Semrock A Unit of IDEX

The Standard in Optical Filters for Biotech & Analytical Instrumentation

Optical Filters:
Coherence and Combining Filters

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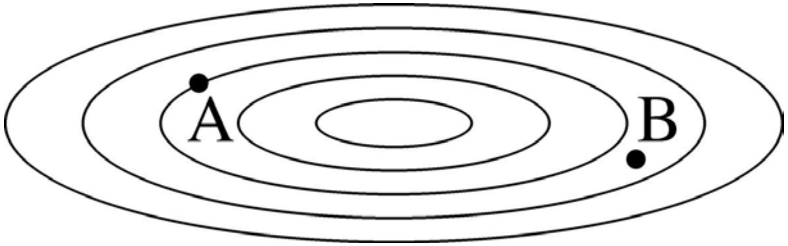
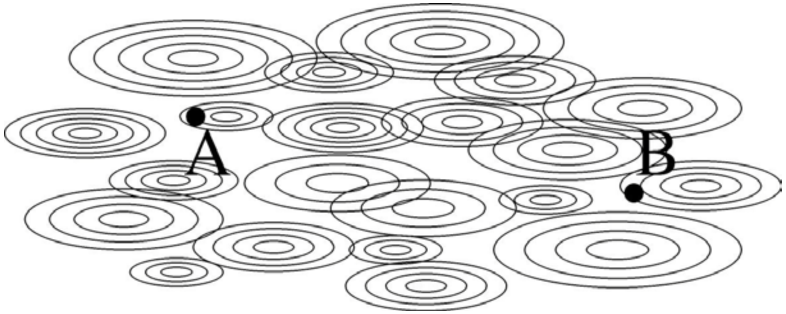
Coherence

- A measure of the correlation between the phases measured at different points on a wave
- Coherence is a property of the wave itself, but it is determined by the characteristics of the source

Coherent source: single stone thrown into a pond; the phases of the waves at points A and B are highly correlated.

Incoherent source: many random rain drops falling on a pond; the phases of the waves at points A and B are not at all correlated.

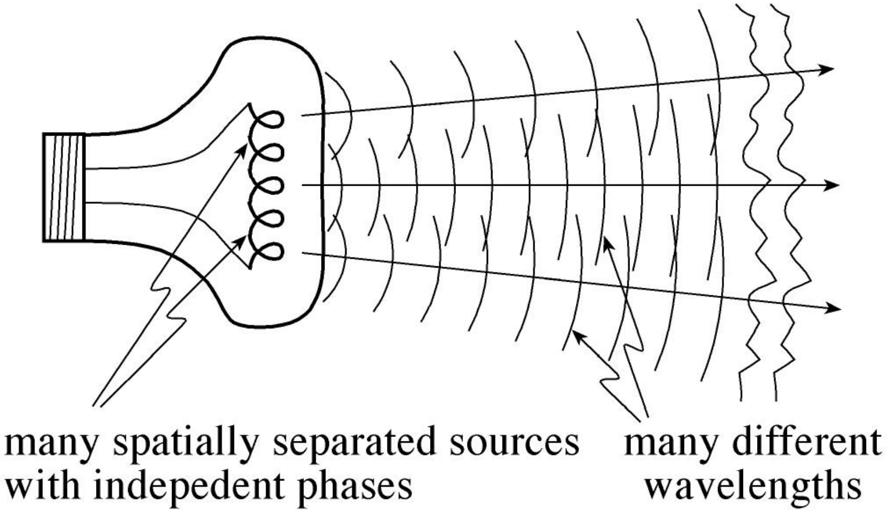




Coherence – two basic types

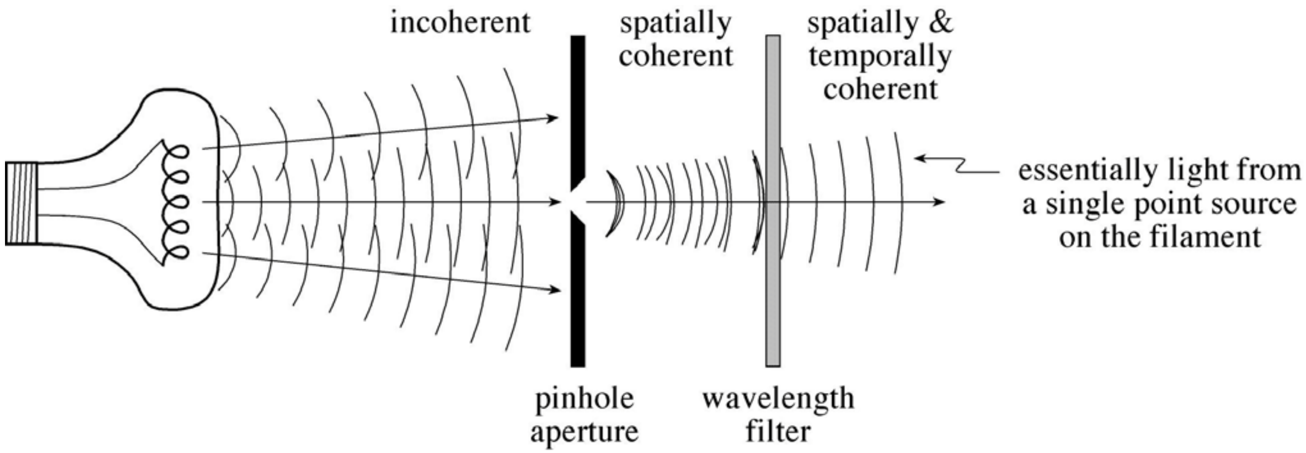
- **Temporal Coherence** is a measure of the correlation of the phase of a light wave at different points along the direction of propagation – it tells how monochromatic a source is
- **Spatial Coherence** is a measure of the correlation of the phase of a light wave at different points transverse to the direction of propagation – it tells us how uniform the phase of the wavefront is

A good example of a temporally and spatially ***incoherent*** source: an incandescent light bulb

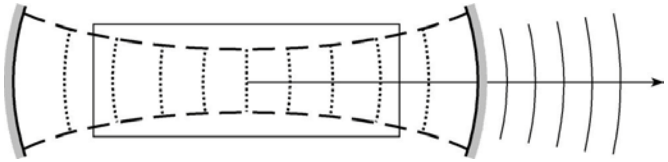


Coherence – how do you achieve it?

