Comparison of “Burn-out” Performance and Reliability of Optical Fluorescence Filters

Document Description

This document describes details of reliability testing done on Semrock’s hard-coated BrightLine® optical fluorescence filters as compared to fluorescence filters made using common manufacturing methods: soft coatings and/or absorbing substrate glass. The testing includes exposure to an intense, continuous light source to determine robustness of the filters to “burn-out,” as well as exposure to extreme environmental conditions over an extended period of time.

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1. Introduction

Tests were performed to evaluate and compare the effects of conventional arc lamp illumination on Semrock's BrightLine® hard-coated fluorescence filters with the effects on soft-coated and absorbing glass filters of a leading competitor.

Fluorescence filters that transmit light in the visible and near-infrared wavelength ranges are frequently manufactured using so-called "soft coating" technology. Typically additional blocking is provided in these filters by one or more absorbing glass substrates as well. Further, fluorescence excitation filters that must transmit at deep-blue or ultraviolet wavelengths are commonly manufactured using a combination of absorbing glass substrates. For both methods, multiple substrates are laminated together using optical adhesives.

By contrast, Semrock manufactures all of its optical fluorescence filters using a "hard-coating" technology called Ion Beam Sputtering (IBS). All filters are comprised of hard thin-films made of oxides of refractory materials and coated on a single glass substrate – so no absorbing glass and no lamination are necessary.

The purpose of the testing performed here is to compare the reliability performance of Semrock's hard-coated optical filters to that of fluorescence filters manufactured using the soft-coated and absorbing glass technology. Both continuous irradiation by lamps similar to those used in fluorescence imaging, and exposure to harsh environmental conditions are explored.

1.a. Testing Overview

The testing performed and reported in this document falls into two categories: optical damage testing and environmental testing. Optical damage testing determines the ability of a component under test to withstand intense optical irradiation for prolonged periods of time. Here we use a continuous wave (cw) optical source (an intense xenon arc lamp) to closely simulate the conditions used in fluorescence imaging instrumentation. Environmental testing exposes the ability of a component under test to withstand changes and/or extreme conditions in its ambient environmental conditions described by temperature and humidity. The environmental conditions tested are taken from accepted standards set forth in the MIL-STD-810F document.

1.b. Optical damage testing conditions

Optical damage tests were performed using an EG&G Model Xenon 300mxt arc-lamp power supply with a Perkin Elmer PE300BUV 300-Watt Cermax Xe arc lamp bulb [1]. This bulb features a parabolic reflector to focus the output light. The continuous source was collimated using a 50 mm focal length fused silica lens. An Ophir thermopile detector head was used to monitor the transmitted power at the position of the filter under test.

For the high-intensity testing of UV filters, the filters were first subjected to an initial 24-hour burst of 15 W of total power over a 15 mm diameter aperture, corresponding to an approximately uniform intensity of 8.5 W/cm^2, followed by a subsequent continuous dose of 2.5 W, corresponding to 1.4 W/cm^2. For the rest of the optical damage tests, a continuous dose of 6.0 W over a 25 mm diameter aperture was used, corresponding to an intensity of 1.2 W/cm^2.

Table 1 lists the filters that were tested, including the manufacturer, part number, and optical coating technology used to make the filter. Samples named "Other" are catalog filters produced by a leading manufacturer of soft-coated fluorescence filters.

Table 1: Description of filters used for optical damage testing.

Sample Name	Filter Type	Part Number	Technology
Semrock DAPI A	Excitation filter	FF01-377/50-25	IBS hard coating
Other DAPI A	Excitation filter	D350/50x	Laminated absorbing glass
Semrock DAPI B	Excitation filter	FF01-377/50-25	IBS hard coating
Other DAPI B	Excitation filter	D350/50x	Laminated absorbing glass
Other DAPI C	Excitation filter	D350/50x	Laminated absorbing glass
Semrock FITC A	Excitation filter	FF01-482/35-25	IBS hard coating
Other GFP A	Excitation filter	D470/40x	Laminated soft coating
Other Texas Red A	Excitation filter	HQ560/55x	Laminated soft coating

All spectral measurements were made on a Perkin Elmer Lambda 950 spectrophotometer.

1.c. Environmental testing conditions

Aggravated humidity cycling of the filters is based upon the U.S. military test specification MIL-STD-810F (507.4)[2]. The basic parameters are given in Table 2. Testing is performed in an industry-standard Thermotron environmental test chamber (model SM-8-7800).

Table 2: Details of MIL-STD-810F aggravated humidity test performed.

Document Number	Test Description	Cycling Temperature		Relative Humidity		Test Duration
		Min	Max	Min	Max	
MIL-STD-810F (507.4)[2]	Aggravated Humidity	30°C (86°F)	60°C (140°F)	85%	95%	10 x 24 hours

Table 3 lists the filters that were tested, including the manufacturer, part number, and optical coating technology used to make the filter.

Table 3: Description of filters used for environmental testing.

