

Optical Filters: Blocking

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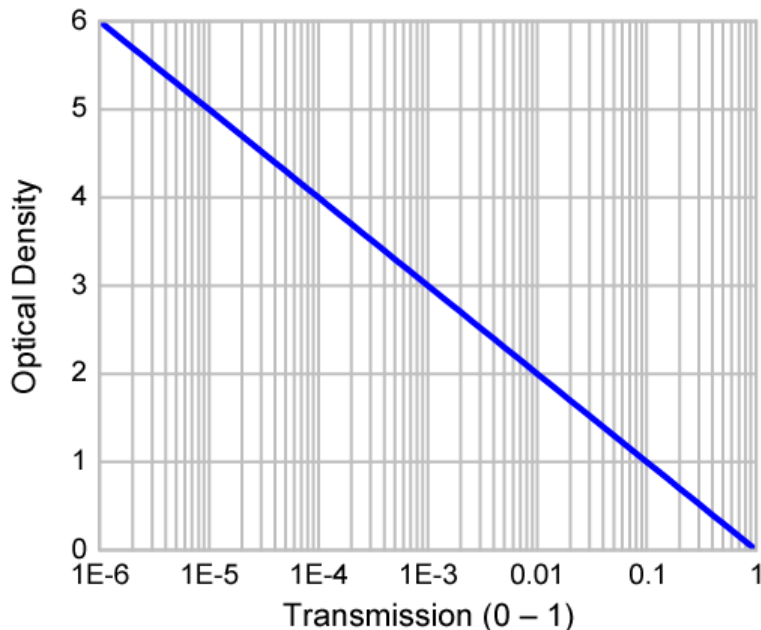
Working with Optical Density (OD)

$$OD = -\log_{10}(T)$$

$$T = 10^{-OD}$$

OD = Optical Density

T = Transmission 0 → 1



Transmission	OD
1	0
0.5	0.3
0.25	0.6
0.2	0.7
0.125	0.9
0.1	1
0.05	1.3
0.025	1.6
0.02	1.7
0.0125	1.9
0.01	2
0.005	2.3
0.0025	2.6
0.002	2.7
0.00125	2.9
0.001	3

The "1" Rule: $T = 1 \rightarrow OD = 0$

The "× 2" Rule: $T \times 2 \rightarrow OD - 0.3$

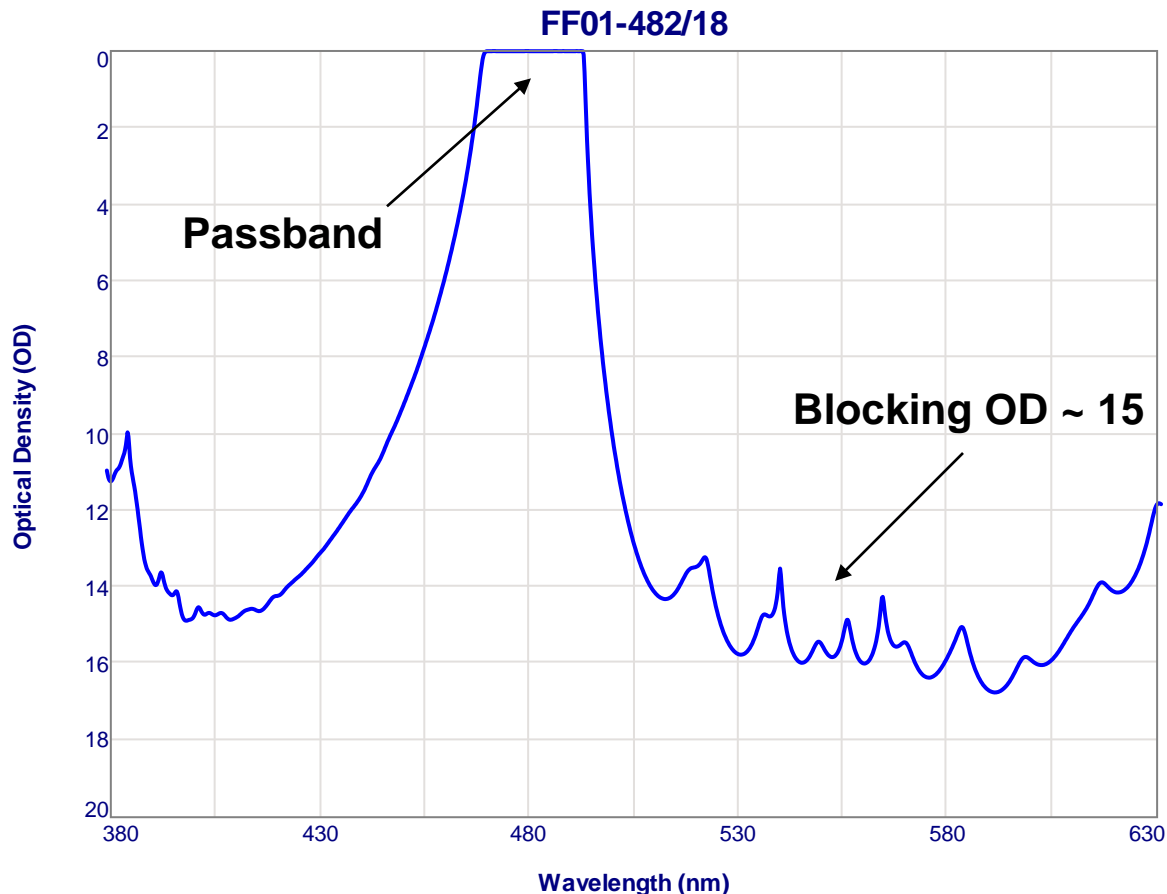
The "÷ 2" Rule: $T \div 2 \rightarrow OD + 0.3$

