting spectrum is an average of the angles of incidence contained within that incident beam.

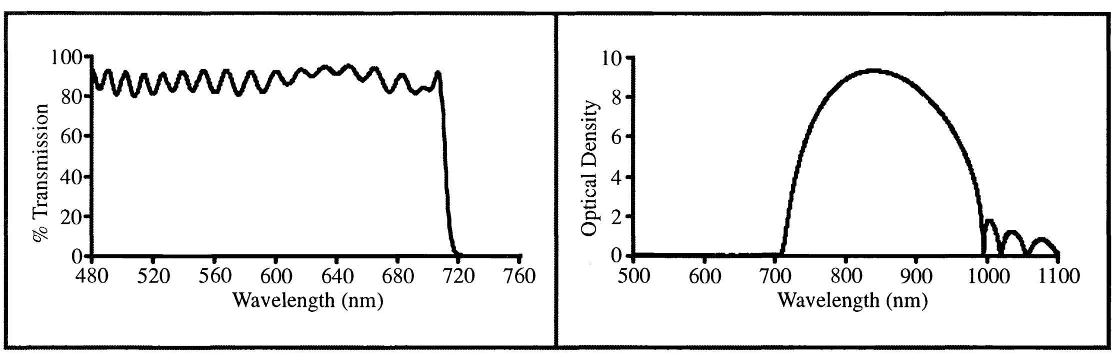


Figure 2: Theoretical transmission and blocking curves for the shortpass filter shown in Figure 1.

Using the same materials it is possible to fabricate a filter that is blocking at lower wavelengths and transmitting to 400 nm. Figure 3 shows the transmission and blocking curves of a shortpass edge filter with 45 layers. Blocking is greater than optical density 5 in the 650-750 nm wavelength range. In this case the absorption edge of ZnS determines the position of the cut-off edge of the filter, at approximately 390 nm. In order to achieve transmission below 400 nm, it would be necessary to use a different material with an absorption edge further into the UV. Hafnium oxide has been shown to be a good high index material for use in the UV[8], with an absorption edge at approximately 215 nm.

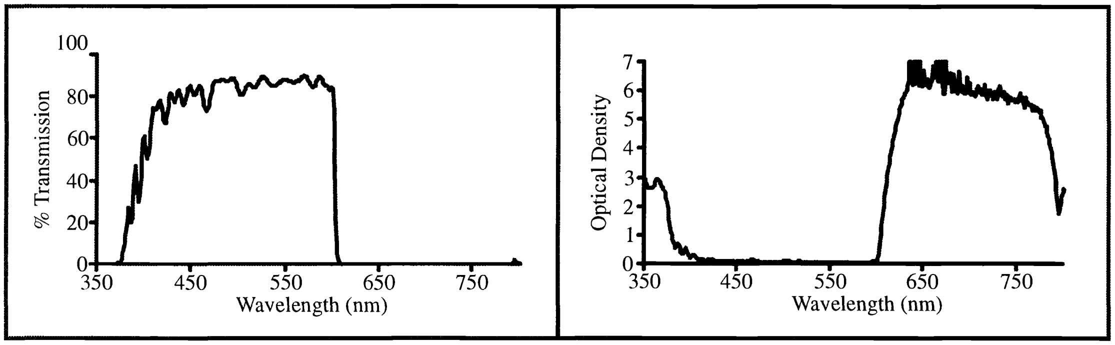


Figure 3: Transmission and blocking curves of shortpass edge filter designed for laser in 650-750 nm region.

## 3. BANDPASS FILTERS

Bandpass filters with high transmission, steep slopes, and deep out-of-band blocking are made by combining Alpha shortpass and longpass edge filters. The center wavelength position of the filter is determined by the placement of the edges. The steepness of each edge may be independently controlled by adjusting the number of layers in the longpass and shortpass component filters. The transmission and blocking curves of such a filter are shown in Figure 4. The center wavelength of the pass band is 468 nm, and the bandwidth (full width at half maximum) is 40 nm. Peak transmission is approximately 80%, and out-of-band blocking is better than optical density 6. This filter is an immersed coating consisting of approximately 100 alternating layers of ZnS and cryolite. Extra blocking in the 700-1100 nm region is provided by a 2 mm thickness of BGG22 absorption glass. A bandpass filter will transmit the fluorescence signal of interest and reject the scattered laser light along with all other interfering wavelengths.