Sample Name	Filter Type	Part Number	Technology
Semrock DAPI C	Excitation filter	FF01-377/50-25	IBS hard coating
Other DAPI D	Excitation filter	D350/50x	Laminated absorbing glass

2. Optical damage tests

All of the tests in the section were performed using the equipment and conditions described above in Section 1.b. The only variables are the total optical power and the exposure time.

2.a. High-intensity testing of UV bandpass filters

Two UV bandpass filters were selected for this test. One was a laminated, multi-substrate, absorbing-glass catalog filter made with the conventional fluorescence filter technology (sample name "Other DAPI A"), whereas the second was a standard catalog BrightLine FF01-377/50-25 hard-coated filter made by Semrock with IBS technology (sample name "Semrock DAPI A"). Both filters are designed to be used as excitation filters for the popular UV-excited, blue-emitting fluorophore DAPI. Because of the challenge of making transmissive optical coatings at ultraviolet wavelengths, the laminated absorbing-glass DAPI exciter is still widely used today even in the highest-end DAPI filter sets from manufacturers other than Semrock.

The filters were first exposed to 15 W of total power (over a 15 mm aperture) for 1 day. The transmission spectrum for each filter was measured before and after the exposure. These are shown in Figure 1. The transmission of the "Other" sample dropped from a peak value of 50.3% to 29.1% – a 42% decrease in peak transmission. However, the transmission of the Semrock filter remained unchanged (within the 0.2% measurement repeatability of the spectrophotometer).

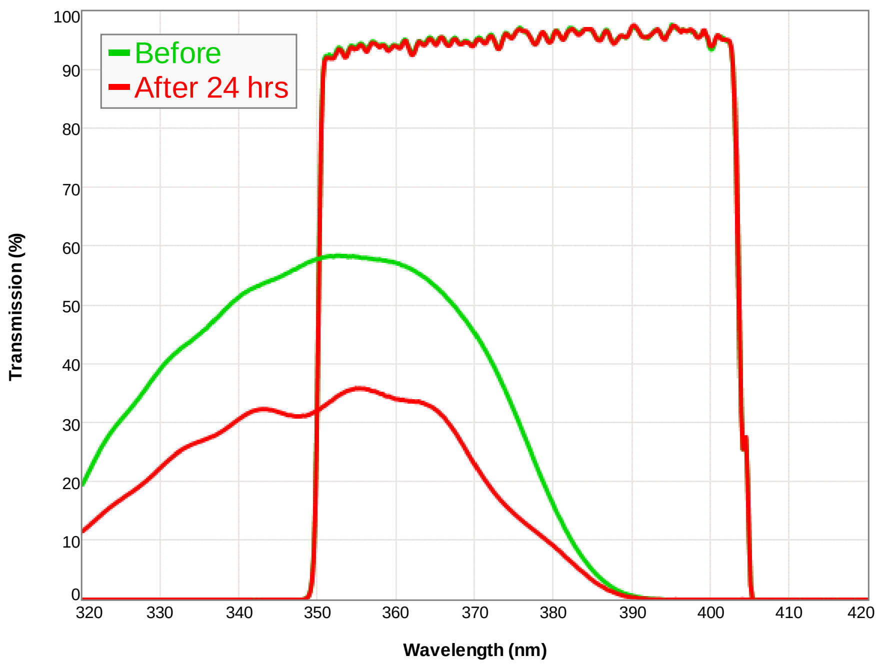

Figure 1: *Transmission spectra of "Other DAPI A" (lower left spectra) and "Semrock DAPI A" (upper right spectra) filters before (green) and after (red) exposure to 15 W of total power for 1 day.*

The appearance of the "Other" filter developed a severe apparent discoloration, visible on both sides, whereas the Semrock filter demonstrated no apparent change in appearance. After the first 24 hour exposure, the total power was reduced to 2.5 W (over a 15 mm diameter). This value approximately simulates the intensity found at rear-most location that an excitation filter might be positioned in a typical fluorescence microscope with a 100 W Hg or Xe arc lamp source (rear-most slider position or excitation filter wheel location). The filters were exposed for a prolonged period of time, and periodically examined and measured spectrally. After about 300 hours of exposure (just over 12 days), the “Other” filter developed a severe crack that propagated across the entire diameter of the filter and through both sides of the filter. The Semrock filter showed no signs of degraded appearance, even after over 1000 hours of exposure, as shown in Figure 3.

Figure 2: *Photographs of both sides of the “Other DAPI A” filter after about 300 hours (just over 12 days) of exposure to first 15 W (1 day) and then 2.5 W of total power. Note severe discoloration in the most intense region of exposure as well as a severe crack that extends across the entire filer through both sides.*

Figure 3: *Photographs of both sides of the “Semrock DAPI A” filter after over 1000 hours of exposure to first 15 W (1 day) and then 2.5 W of total power. The filter looks identical to its appearance before exposure.*

2.b. Prolonged testing of UV bandpass filters

In this test three new UV bandpass (DAPI excitation) filters were selected. Spectra were measured as a function of time. Two of the filters were laminated, multi-substrate, absorbing-glass catalog filters made with the conventional fluorescence filter technology (sample names "Other DAPI B" and "Other DAPI C"), while the third was a standard catalog BrightLine FF01-377/50-25 hard-coated filter made by Semrock with advanced IBS technology (sample name "Semrock DAPI B").

