USING OPTICAL FILTERS IN LIFE SCIENCE APPLICATIONS

Jeff Carmichael
John Atkinson
Chroma Technology Corp.
SOME TERMS AND DEFINITIONS

ANGLE OF INCIDENCE (AOI): The angle between the optical axis of the light incident on the surface of a filter and the axis normal to this surface.

An AOI of 0 degrees indicates the light falling on the filter is perpendicular to the surface of the filter. Most filters are used in this 0 degree (normal incidence) configuration. Beamsplitters are most often used at 45 degree AOI. A wide range of angles can be accommodated by optical design for custom or non-standard applications.

CONE ANGLE or HCA (half cone angle): The range of angles impinging on a filter. Applies to non-collimated light. Half cone angle refers to the absolute angle in any direction relative to the normal incidence. HCA is more useful as it indicates the range of AOI to accommodate in optical design of thin film filter coatings.

CUT-ON or CUT-OFF: The wavelength on a transition slope which is 50% of the max. transmission of that particular filter. A single bandpass filter has one cut-on and one cut-off. A multi bandpass filter or dichroic has multiple cut-ons and cut-offs. A shortpass or longpass filter or single edge dichroic has one cut-on.

CENTER WAVELENGTH (CWL): The arithmetic mean of the cut-on and cut-off wavelengths.

BANDWIDTH or FULL WIDTH/HALF MAX (FWHM): The full width of the pass band at half of maximum transmission.

BLOCKING LEVEL: Also “Attenuation” level. A measure of the out-of-band attenuation of an optical filter over an extended range of the spectrum. The attenuation level is often defined in units of optical density.

BLOCKING RANGE: The range of wavelengths over which an optical filter maintains a specified attenuation level.

BANDPASS FILTER: Filter with a well-defined short wavelength cut-on and long wavelength cut-off. Can be single band with one cut-on/one cut-off, or multiband with multiple cut-on/cut-off.

LONGPASS FILTER: An optical filter that attenuates shorter wavelengths and transmits longer wavelengths over the active range of the spectrum (which depends on the specific application). LP filters are denoted by the cut-on wavelength at 50% of peak transmission.

SHORTPASS FILTER: An optical filter that attenuates longer wavelengths and transmits shorter wavelengths over the active range of the spectrum (which depends on the specific application). SP filters are denoted by the cut-off wavelength at 50% of peak transmission.
SOME TERMS AND DEFINITIONS

DICHROIC BEAMSPLITTER: Also Dichroic mirror, Dichromatic beamsplitter. A type of beamsplitter that selectively reflects one range of wavelengths and transmits a different range of wavelengths.

Dichroic beamsplitters can be found in any part of an optical system where light needs to be split or combined according to wavelength (color).

NOTCH FILTER: Looks like an inverted bandpass filter. Blocks a range of wavelengths while transmitting shorter and longer wavelengths.

CROSS-TALK: The blocking (attenuation) level (over a specified wavelength range) between an excitation and emission filter in a fluorescence application. This measurement is usually stated in units of optical density (OD = -log(\%T). OD 1.0 = 10\%T, OD 6.0 = 0.0001\%T). The greater the attenuation the lower the noise level.

OPTICAL DENSITY: A logarithmic unit of transmission: OD = -log (T), where T is the transmission (0 ≤ T ≤ 1).

WEDGE: Also Parallelism. A measure of the deviation of the outer surfaces of an optical element from perfect parallelism, usually measured in arc-minutes or arc-seconds of angle.

TRANSMITTED WAVEFRONT DISTORTION (TWD or TWE): A measure of the distortion (or “error”) a plane wavefront of light undergoes when transmitted through an optical element, measured in fractions or multiples of a wavelength of visible light (usually 633 nm, ISO uses 546nm conversion).

REFLECTED WAVEFRONT DISTORTION (RWD or RWE): A measure of a wavefront’s deviation (or “error”) from perfectly planar after reflecting off the surface of an optic (usually 633 nm, ISO uses 546nm conversion).