- An incoherent source can be filtered to produce coherent light, but you have to throw away most of the light!

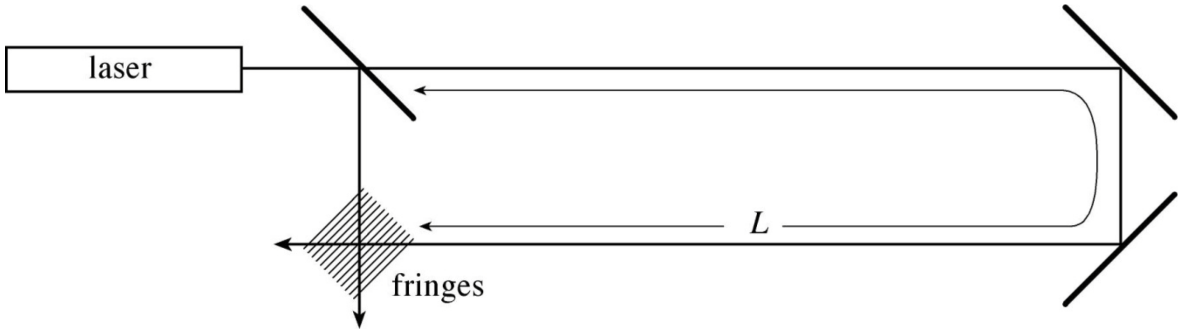


- However, a laser naturally produces a lot of coherent light!



Coherence – how do you measure it?

- **Temporal coherence** is characterized by the **coherence length L_c**
  - L_c is the maximum separation of two points along the propagation direction at a fixed time such that the two points still have a well-defined phase relationship (and hence are able to produce interference fringes)

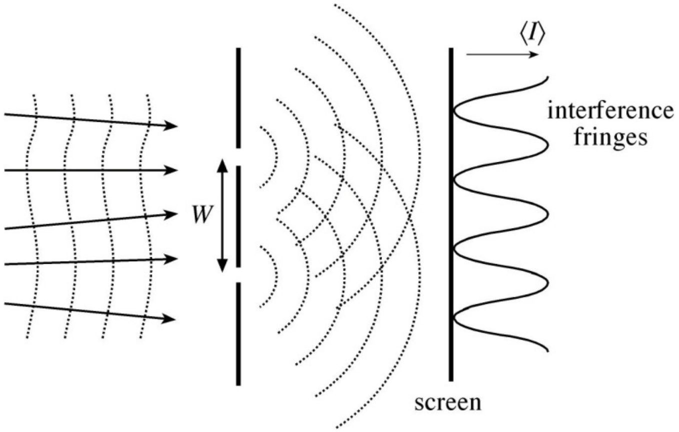


  - For a source centered at wavelength λ and with a total spectral width Δλ, a good approximation is:

$$\boxed{L_c \cong \frac{\lambda^2}{\Delta\lambda}}$$

Coherence – how do you measure it?

- **Spatial coherence** is characterized by the **coherence width W_c**
  - W_c is the maximum separation of two points across the wavefront at a fixed time such that the two points still have a well-defined phase relationship (and hence are able to produce interference fringes)

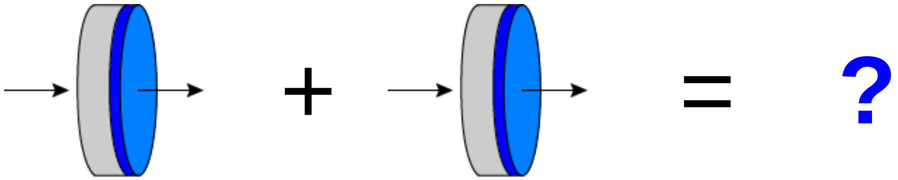


  - W_c can also be measured from the beam divergence; since the source behaves like a bunch of independent sources of aperture size W_c, then