The three filters were exposed to 6.0 W of total power over a 25 mm aperture. For these experiments the beam overfilled the filter clear aperture of about 21 mm. This intensity value approximately simulates the intensity found at the rear-most location at which an excitation filter might be positioned in a typical fluorescence microscope with a 100 W Hg or Xe arc lamp source (rear-most slider position or excitation filter wheel location). The transmission spectrum for each filter was measured periodically throughout the exposure. As an example, spectra before exposure and after 120 hours (5 days) are shown in Figures 4 through 6. Note that this time period would correspond to about 3 weeks of usage on a well utilized microscope of other fluorescence instrument, assuming it is used 8 hours per day and 5 days per week.

Figures 4 and 5 show the before and after spectra of the two "Other" filters made with the conventional, absorbing-glass technology. Clearly, even with standard fluorescence microscope illumination conditions for an exciter filter, there is a significant reduction in the transmission of these filters after only 5 days of exposure. Figure 6 shows the before and after spectra of the Semrock hard-coated filter, demonstrating that there is essentially no change in the transmission. Note that there appears to be a very slight reduction in transmission on the short-wavelength side of the passband (~ 1%); however, as the exposure continued the transmission of subsequent spectra actually showed an increase again (see Figure 7), and thus this initial decrease is believed to be a measurement artifact, possibly resulting from contamination on the filter that was later cleaned (all filters were cleaned periodically when any dirt or contamination was apparent).

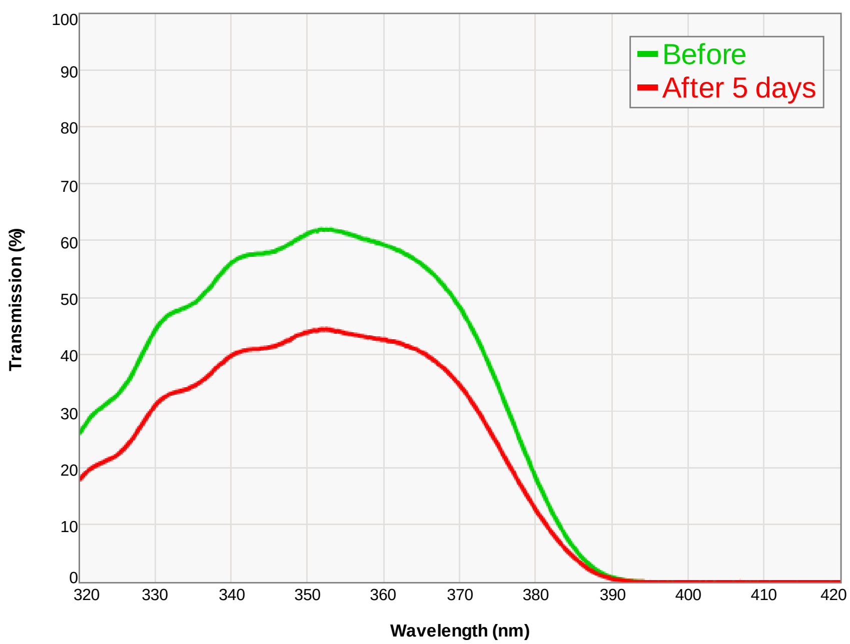

Figure 4: *Measured transmission spectra of the "Other DAPI B" filter before exposure and after 5 days.*

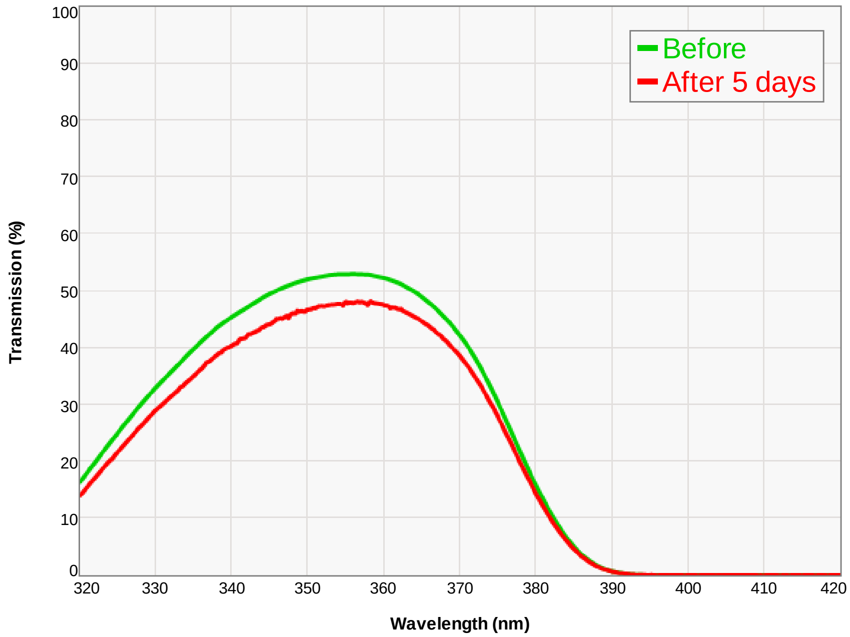

Figure 5: *Measured transmission spectra of the "Other DAPI C" filter before exposure and after 5 days.*

