# Optical Filters: Blocking

Turan Erdogan, PhD (CTO and Co-founder)
Semrock, A Unit of IDEX Corporation

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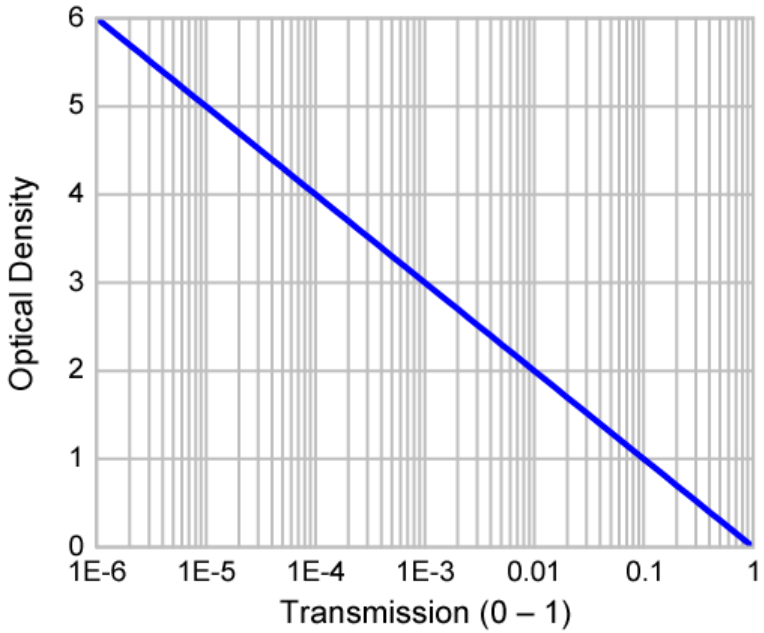
# Working with Optical Density (OD)

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

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

OD = Optical Density

T = Transmission $0 \rightarrow 1$




| 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 : $T \times 2 \rightarrow OD - 0.3$

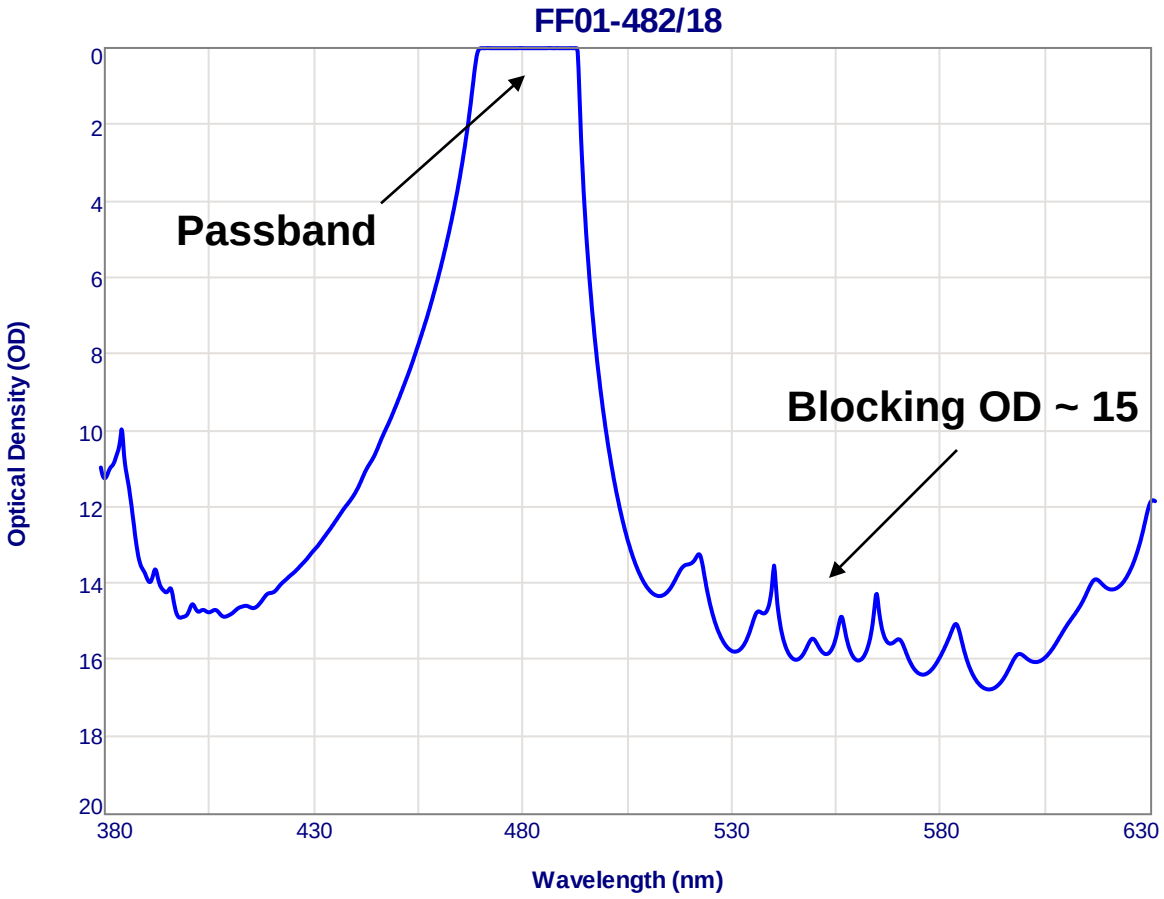
The "÷ 2" Rule : $T \div 2 \rightarrow OD + 0.3$