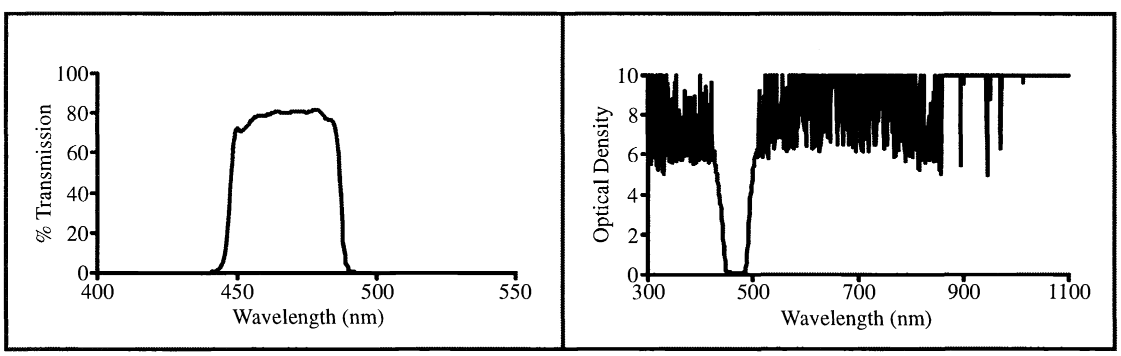


Figure 4: Transmission and blocking curves of bandpass filter centered at 468 nm.

## 4. DICHROIC FILTERS

A dichroic mirror for multiphoton fluorescence microscopy applications is illustrated in Figure 5. This filter is designed for use at a 45° angle of incidence to reflect the laser line and transmit the fluorescence signal. It is highly reflecting at wavelengths longer than 750 nm, and highly transmitting down to approximately 400 nm. This filter is an exposed coating composed of $TiO_2$ and $SiO_2$, which are hard oxide coatings. Since deep blocking is not required, a standard quarter wave stack design is used. A hard oxide antireflection (AR) coating has been applied to the uncoated side of the substrate to boost visible transmission. To insure minimal autofluorescence, the filter is deposited on a fused silica substrate. By changing the deposition monitor wavelength it is possible to shift the edge position to accommodate other laser lines. However, the absorption edge of titania is located at approximately 400 nm. In order to achieve transmission further into the UV, a material with an absorption edge lower than that of $TiO_2$ must be used.

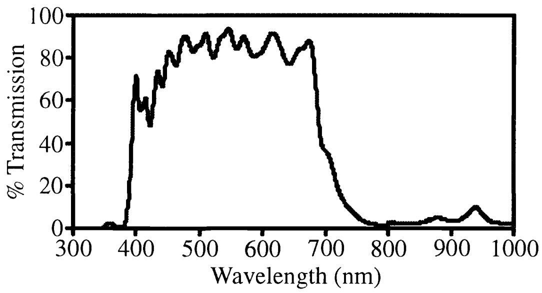


Figure 5: Transmission curve of dichroic mirror measured at 45° angle of incidence.

## 5. SUMMARY

A new deposition technology has been applied to the production of shortpass edge and bandpass filters for multiphoton fluorescence applications. This technology allows for the deposition of a large number of dielectric layers, so deep blocking of the scattered laser light is achieved. The shortpass edge filters have wide regions of transmission and approximately 100 nm wide regions of blocking, providing flexibility with tunable laser sources. A bandpass filter with high transmission in the pass band and deep out-of-band blocking has been presented. This would be ideal for applications in which the laser excitation wavelength is fixed and there is only one fluorescence signal of interest. An example of a dichroic mirror with high reflection throughout the near infrared and high transmission throughout the visible has been presented for microscopy applications.

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