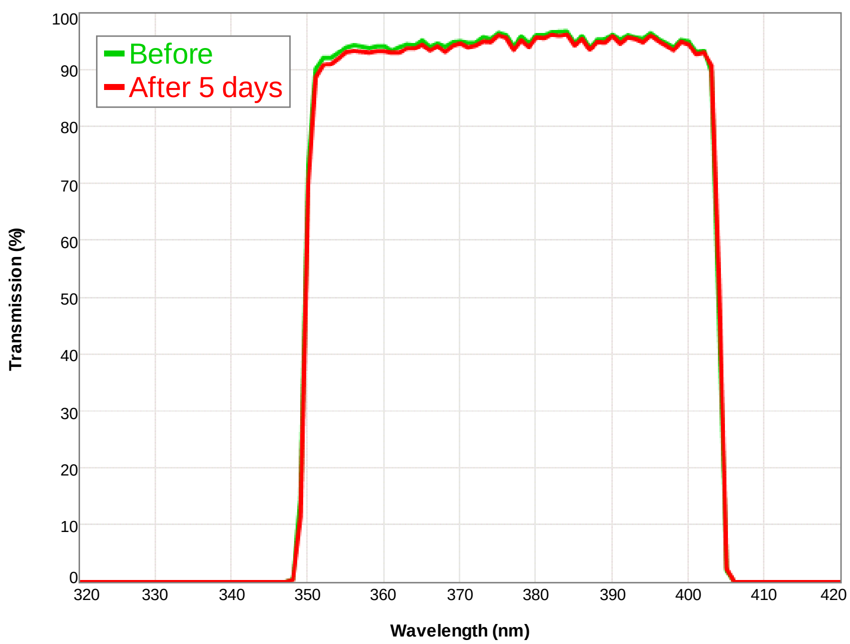

Figure 6: *Measured transmission spectra of the "Semrock DAPI B" filter before exposure and after 5 days. The slight decrease in transmission is believed to be a measurement artifact, as it later increased again.*

Figure 7 summarizes the results with a graph of the average transmission over the passband of each filter as a function of time over a period of 300 hours (12.5 days). To calculate average transmission, the passband is taken to be the wavelength interval between 1.005 times the short-wavelength half-maximum edge point and 0.995 times the long-wavelength half-maximum edge point. In other words, the passband bandwidth is 1% of the center wavelength narrower than the full-width-at-half-maximum (FWHM). While the rate of decrease in transmission as a function of time for the "Other" filters slows down, the decrease does continue to occur.

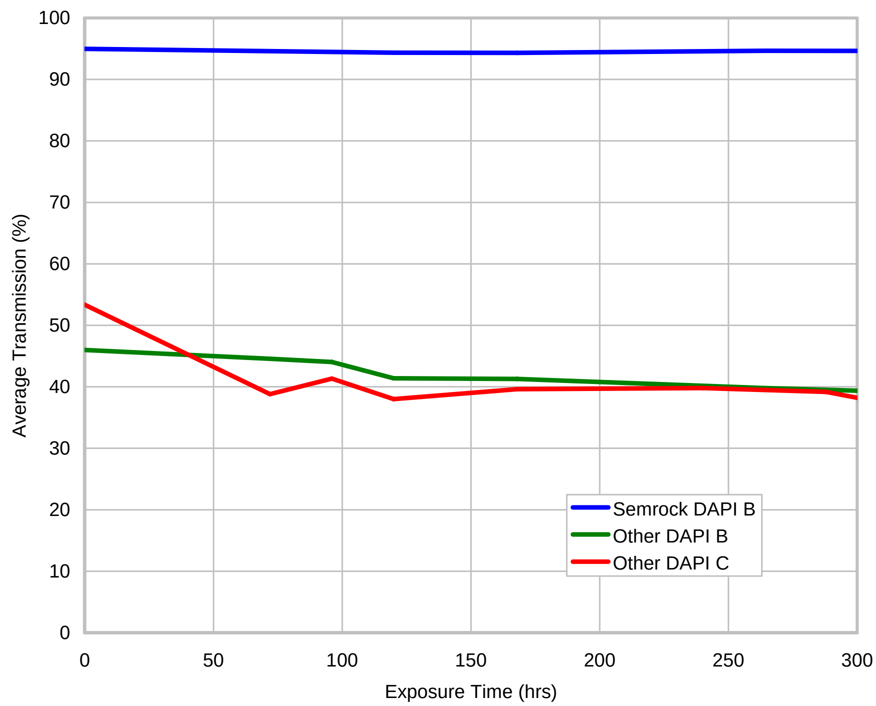

Figure 7: *Measured average transmission over the passband of the "Other DAPI B," "Other DAPI C," and "Semrock DAPI B" filters. The fluctuations in transmission of the "Other" filters are believed to be real, and do not result merely from measurement error.*