$$\boxed{W_c \cong \frac{\lambda}{\theta}}$$

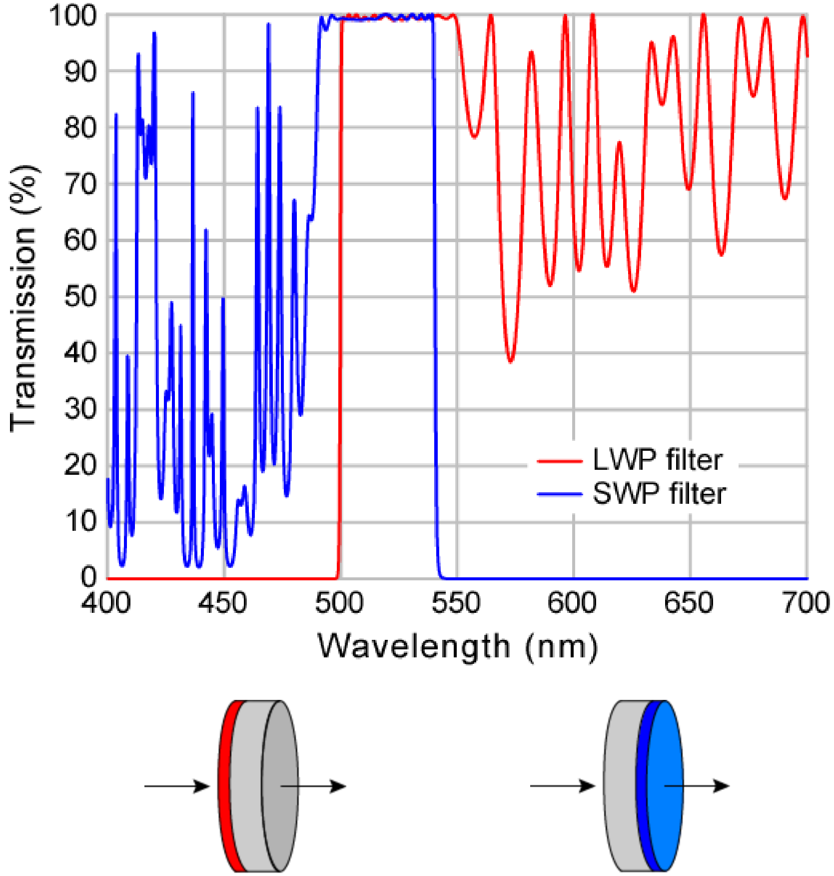
Combining 2 or more filters together

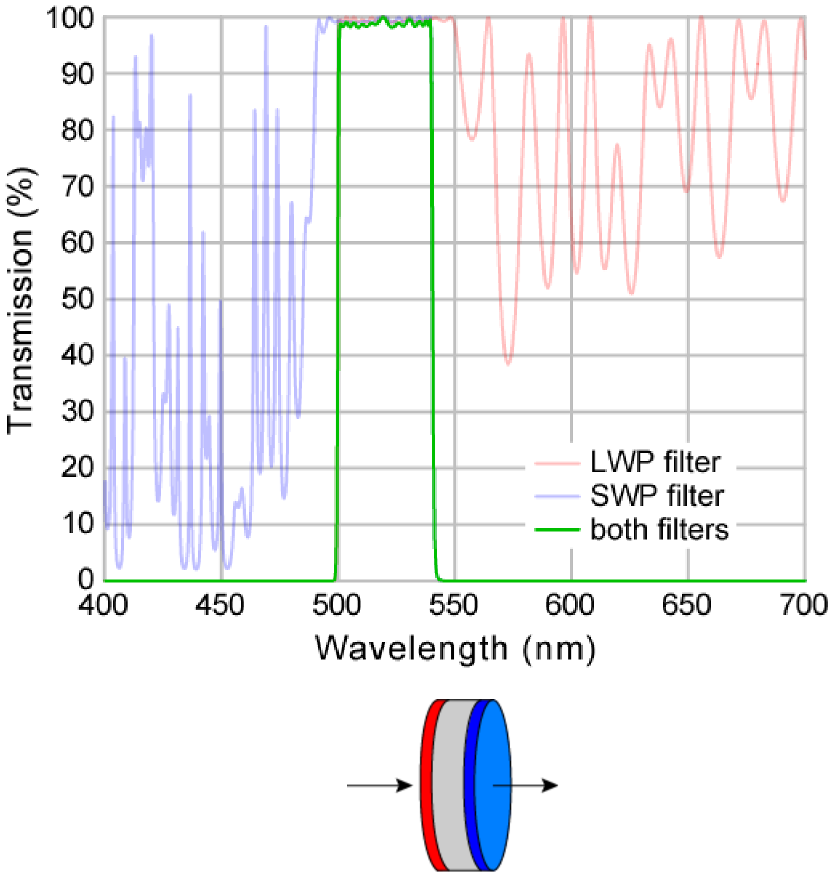
- Often it is desirable to combine two or more filters in sequence to…
    - … increase the wavelength range of blocking, or
    - … increase the blocking level at particular wavelength ranges
- Does “1 + 1 = 2” when filters are combined this way?



Bandpass filter by combining LWP-SWP

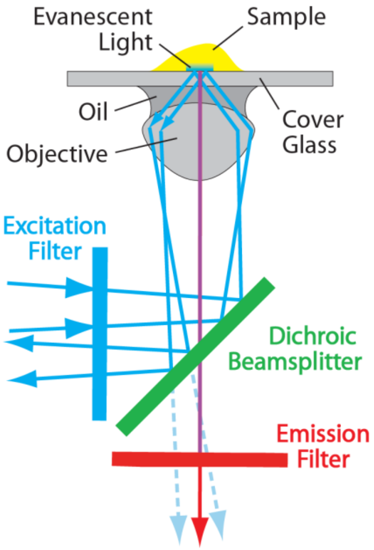
- For **incoherent light**, the combination of two edge filters – an LWP (long-wave-pass) and an SWP (short-wave-pass) – “looks like” a single-coating bandpass filter*



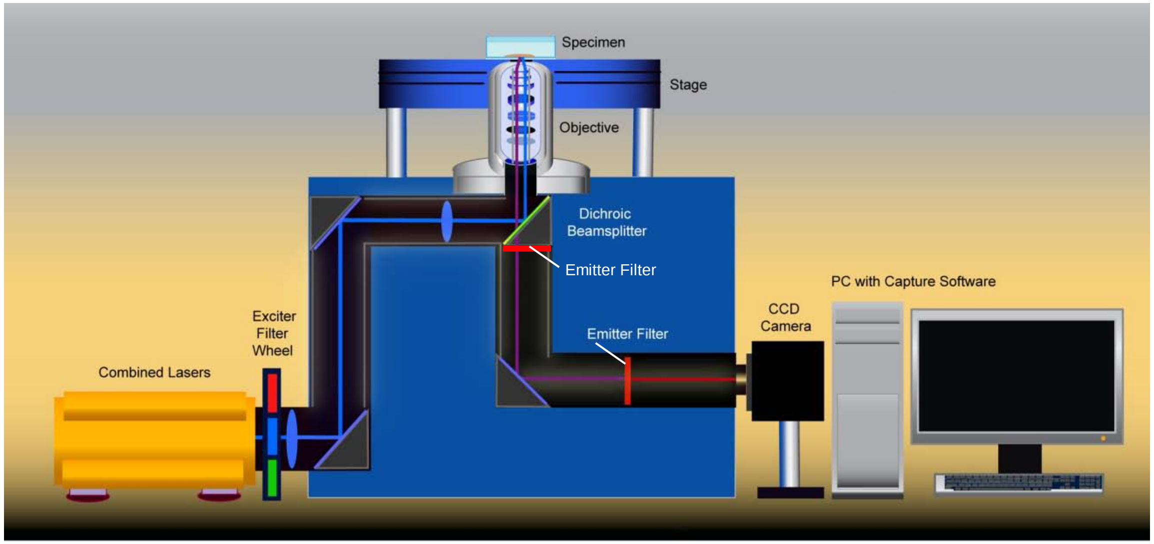


TIRF microscopy

- TIRF = Total Internal Reflection Fluorescence
- The TIR process redirects *ALL* of the laser excitation light back toward the camera, and therefore exceptional blocking by emission filters is critical