SURFACE FLATNESS: Also called Surface Form Tolerance. A measure of a surface’s deviation from perfectly flat

SLOPE: The slope of the transition from either transmission to blocking for a filter, or from transmission to reflection for a beamsplitter. Typically measured in %. As an example, one way of describing the slope of a bandpass filter could be 1%, where this may refer to a distance of 1% of the wavelength of light between the bandpass cut-on/cut-off and where it reaches a characteristic Optical Density.
Optical methods are preferred for real-time imaging applications - high spatial resolution.

**Fluorescence** is a molecular phenomenon in which a substance absorbs light of some color and almost instantaneously radiates light of another color.

**Stoke’s Law** states that the wavelength of emitted light is ‘almost always’ longer than the wavelength of the excitation light.
Excited Electronic State

Ground Electronic State

- Single-photon excited fluorescence
- Two-photon excited fluorescence (TPEF)
- Second Harmonic Generation (SHG)
- Coherent anti-stokes Raman scattering (CARS)

**Excited State**
- $h\nu_P$
- $h\nu_{\text{Fluo}}$

**Ground State**
- $h\nu_P$
- $h\nu_{\text{Fluo}}$
- $h\nu_{\text{SHG}}$
- $h\nu_S$
- $h\nu_{\text{CARS}}$

**Energy Level Diagram for Fluorescence/SHG/CARS Microscopy**

- **Confocal Microscope**
- **Two-photon Microscope**
- **SHG Microscope**
- **CARS Microscope**
Jablonski Energy Diagram

Excitation (Absorption)
$10^{-16}$ Seconds

Internal Conversion and Vibrational Relaxation
$(10^{-14} - 10^{-11}$ Sec)

Fluorescence
$(10^{-3} - 10^{-7}$ Sec)

Intersystem Crossing

Quenching

Non-Radiative Relaxation

Excited Singlet States

Vibrational Energy States

Ground State

Figure 1
WHY USE FLUORESCENCE?

SENSITIVITY
- Is sensitive to the local “micro” environment, which includes factors such as pH, osmolality (concentrations of solutes), polarity of solvent

SIGNAL-TO-NOISE
- We really want more signal, and less noise...

SPECIFICITY/RESOLUTION
- Can see single molecules, in both spatial and temporal terms
WHY USE FLUORESCENCE?

TAGGING BIOLOGICAL COMPOUNDS

VIABILITY
- labels for live cells/tissues

QUANTIFICATION
- linear with number of molecules…
Filters do two things:

1. **TRANSMIT** (allow to “pass” through) the desired wavelengths of light (color), and the desired amount (brightness) of light

2. **REJECT** (block) undesired light to a very high degree (high OD, or Optical Density)

The combined effect is a filter with a very high signal/noise ratio
Two of these filters in a set work together to visualize fluorescence:

- **Excitation** Filter
  
  Selects and transmits the optimal wavelengths of light to “excite” a FISH probe(s)

- **Emission** Filter
  
  Selects and transmits the optimal wavelengths of fluorescence emitted by a FISH probe(s)

By combining the right amount and color of excitation light with the right amount and color of fluorescence emission, you can visualize the desired FISH probe(s)
Here's How It Works:

**EXCITATION FILTER:**
selects and transmits the optimal wavelengths of light to excite a FISH probe(s)

- VIOLET
- BLUE
- CYAN
- GREEN
- YELLOW
- ORANGE
- RED
- DARK RED

**EMISSION FILTER:**
blocks excitation light and transmits the optimal wavelengths of fluorescence emitted by a FISH probe(s)

- GREEN ONLY
- GREEN/CYAN

**DICHROIC MIRROR:**
reflects excitation light towards sample and transmits longer wavelengths, such as fluorescence emission towards eye or camera

$$h \nu_{Ex} > h \nu_{Em} - \text{Stokes Shift}$$

White Light

Specimen labeled with Green FISH probe
By transmitting the most effective wavelengths of light, fluorescence filters influence the **4 most important aspects of fluorescence images:**

- **Contrast**
- **Brightness**
- **Color Separation**
- **Registration** (or alignment)
FLUORESCENCE SIGNAL VS. BACKGROUND NOISE

This is the Signal/Noise ratio of the image.