Figures 8 to 10 show photographs of the filters measured in this section after the prolonged exposure. Figure 8 shows images of one side of the "Other DAPI B" filter before and after about 500 hours of exposure. The apparent "burning" and several large surface point defects are real, and are a direct result of the exposure (not handling related). Figure 9 shows the opposite side of the "Other DAPI C" filter after exposure of about 300 hours. At 300 hours the transmission began to increase again substantially, almost returning to the starting value of just over 50%. This behavior is explained by the fact that the filter developed a crack which allows light of all wavelengths to leak through the filter. When held up to a standard room light, visible light can be seen clearly through the filter in the vicinity of the crack. Because this filter failed catastrophically, exposure was terminated at 300 hours. The filter also shows signs of "burning" near the edges of the filter.

Figure 10 shows images of both sides of the "Semrock DAPI B" filter after 500 hours of exposure. The filter showed no signs of degradation.

Figure 8: *Photographs of the "Other DAPI B" filter before and after about 500 hours of exposure to 6 W of total power over a 25 mm diameter. The dig defects and apparent "burning" are real.*

Figure 9: *Photograph of the "Other DAPI C" filter after about 300 hours of exposure to 6 W of total power over a 25 mm diameter. At this point it developed a severe crack which allows light to leak through the filter.*

Figure 10: *Photographs of both sides of the "Semrock DAPI B" filter after about 500 hours of exposure to 6W of total power over a 25 mm diameter. The filter showed no signs of degradation.*

2.c. Prolonged testing of visible bandpass filters

In this test three new visible bandpass filters were selected. Spectra were measured as a function of time. Two of the filters were laminated, multi-substrate, soft-coated catalog filters made with the conventional fluorescence filter technology (sample names “Other GFP A” and “Other Texas Red A”), while the third was a standard catalog BrightLine FF01-482/35-25 hard-coated filter made by Semrock with advanced IBS technology (sample name “Semrock FITC A”). The soft-coated filters are exciters optimized for green fluorescent protein (GFP) and the fluorophore Texas Red, while the Semrock filter is an exciter optimized for fluorescein (or FITC).

The three filters were exposed to 6.0 W of total power over a 25 mm aperture. For these experiments the beam overfilled the filter clear aperture of about 21 mm. This intensity value approximately simulates the intensity found at the rear-most location at which an excitation filter might be positioned in a typical fluorescence microscope with a 100 W Hg or Xe arc lamp source (rear-most slider position or excitation filter wheel location). The transmission spectrum for each filter was measured periodically throughout the exposure. Spectra before exposure and after 120 hours (5 days) are shown in Figures 11 through 13.

Figures 11 and 12 show before and after spectra of the two “Other” filters made with the conventional, soft-coating technology. Clearly, *even with standard fluorescence microscope illumination conditions* for an exciter filter, there is a significant reduction in the transmission of these filters after only 5 days of exposure. Figure 13 shows the before and after spectra of the Semrock hard-coated filter, demonstrating that there is no change in the transmission spectrum.

Figures 14 and 15 summarize the changes in spectral features as a function of exposure time. Figure 14 shows the average transmission, illustrating the appreciable decrease in transmission for both of the soft-coated filters, and no change for the Semrock hard-coated filter. Figure 15 shows the shift of the short-wavelength passband edge with exposure time for the three filters plus the Semrock DAPI exciter from section 2.a. While the soft-coated filters exhibit shifts as high as 0.7% of the edge wavelength (over 3 nm), the Semrock hard-coated filter edges shift less than about 0.2 nm, which is approximately the measurement uncertainty.