The "× 10" Rule: $T \times 10 \rightarrow OD - 1$

The "÷ 10" Rule: $T \div 10 \rightarrow OD + 1$

How much blocking / optical density is enough?

- First, it is important to recognize that the actual optical density (at any given wavelength) is rarely as high as the design spectrum indicates



- This design spectrum indicates blocking of OD ~ 15
- Actual blocking is more likely OD ~ 8 – 10
- Could be even lower if there are observable “pinholes” or substantial defects in the mounting of the filter

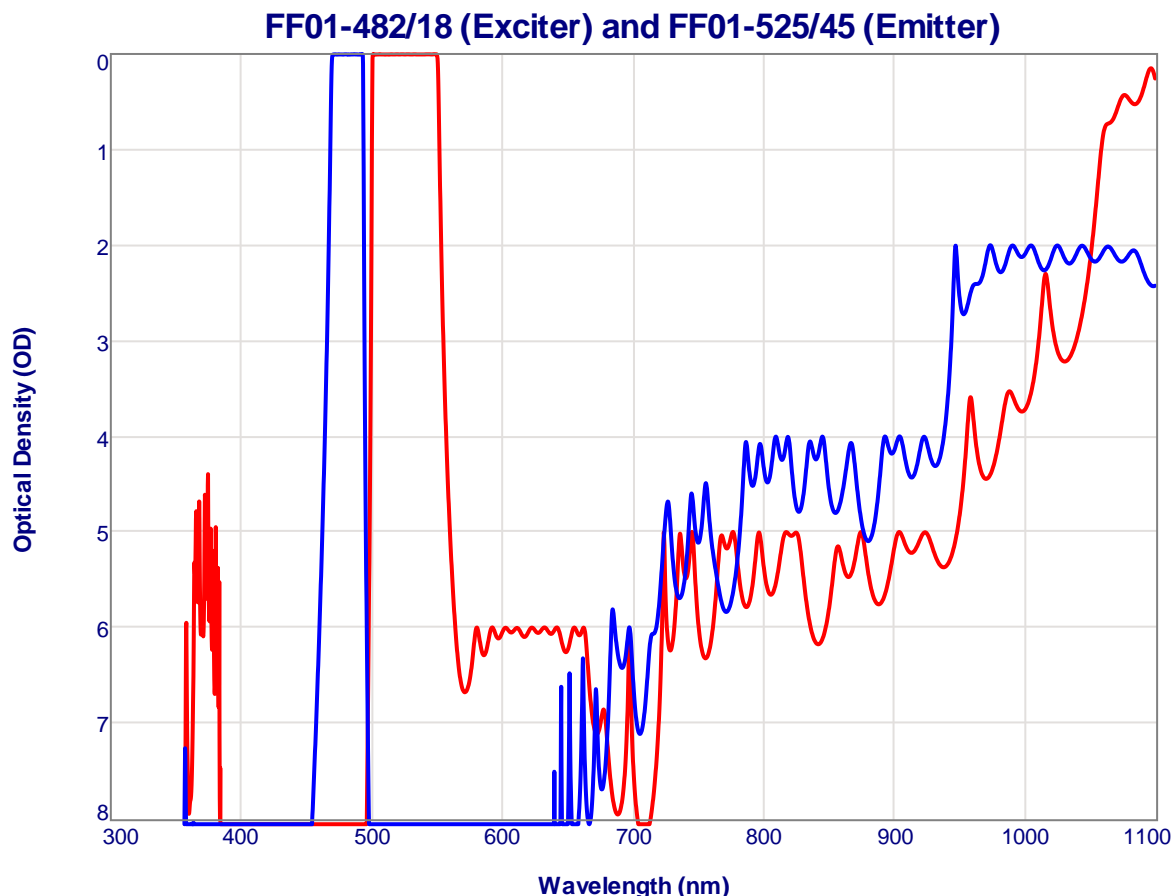
So how much blocking is necessary?