Totally reflected excitation light directed back toward the camera

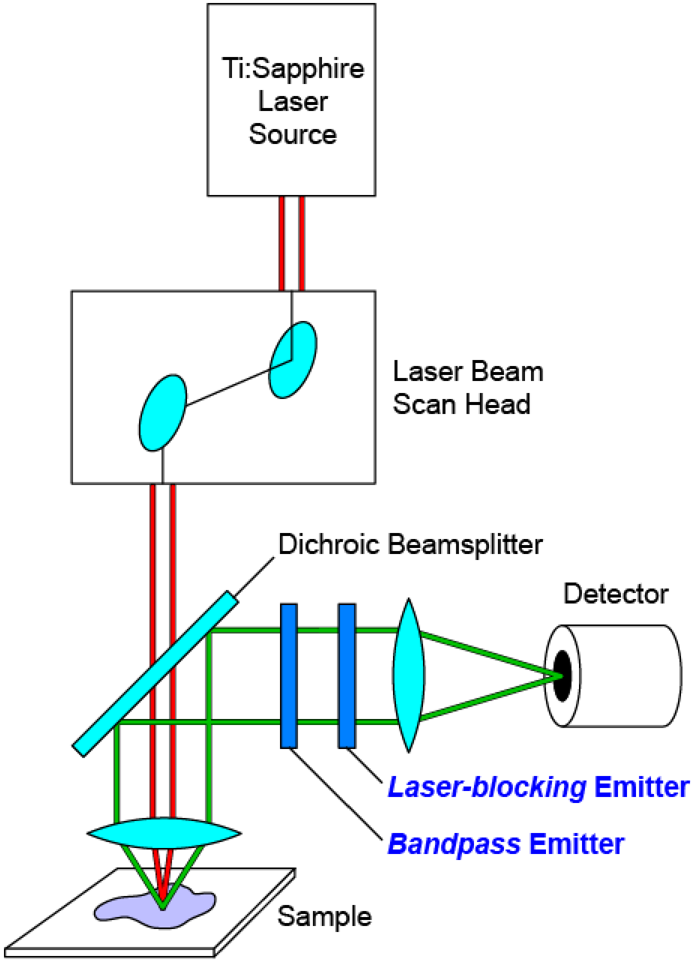


Result: often 2 emission filters are required for sufficient contrast

- Often 2 emission filters – spatially separated – are required to provide sufficient blocking of the laser excitation light

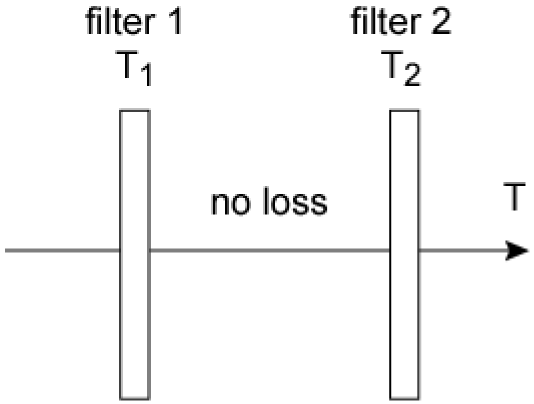
Multiphoton fluorescence microscopy

- A high-peak-intensity (but moderate average intensity) pulsed laser source is focused on the sample and raster-scanned, just as in confocal microscopy
- With appropriate filters it is possible to exclude excitation light from the fluorescence signal and thus obtain a very high signal-to-noise ratio
- The result: *very high resolution 3D imaging of dynamic processes in very thick, live samples*
  - Often it is desirable to use a fixed **short-wave-pass emitter for laser-blocking**, in addition to an exchangeable **bandpass emitter to isolate different fluorophores**



Combining filters for **coherent** light

- Because of multiple-path interference, the transmission of coherent light (e.g., **a laser beam**) through two filters is ***not*** simply the product of the individual transmissions (**$T \neq T_1 * T_2$**)



$$\boxed{T = \frac{T_1 T_2}{1 + (1 - T_1)(1 - T_2) - 2\sqrt{(1 - T_1)(1 - T_2)}\cos(2\pi L/\lambda)}}$$

(L is the separation between the two filters)