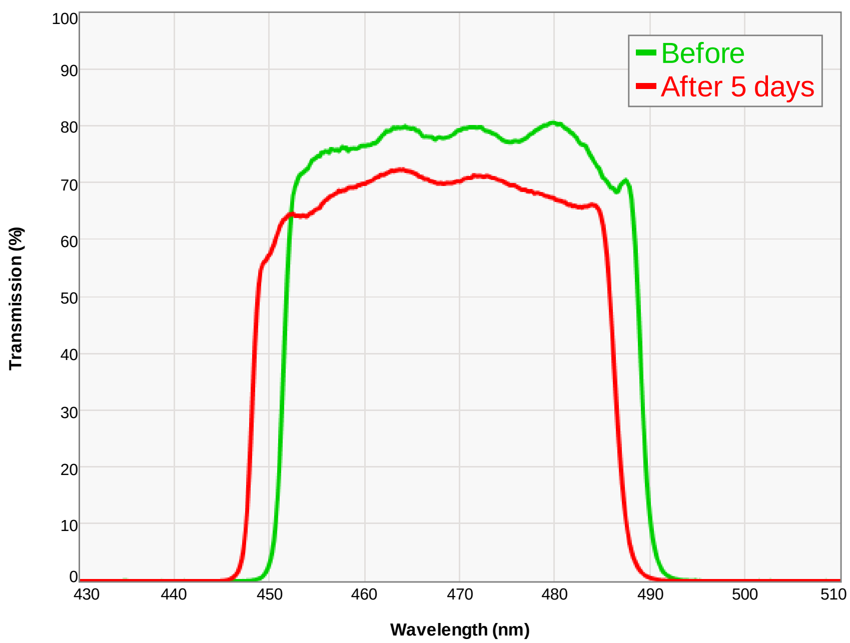

Figure 11: *Measured transmission spectra of the “Other GFP A” filter before exposure and after 5 days. Note the significant loss of transmission and blue shift of the spectrum.*

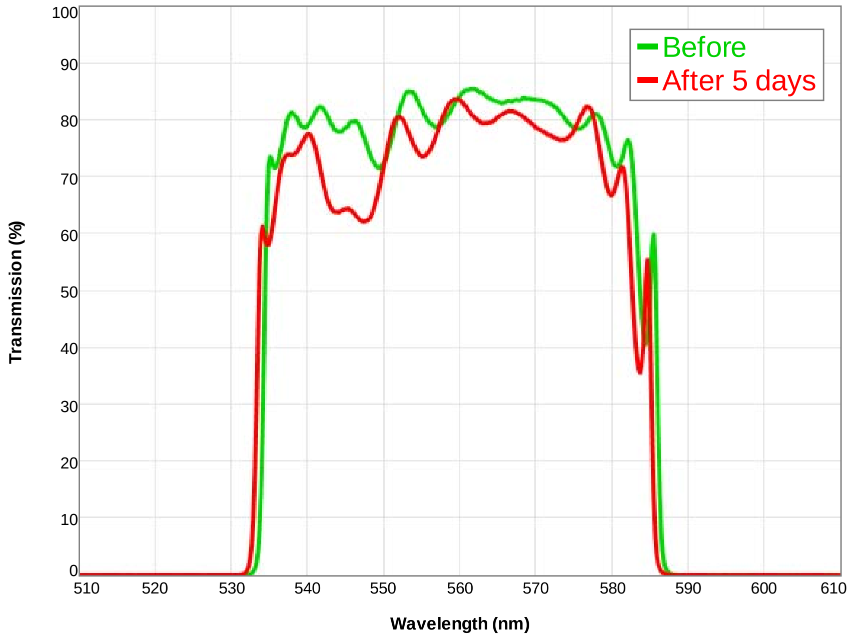

Figure 12: *Measured transmission spectra of the "Other Texas Red A" filter before exposure and after 5 days. This filter exhibited a blue shift and a significant change in the passband ripple, resulting in a loss of average transmission.*

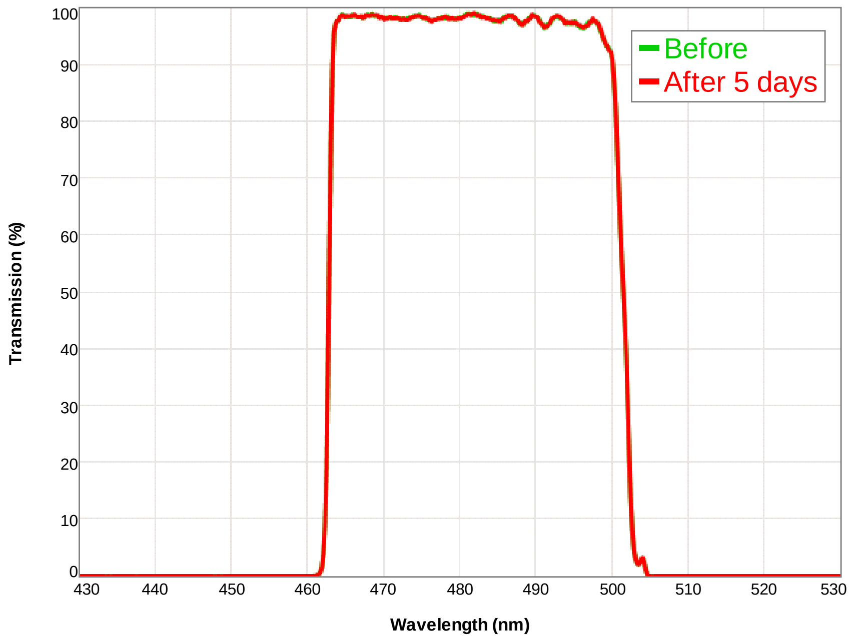

Figure 13: *Measured transmission spectra of the "Semrock FITC A" filter before exposure and after 5 days. The filter exhibited no measurable change.*