Problem:
Low Contrast, Difficult to score slides

Solution:
Maximize Contrast

High Signal/Noise

Benefit:
Allow you to more easily see the difference between real signal and background
Good Contrast

Inadequate Contrast
A TALE OF TWO FILTER SETS

**A**

Good Contrast

(more signal to noise) - easy to score.

Overlap of filter set 'A' spectra with probe spectra
(Grey-shaded area)

**B**

Brighter

(more shaded area) but less contrast - more difficult to score.

Overlap of filter set 'B' spectra with probe spectra
(Grey-shaded area)

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Excitation filter transmission spectra

Emission filter transmission spectra

Green FISH probe excitation and emission spectra
IF IMAGE ‘B’ IS BRIGHTER, WHY IS THE CONTRAST LOWER?

Because the noise is higher...

There is always background fluorescence from various sources:
- Cells and tissues themselves are autofluorescent
- Fixatives and other reagents are autofluorescent

Fluorescent probes “stick” non-specifically.
‘A’ has better signal/noise ratio = more contrast

‘B’ will detect 91% more signal than ‘A’ but...

‘B’ will detect 226% more noise than ‘A’
Filters out all but the desired wavelengths of excitation light which are emitted from a light source (such as an Hg lamp) in order to effectively excite a particular fluorochrome. The excitation filter bears the burden of attenuating a very broad spectrum at a very high level. Used at 0 degrees AOI. Named using CWL and bandwidth at FWHM. ET470/40x = 470nm CWL, 40nm FWHM.
Measured absorbance data, expressed in Optical Density units (OD = -log[%T]).

OD 1.0 = 10%T, OD 6.0 = 0.0001%T
The emission filter (also called the barrier filter) bears the burden of blocking or attenuating the excitation light sufficiently, thereby allowing the detection of weak fluorescence signals. This is its primary function. Secondary to this function is maximizing the level of transmission of the desired wavelengths. Used at 0 degrees AOI. Named using CWL and bandwidth at FWHM. ET525/50m = 525nm CWL, 50nm FWHM.
EMISSION FILTER “BLOCKING” OF WAVELENGTHS TRANSMITTED BY THE EXCITATION FILTER

OD 1.0 = 10%T, OD 6.0 = 0.0001%T
The dichroic beamsplitter (also called dichroic mirror or dichromatic beamsplitter) is a thin piece of coated substrate, typically set at a 45 degree angle to the optical path of the microscope. The coating reflects a particular range of wavelengths (typically excitation) and transmits a different range of wavelengths (typically emission). A longpass dichroic reflects excitation wavelengths to the sample, and transmits the longer wavelength fluorescence emission to the detector. A shortpass dichroic reflects longer wavelengths, while transmitting shorter.
Dichroics do not necessarily reflect “everything below” the cut-on and transmit “everything above”. The “cut-on” wavelength of any filter (including ex. and em. filter) is the wavelength at which transmission is 50% of maximum transmission. All of the following are approx. “600nm” dichroics:
Q: WHY NOT PLACE FILTERS CLOSER TOGETHER SPECTRALLY?
A: INSUFFICIENT BLOCKING

OD 1.0 = 10%T, OD 6.0 = 0.0001%T
TRANSMISSION DIFFERENCES BETWEEN FILTER COATINGS
BLOCKING DIFFERENCES BETWEEN FILTER COATINGS:
Sample Light Source

Unfiltered Excitation Light

Unfiltered Fluorochrome Excitation and Emission

“Cleaned-up” Fluorochrome Emission

Camera/Eye
COMMON MICROSCOPE LIGHT SOURCES

Mercury
Metal Halides

Xenon
• **Light Emitting Diodes (LED)**
  - Huge variety of choices now and growing
  - Getting more powerful/useful
  - Still have to worry about the cone angle...

• **Lasers**
  - Plasma
  - Diodes
  - DPSS (diode pumped solid state)
  - 2-photon/nir and CARS...
Back reflected laser beam can lead to the ratio of illumination/fluorescence signal as high as $10^{15}:1$.

Additional blocking is most often required for optimal image quality by pairing emission filters.