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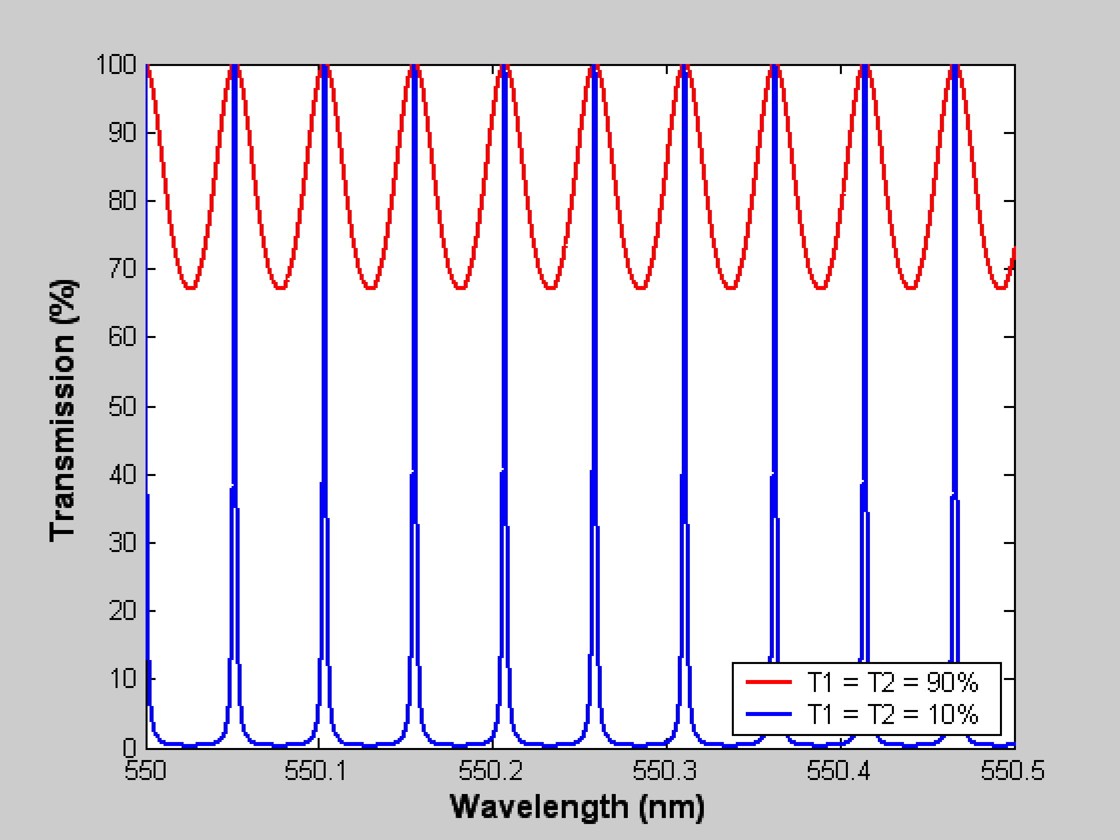


Examples:

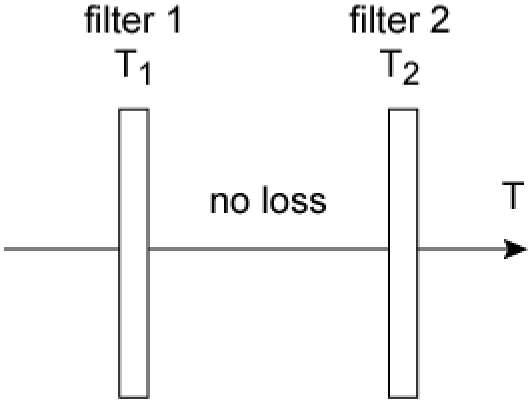
$T_1 = T_2 = 90\%$

$T_1 = T_2 = 10\%$

(L = 2 mm)

Combining filters for **incoherent** light

- Because of averaging due to multiple-path interference, the transmission of incoherent light (e.g., **fluorescence**) through two filters is ***not*** simply the product of the individual transmissions (**$T \neq T_1 * T_2$**)



$$\boxed{\frac{1}{T} = \frac{1}{T_1} + \frac{1}{T_2} - 1}$$

Combining filters for **incoherent** light

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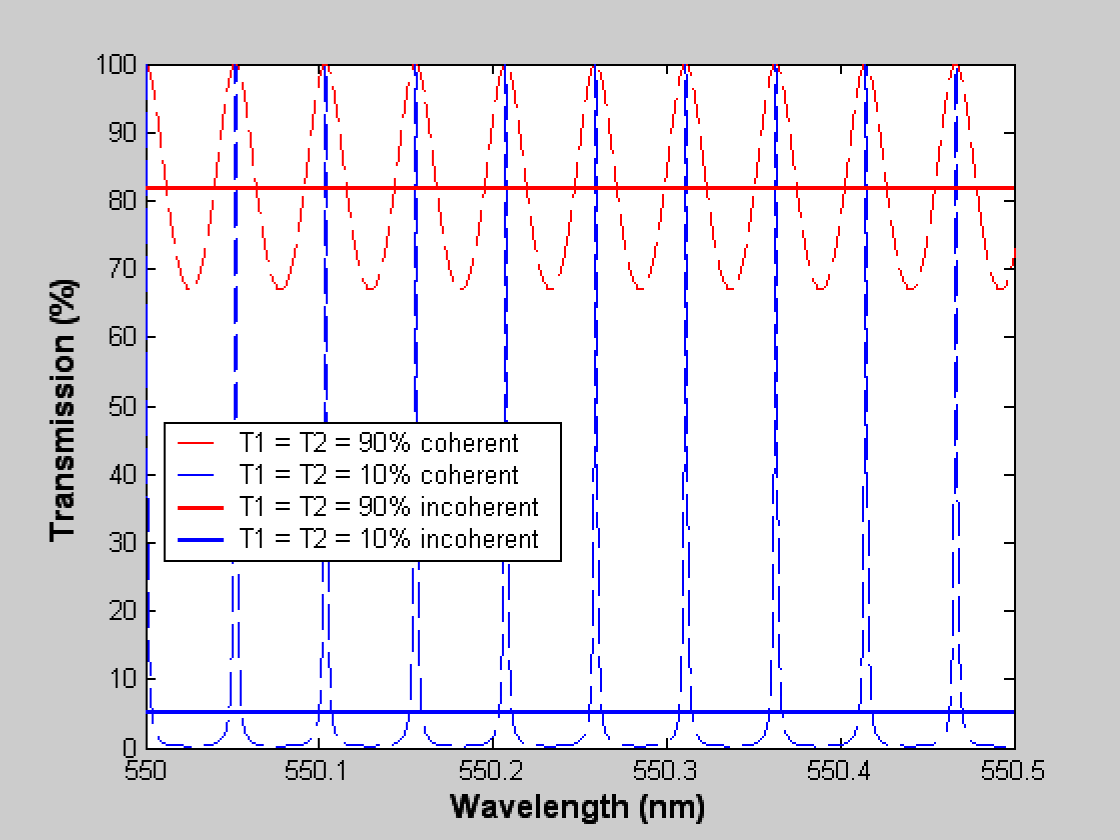