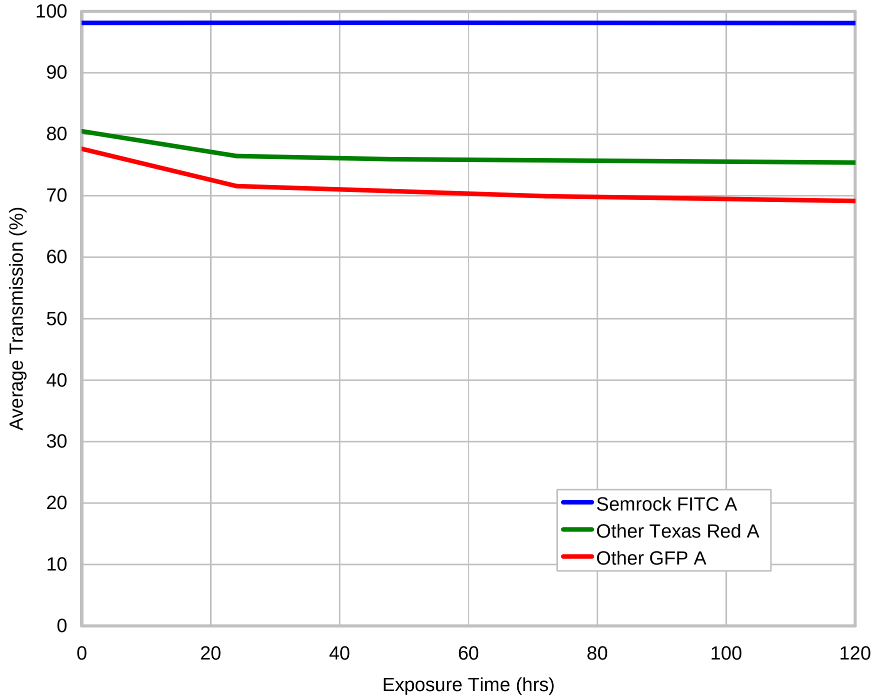

Figure 14: *Measured average transmission over the passband of the "Other GFP A," "Other Texas Red A," and "Semrock FITC A" filters. The Semrock filter shows no change.*

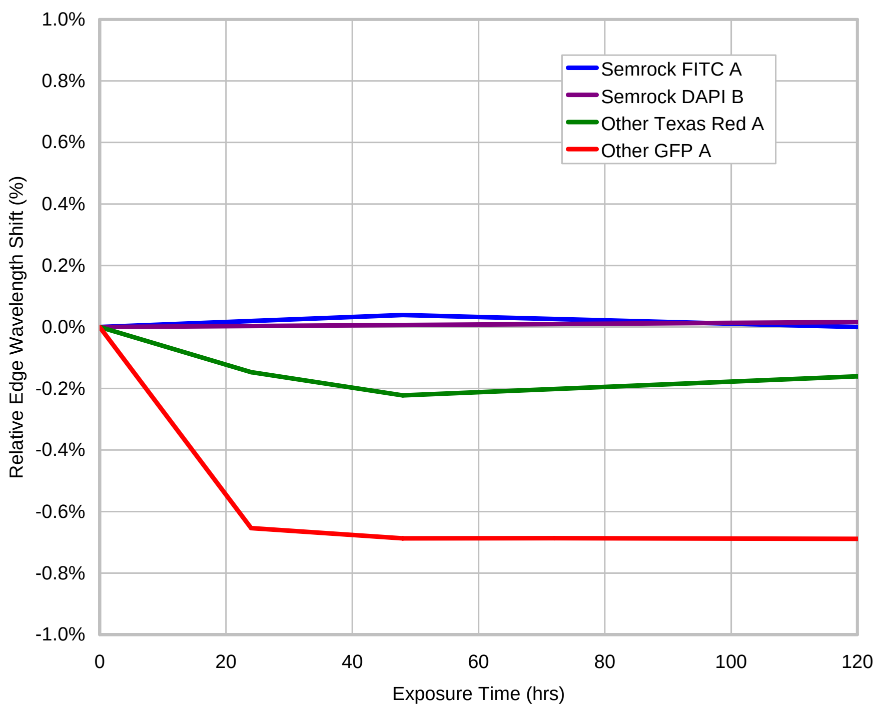

Figure 15: *Measured wavelength shift of the short-wavelength edge on the "Other GFP A," "Other Texas Red A," "Semrock FITC A," and "Semrock DAPI B" filters.*

None of the filters in this test exhibited appreciable changes in the cosmetic appearance, so no photographs are shown.

3. Environmental test

In this test two new UV bandpass (DAPI excitation) filters were selected and then subjected to harsh environmental testing as outlined in Section 1.c. One of the filters was a laminated, multi-substrate, absorbing-glass catalog filter made with the conventional fluorescence filter technology (sample name "Other DAPI D"), while the other was a BrightLine FF01-377/50-25 hard-coated catalog filter made by Semrock with advanced IBS technology on a single substrate (sample name "Semrock DAPI C").