- It depends on your instrument and your sample conditions!
- In this example the fluorophore concentration is high enough for the Signal to be ~ 800X larger than both Excitation Light Noise (unblocked light from the source) and Autofluorescence Noise
- Note that almost all of the Signal comes from the passband of the emitter (500 – 550 nm), whereas the Noise is integrated over a very wide range of wavelengths covering the full range of the detector (out to even 1100 nm)



Typical filter blocking (from previous example)

- The design spectra below show typical blocking levels that are optimal for most fluorescence imaging applications



- Exciter blocking
 - > OD 6 UV – 700 nm
 - >> OD 6 in Em passband
 - > OD 4 700 – 925 nm
 - > OD 2 925 – 1100 nm
- Emitter blocking
 - > OD 4 UV to Ex passband
 - >> OD 6 in Ex passband
 - > OD 6 to 700 nm
 - > OD 5 700 – 925 nm

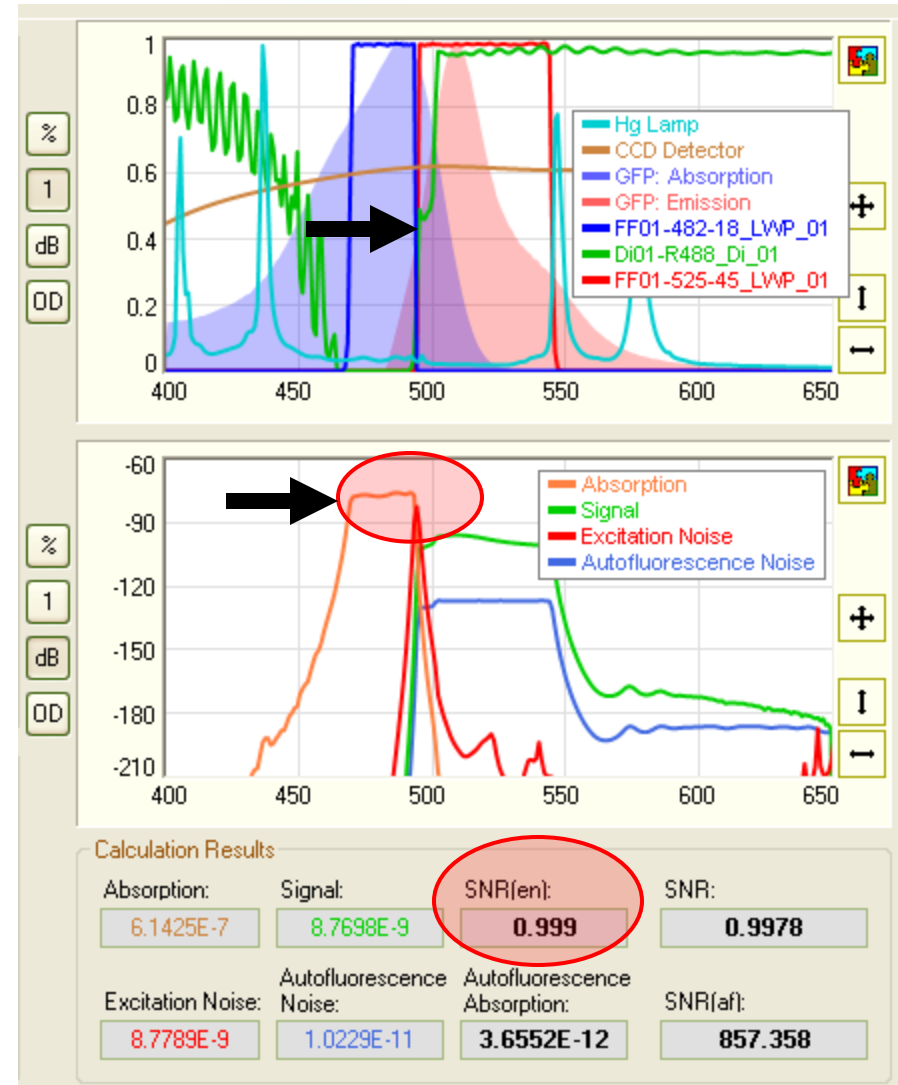
More blocking needed for very low signal levels