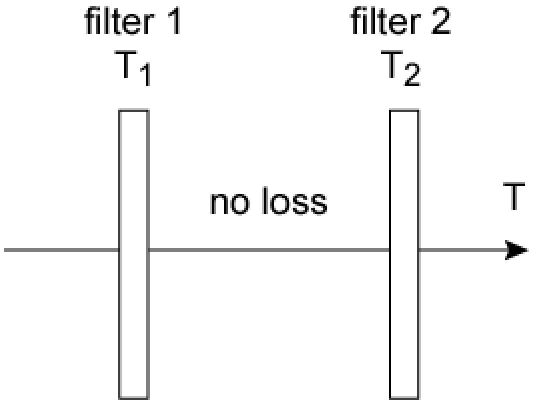
Examples:

$T_1 = T_2 = 90\%$
so $T = 81.82\%$

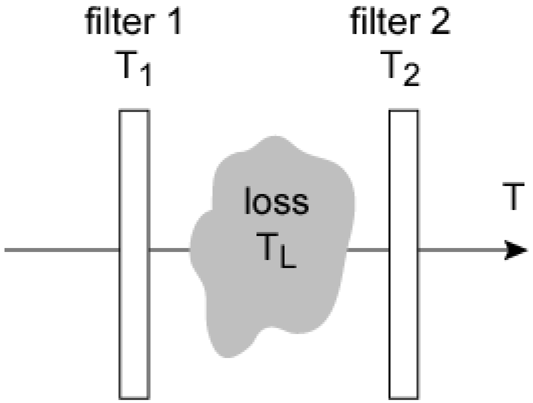
$T_1 = T_2 = 10\%$
so $T = 5.26\%$

Combining filters for **incoherent** light – with loss

- Because of averaging due to multiple-path interference, the transmission of incoherent light (e.g., **fluorescence**) through two filters is ***not*** simply the product of the individual transmissions (**$T \neq T_1 * T_2$**)



$$\boxed{\frac{1}{T} = \frac{1}{T_1} + \frac{1}{T_2} - 1}$$

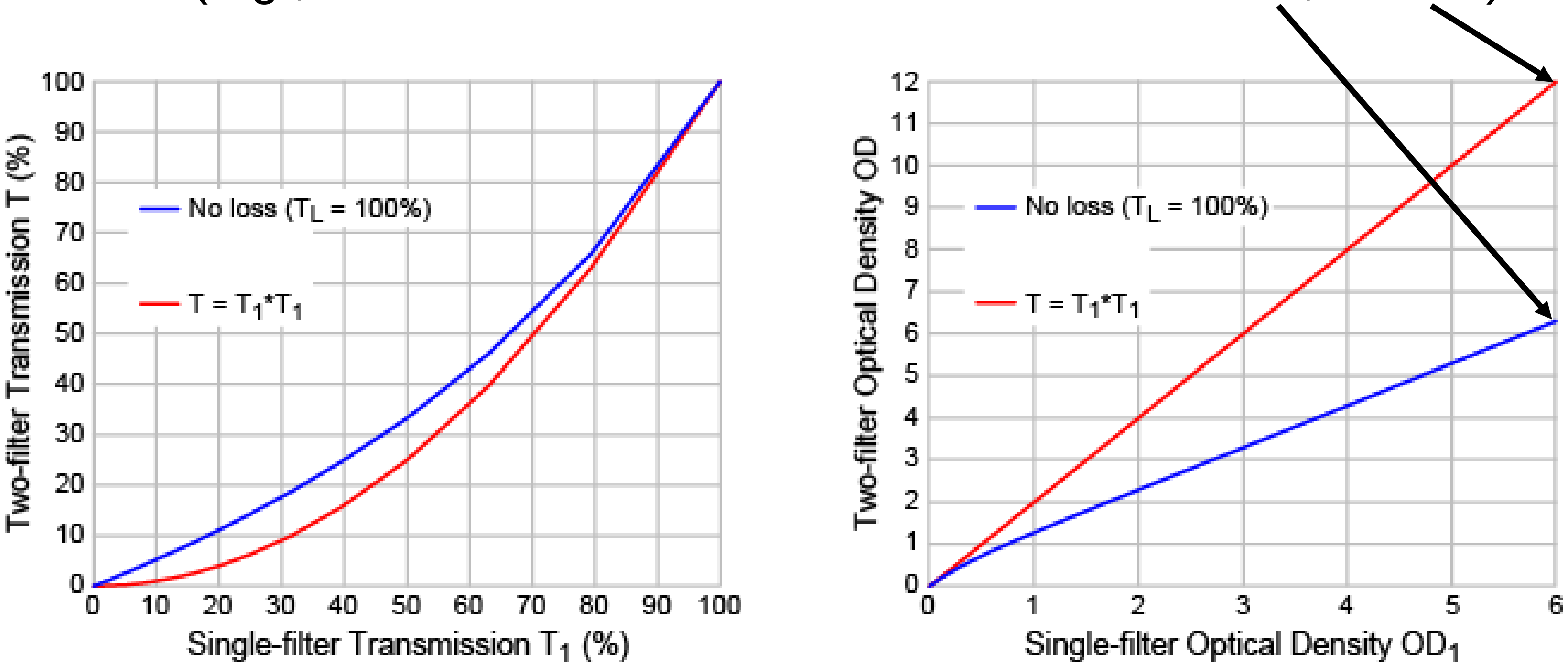


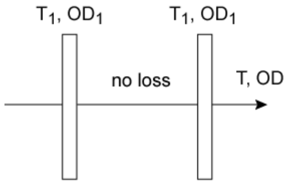
$$\boxed{\frac{1}{T} = \frac{(1 - T_L)}{T_L T_1 T_2} + \frac{1}{T_1} + \frac{1}{T_2} - 1}$$