The two filters were photographed and measured in the spectrophotometer before and after the 10-cycle test. The results are shown in Figures 16 and 17.

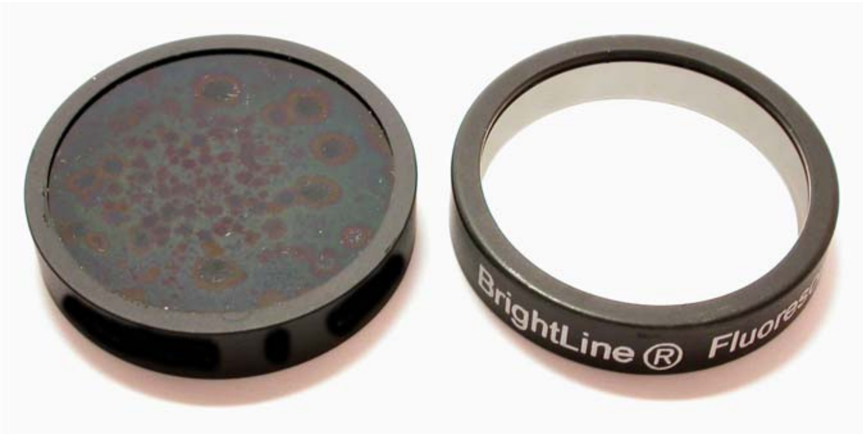

Figure 16: *Photographs of the filters "Other DAPI D" (left) and "Semrock DAPI C" (right) after 10 x 24 hour cycles of aggravated humidity testing according to the MIL-STD-810F. Note the significant degradation of the "Other" filter. The Semrock filter showed no apparent change in appearance.*

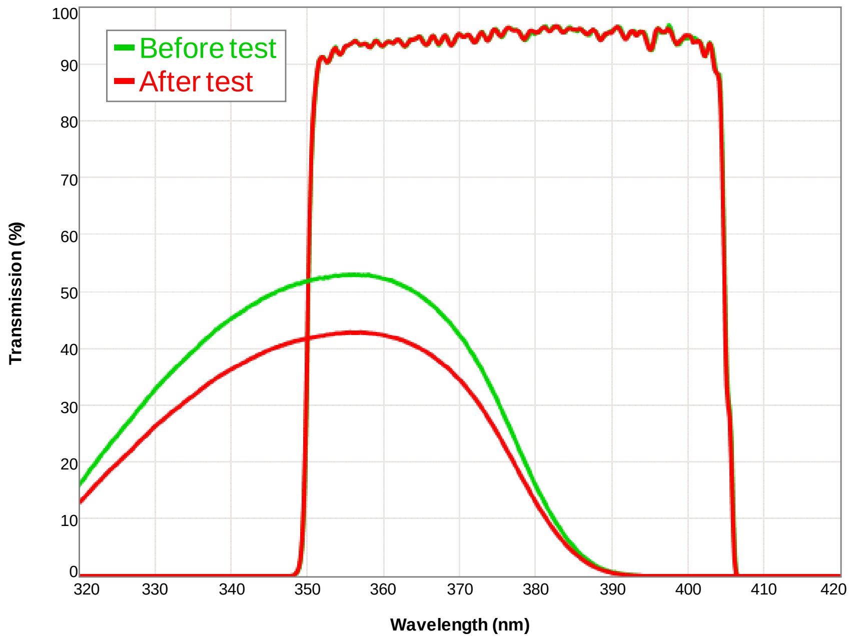

Figure 17: *Transmission spectra of "Other DAPI D" (lower left spectra) and "Semrock DAPI C" (upper right spectra) filters before (green) and after (red) 10 x 24 hour cycles of aggravated humidity testing according to the MIL-STD-810F. Note the significant decrease in transmission of the "Other" filter. The Semrock filter did not exhibit any measurable spectral change.*

4. Conclusions

This document describes details of reliability testing done on Semrock's hard-coated BrightLine® optical fluorescence filters as compared to fluorescence filters made with using the more common manufacturing approaches: soft coatings and/or absorbing substrate glass. Results show that Semrock's optical filters are not affected by optical irradiation equivalent to and even significantly exceeding that found in standard fluorescence instrumentation, whereas the conventional filters show appreciable optical damage that results in loss of transmission and in some cases catastrophic failure leading to compromised blocking. Furthermore, an absorbing glass type filter exhibited severe degradation when exposed to aggravated humidity environmental cycling, whereas the hard-coated Semrock filter was not affected by these conditions.

5. References

1. Detailed data sheet for Perkin-Elmer PE300BUV 300-Watt Cermax Xe arc lamp bulb with parabolic reflector: http://optoelectronics.perkinelmer.com/content/RelatedLinks/TypeBParabolic.pdf.

2. Department of Defense, "Test Method Standard for Environmental Engineering Considerations and Laboratory Tests," MIL-STD-810F, January 2000.