- Bandpass emission filter paired with a longpass emission filter in a cube.
- A multi-band emission filter in a cube paired with single bandpass emission filters in a filter wheel.
- Minimize reflected wave front distortion very important.
MULTIPHOTON MICROSCOPY

One-Photon Excitation

- Reduced absorption and scattering
- Better penetration depth
- Less Photobleaching and Phototoxicity
  - live animal imaging

Multi-Photon Excitation

NIR
• Post-coating flatness of the dichroic mirror is critical for maintenance of RWD
• Use of 5-6 mm thick dichroic with 0.1 wave/inch RWD peak-to-valley

http://www.anes.ucla.edu

Berning et al., Science, 2012
LASERS VS. LEDS

Lasers
- High Intensity, coherent light source
- Narrow spectral range
- Collimated output
- Many types of lasers
  - Gas Lasers
    - Ex. HeNe (633nm), Argon-ion (488 nm & 514 nm)
  - Solid State Lasers
    - Ex. Nd: YAG (1064 nm, 532, 355, 266 nm)
  - Dye Lasers
  - Tunable lasers
  - Diode Laser
    - Semiconductor lasers (375-3000 nm)
    - Can drift with temperature

Light Emitting Diodes (LEDs)
- Lower Intensity, non-coherent light source
- Broader spectral range
- Generally not collimated
- All types are of a similar package compared to the variety of lasers
  - On a chip, with or without dome or reflector
LEDs and Lasers have very different intensity distributions

- LEDs by themselves tend to throw light out in a very broad distribution
  - Domes or back reflectors can be added to partly focus the light, but the resulting beams are by no means collimated – a lens or system of lenses is usually needed to collimate an LED
- Lasers are the opposite, putting out a very concentrated and collimated beam
SOFT (THERMAL) COATING

Glass Substrates

Powder material, low index

Optical monitor system, with light path, lamp at the top

Powder material, high index

Resistive heater, tungsten filament
ELECTRON BEAM COATING

- Pellet material, low index
- Pellet material, high index
- Glass Substrates
- Optical monitor system, with light path, lamp at the top
- electron beam with sweep
ION ENHANCED E-BEAM COATING

Glass Substrates

Pellet material, low index

Pellet material, high index

Optical monitor system, with light path, lamp at the top
MODIFIED MAGNETRON SPUFFERING

- Metal target, low index
- Metal target, high index
- Optical monitor system, with light path, lamp at the top
- Glass Substrates
- Ion gun for oxidation
New, brighter fluorochromes and optimized filter sets make multi-color imaging simpler and more accessible.
• Novel polymer chemistry led to the discovery and development of conductive organic polymers.*

These polymers led to the development of a new class of extremely bright fluorescent molecules which have now become the BD Horizon Brilliant™ family of fluorochromes.

* Nobel Prize in Chemistry, 2000 (Alan J. Heeger, Alan G. MacDiarmid and Hideki Shirakawa)
• Brightness and spectral properties of Brilliant™ Violet Fluorochromes enable:

  • Lower levels of excitation energy, minimizing photodamage
  • Fewer numbers of molecules (increased sensitivity)
  • Shorter exposures, increasing speed
  • Increased parameters per sample
- BV421 and BV480 fill a gap in the visible spectrum. This is an opportunity to make 5-color imaging easily accessible to anyone working with a basic fluorescence microscopy stand.
Existing, reliable and popular fluors provide the longer wavelengths. 5 colors, with excitation wavelengths from approx. 390-650nm, and emission wavelengths from approx. 420-700nm allow for the use of standard light sources and cameras:
THE EQUIPMENT YOU NEED:

- Standard Microscope
- Standard Light Source
- Standard Camera

What You Don’t Need:

- Advanced imaging expertise
- Sophisticated technologies or equipment
Because these are all very bright fluors, we are able to use narrow-band excitation and emission filters to maximize spectral separation.

Shown here are separate excitation and emission filter graphs for ease of illustration.