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?

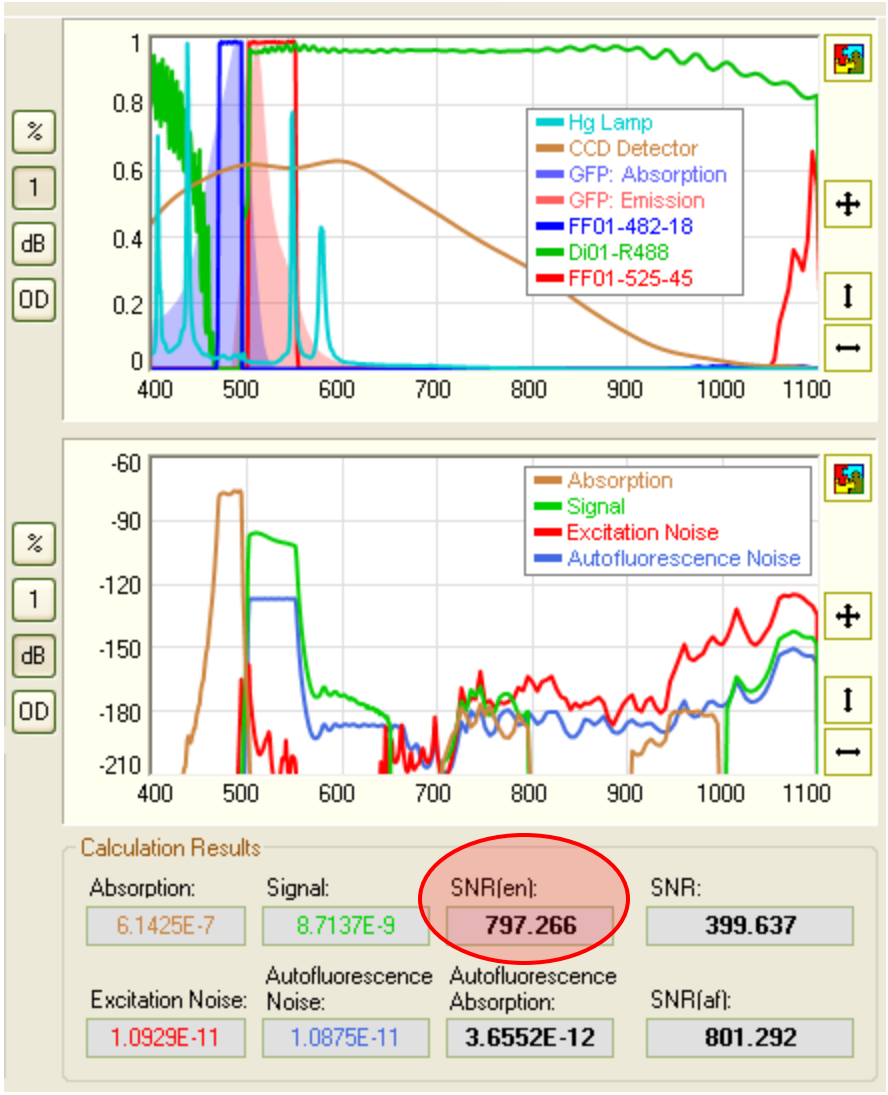
- First, it is important to recognize that the actual optical density (at any given wavelength) is rarely as high as the design spectrum indicates