$$\boxed{OD = \log_{10}\left(10^{OD_1} + 10^{OD_2} - 1\right)}$$

Combining filters for incoherent light – no loss

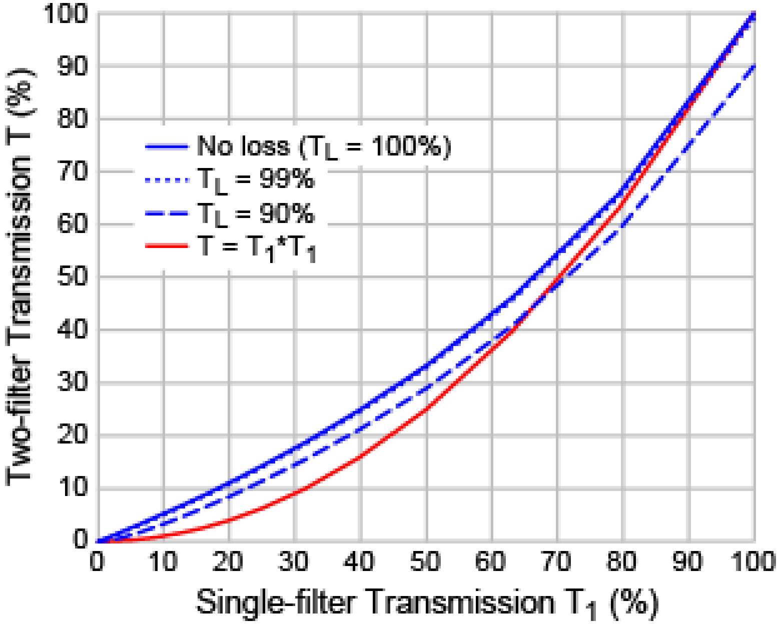
- For high values of transmission (> 80%) the product of the individual transmissions is *approximately* correct
- For very low transmission values (measured in OD), use the correct formula! (e.g., two OD 6 filters have a combined OD of 6.3, *not* 12!)

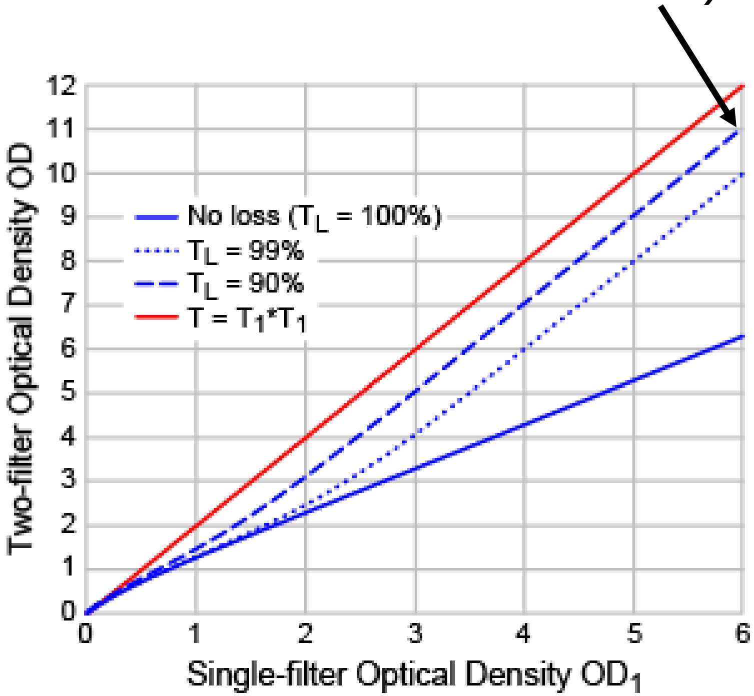


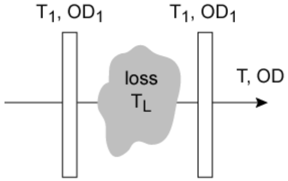


Combining filters for incoherent light – with loss

- Adding even a little bit of loss between the two filters very rapidly cancels the multiple-path interference effects
- For very low transmission values (measured in OD), a little loss greatly increases the combined OD (10% loss makes the combined OD 11!)