**THE FILTERS YOU NEED:**

- BV421
- BV480
- *488
- *568
- *647
Multi band filter sets allow for fast, efficient imaging, including z-stacks. They also ensure precise image registration. Chroma offers a 5-band filter set for use with external filter wheels to house 5 excitation and 5 emission filters with one multi band dichroic mirror in the cube. These filters are plotted below, excluding emission filters for ease of illustration.
EXAMPLE OF 5-BAND SET WITH 5-BAND EX. FILTER, 5-BAND EM. FILTER AND 5-BAND DICHROIC:
BD HORIZON BRILLIANT™
FLUOROCHROMES EXCEL IN
FLUORESCENCE IMAGING APPLICATIONS:

**Immunocytochemistry**
- BV421
- Draq5

**Immunohistochemistry**
- BV480
- Draq5

**FISH**
ADDITION OF BV421 AND BV480 ENABLES MORE EFFECTIVE MULTIPLEXING AND FLEXIBILITY:

5-Channel Options

- **BV421**
  - BA480
  - 488 fluor
  - OR
  - Various Green FPs
  - 568/555/546 fluor
  - OR
  - Various Red FPs
  - Draq5 (DNA)

- **BV421**
  - BA480
  - 488 fluor
  - OR
  - Various Green FPs
  - 568/555/546 fluor
  - OR
  - Various Red FPs
  - 647 fluor

- **DAPI (DNA)**
  - BA480
  - 488 fluor
  - OR
  - Various Green FPs
  - 568/555/546 fluor
  - OR
  - Various Red FPs
  - 647 fluor
- Robust 5-color imaging:
- DAPI, BV480, Alexa Fluor® 488, Alexa Fluor® 555, Alexa Fluor® 647
• Robust 5-color imaging:
• BV480, Alexa Fluor® 488, Alexa Fluor® 555, BV421, DRAQ5
• Robust 5-color imaging:
• GFP, RFP, Alexa Fluor® 647, BV480, BV421

Thank you to collaborators in the Catherine Hedrick lab at La Jolla Institute of Allergy and Immunology for data.
EXAMPLES OF FILTER TYPES
AOI 0° Bandpass filter in %T

![Graph showing transmission percentage vs wavelength for AOI 0° Bandpass filter. The graph peaks at approximately 60% transmission around 500 nm.]
AOI 0° Bandpass filter in OD
AOI 0° Long-pass filter in OD

Optical Density

Wavelength (nm)

ET500lp in %T OD
AOI 0° Short-pass filter in %T
AOI 0° Short-pass filter in OD
AOI 45° Dichroic Beamsplitter in OD

[Graph showing Optical Density versus Wavelength (nm)]

- Optical Density
- Wavelength (nm)
- 290832 OD
AOI 0° Green-Red Dualband in %T
AOI 0° Green-Red Dualband in OD
Quadband Set in %T

[Graph showing transmission (%T) vs. wavelength (nm) for different filters: Quad Laser Emission filter, Quad Laser Clean-up filter, Quad Laser Polychroic beamsplitter]
Quadband Set in OD
AOI 0° Notch Filter in %T

Transmission (%)

Wavelength (nm)

ZET561NF
AOI 0° Notch Filter in OD
40nm-wide bandpass filters: Transmission
10nm-wide bandpass filters: Transmission
10nm-wide bandpass filters: Optical Density
IR Bandpasses w/ sputtered amorphous Silicon
UV-Metal Induced transmission BPs with Al metal
All-dielectric UV Narrow bands with alumina and silica
WHAT IS LIGHT?

Light is a particle … … but doesn’t always behave like one:

Photon:
• A fundamental particle with a discreet bundle of energy
  • has no mass
• travels at the speed of light
• carries electromagnetic energy and momentum
  • has intrinsic angular momentum, or spin

Thomas Young’s Double-slit experiment (1803)
Light is also a wave.

Young’s Double-slit experiment:

Thomas Young’s Double-slit experiment (1803)

Animation: http://www.itp.uni-hannover.de/~zawischa/ITP/diffraction.html
Light waves of different wavelengths carry different amounts of energy.

Our eyes are only sensitive to a relatively small range of wavelengths in the electromagnetic spectrum.

Most objects output a light signal that is “broadband” in nature; they emit a spectrum or range of wavelengths of light.
Eyes detect light using cone and rod photoreceptors.