- 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

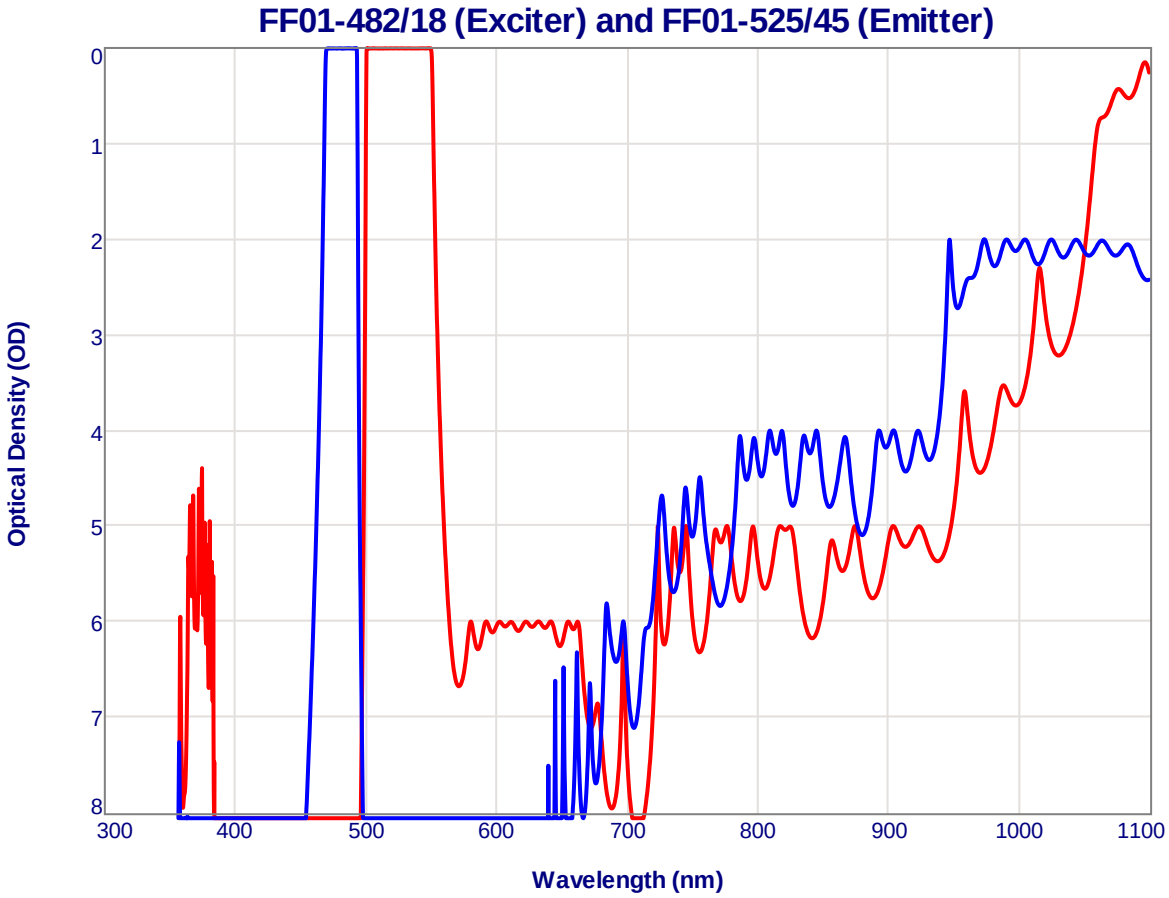
# So how much blocking is necessary?