- In this example we assume the same light source and filters, but a much lower fluorophore (and autofluorescence) concentration
- The Signal is now actually below the Excitation Light Noise – more blocking is needed for this case!



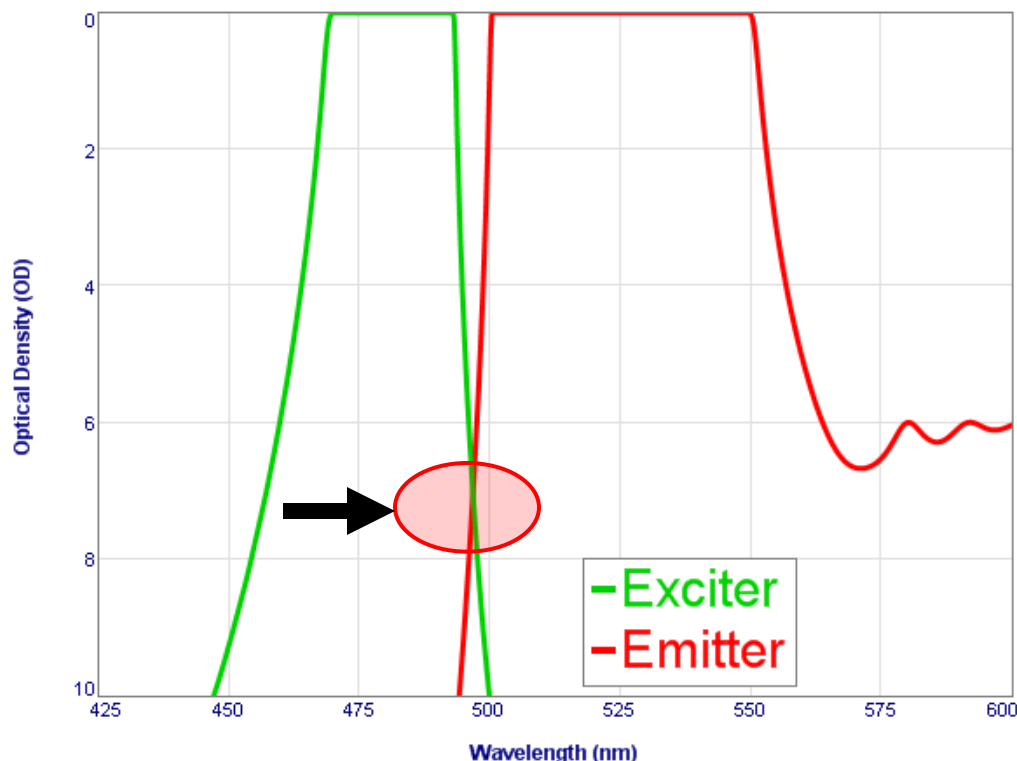
Exciter-emitter overlap also critical for low signal

- When the excitation filter and the emission filter have too much overlap, the excitation noise peak can rival or exceed the Signal in the emission band
- Overlap is especially important for low Signal levels (that is, low fluorophore concentration)
- For most cases, a combined OD of 7 – 8 is generally sufficient (guaranteed blocking for each filter of OD 3.5 – 4 at the actual crossover point)



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Thank you!