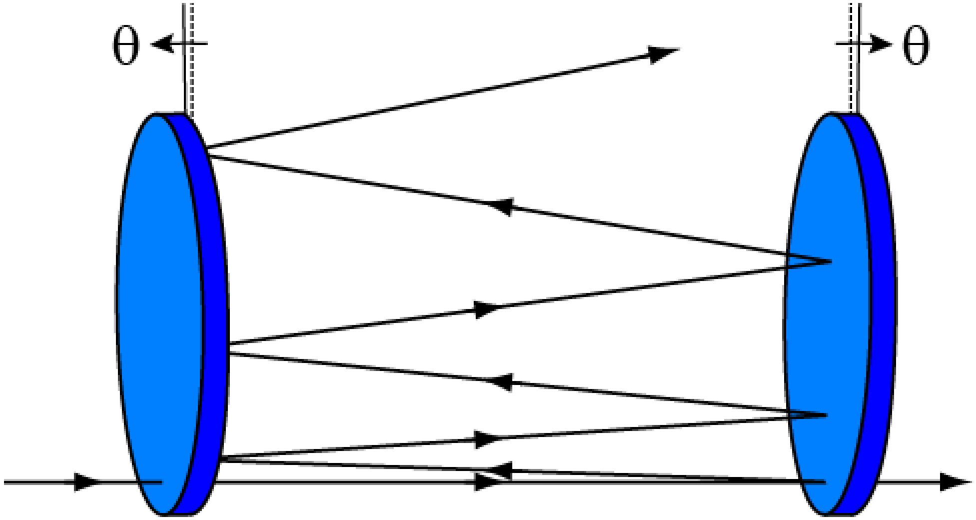






Tilting two filters with respect to one another

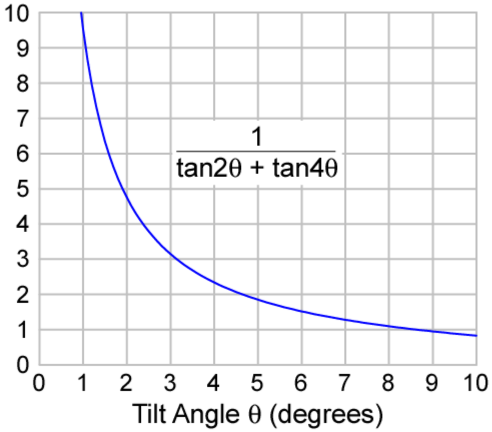
- An easy way to eliminate multiple reflections between two filters (and thus add loss) is to tilt them with respect to one another
- Multiple reflections can be completely eliminated if the filter separation is larger than the filter diameter for tilt angles θ of at least a few degrees
  - Then transmission *is* given by the product of the individual values (**$T = T_1 * T_2$**)

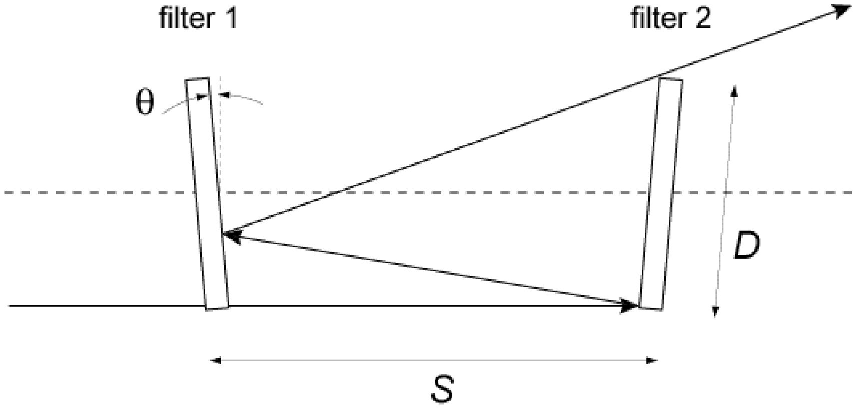


Tilting two filters with respect to one another

- An easy way to eliminate multiple reflections between two filters (and thus add loss) is to tilt them with respect to one another
- Multiple reflections can be completely eliminated if the filter separation *S*, diameter *D*, and tilt angle θ obey the relation below – in this case the transmission *is* given by the product of the individual values (**$T = T_1 * T_2$**)

$$\boxed{S > \frac{D}{\tan 2\theta + \tan 4\theta}}$$



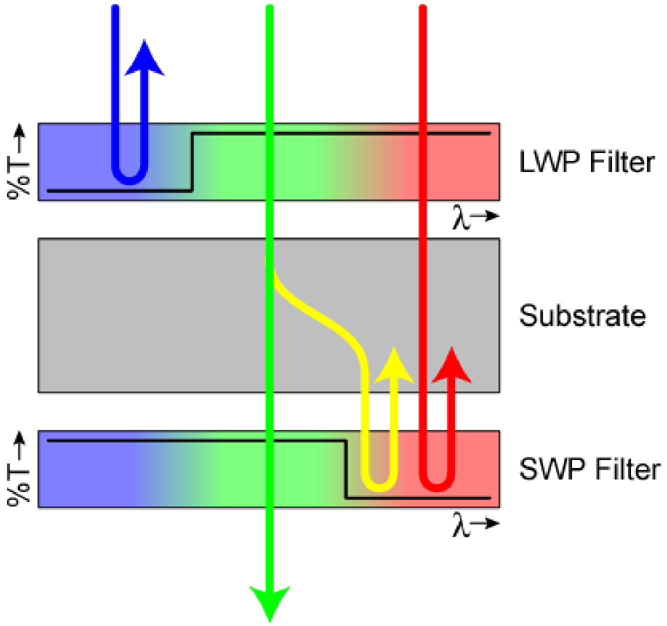


Minimizing substrate autofluorescence

- Filters made from more than one coating can yield a different amount of substrate autofluorescence depending on how they are oriented
- For a LWP-SWP filter, the light should travel from the LWP to the SWP

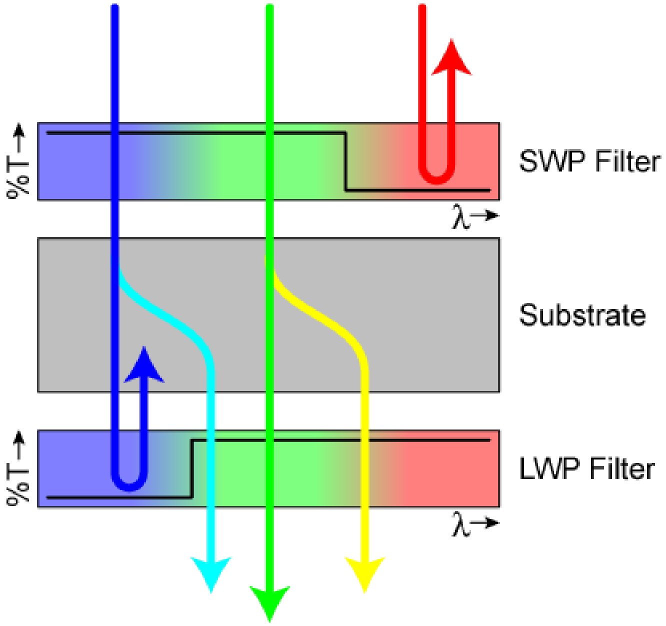
Correct Orientation

- *LWP first*
- No autofluorescence leaks through the filter



Incorrect Orientation

- *SWP first*
- Autofluorescence *can* leak through the filter



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Thank you!