- **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)

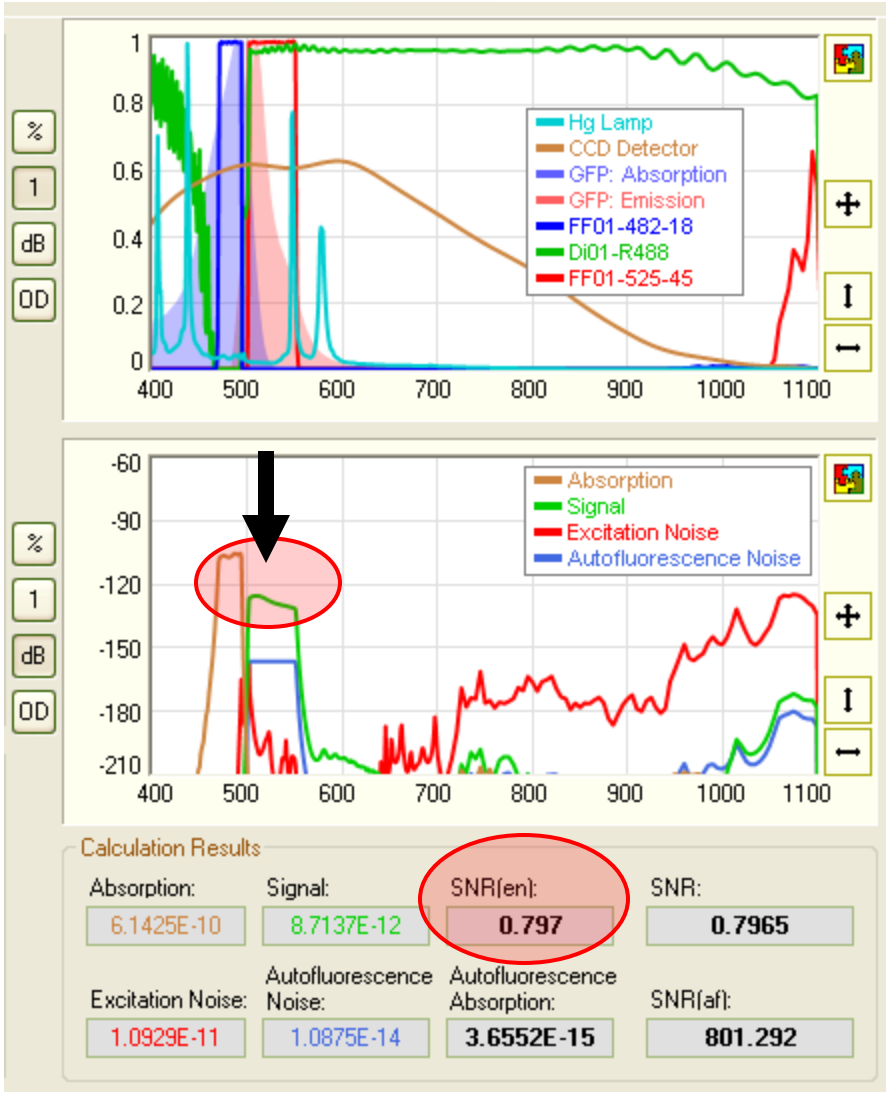
- The design spectra below show typical blocking levels that are optimal for most fluorescence imaging applications




- 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

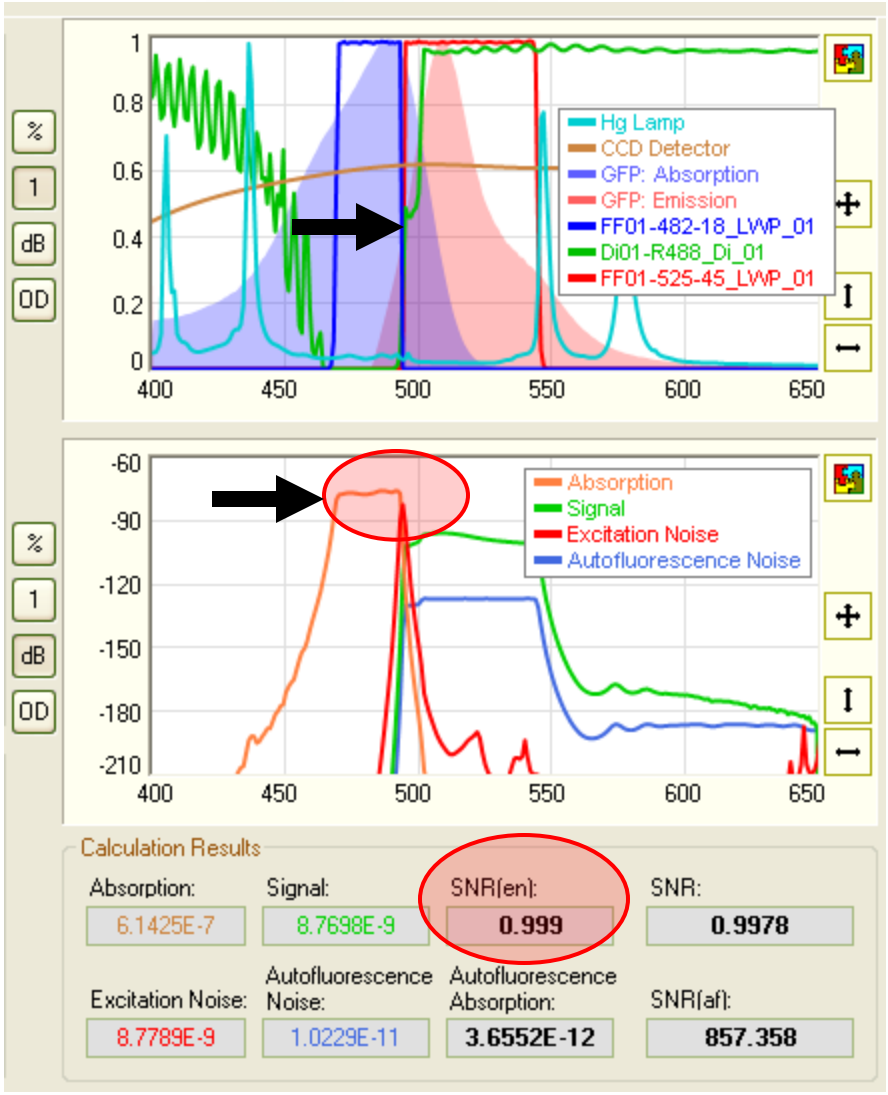
# More blocking needed for very low signal levels

- 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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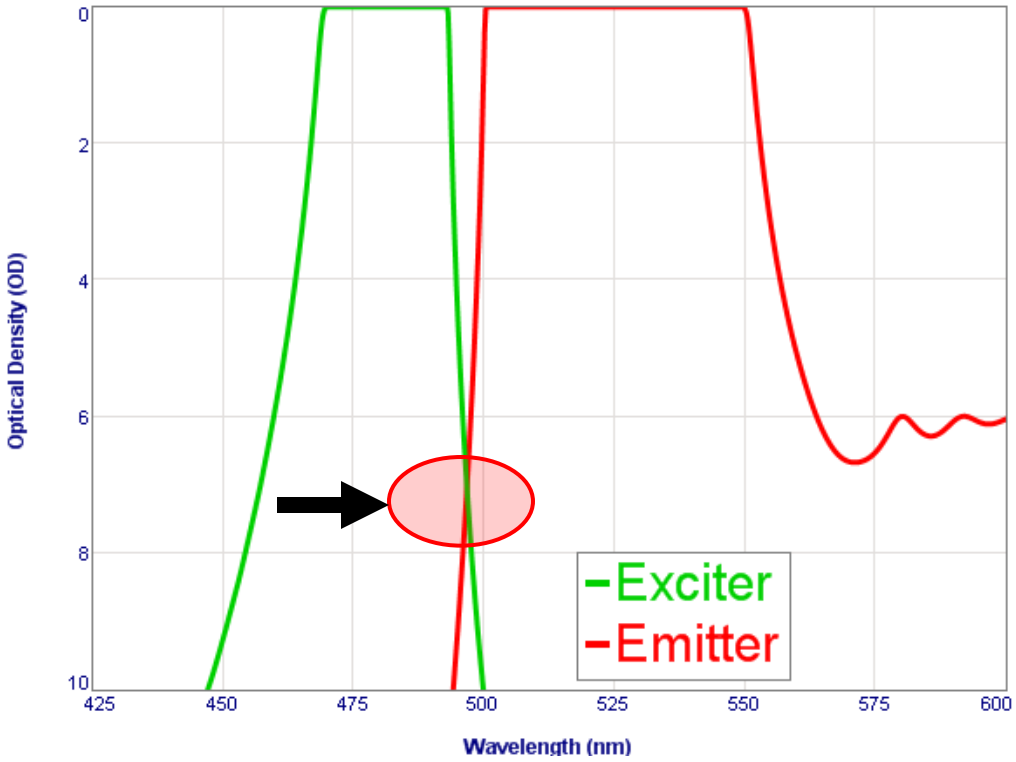

# Thank you!