Cones function with medium-bright light levels (photopic vision) while rods function at low light levels (scoptopic or “night” vision).

Cones are highly concentrated at the fovea, or best focus of the eye, so they are responsible for spatial acuity (or image sharpness).

Rods are more distributed throughout the retina, so they pick up light from a wider angle (peripheral vision).
There are three types of cones, L, M, and S (for short medium and long), each with a different photopigment, and having a different sensitivity to wavelength – cones are therefore responsible for color vision.

There is only one kind of rod photopigment, with a peak sensitivity around 500nm (blue-green), but because there is only one, rod vision is monochromatic.
Cones/ Photopic Vision

The plot of normalized cone sensitivity shows three distinct peaks ...

… but taking the log shows there is significant overlap, and in fact the L and M cones each cover almost the entire visible spectrum.

The weighted response given the relative number of L, M, S cones in the retina gives a more accurate view of how our color vision is influenced by cone sensitivity.

- L & M cones dominate, so our eyes are overall more sensitive to green light!
HUMAN EYE SPECTRAL SENSITIVITY (AKA PHOTOPIC RESPONSE FUNCTION)

- Represents the spectral luminous efficiency for the human eye
HUMAN EYE SPECTRAL SENSITIVITY
(AKA PHOTOPIC RESPONSE FUNCTION)

- Represents the spectral luminous efficiency for the human eye
Rod photoreceptors are overall more sensitive to incoming energy and also blue shifted compared to photopic (cone) vision.

Once the eye is dark-adapted, rods can detect very low light levels (high gain).

Rod signals are integrated or ‘summed’ before leaving the retina, meaning slower response – this also makes it difficult to see fine detail no matter how close an object is brought to the eye.

Even the dark-adapted eye can use cones to see color if the source is colored and bright enough.
Color perception is achieved by a complex process that starts with the differential output of the cone photoreceptors in the retina and is finalized in the visual cortex of the brain.

In 1931 (later revised in 1964) the Commission Internationale de l’Eclairage (or CIE) gave us a construct to model color perception.

The \( y \) function is just the photopic response function, as it determines the overall sensitivity to brightness, or luminance.

RGB color spaces are additive models based on 3 distinct primaries. The CIE’s RGB color space is based on standardized monochromatic primaries at wavelengths of 700 nm (red), 546.1 nm (green) and 435.8 nm (blue).

The RGB color matching functions are the amounts of primaries needed to match the monochromatic test primary at the wavelength shown on the horizontal scale (Wikipedia).
STANDARD ILLUMINANT A, INCANDESCENT LAMP
SPECTRAL RESPONSE OF SILICON PHOTODIODES
### THE UNITS OF MEASURE LIGHT...

<table>
<thead>
<tr>
<th>Radiometric quantity</th>
<th>Photometric quantity</th>
<th>Physical Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>radiant energy (J)</td>
<td>luminous energy (lm s), $Q$</td>
<td>energy</td>
</tr>
<tr>
<td>radiant flux (W)</td>
<td>luminous flux (lm), $\Phi$</td>
<td>power, flux - rate of transfer of energy</td>
</tr>
<tr>
<td>radiant intensity (W/sr)</td>
<td>luminous intensity (lm/sr = cd), $I$</td>
<td>intensity - power per unit solid angle from a source</td>
</tr>
<tr>
<td>radiance (W/sr/m²)</td>
<td>luminance (lm/sr/m² = cd/m²), $L$</td>
<td>power per unit solid angle per unit area from a source</td>
</tr>
<tr>
<td>irradiance (W/m²)</td>
<td>illuminance (lm/m² = lx), $E$</td>
<td>flux density - power per unit area incident on a surface</td>
</tr>
<tr>
<td>radiant exitance or emittance (W/m²)</td>
<td>luminous exitance (lm/m²), $M$</td>
<td>flux density - power per unit area emitted from a surface</td>
</tr>
</tbody>
</table>

### Approximate Luminance Levels

<table>
<thead>
<tr>
<th>Source</th>
<th>Luminance [cd/m²]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sun’s disc</td>
<td>1,500,000,000</td>
</tr>
<tr>
<td>100-W soft light bulb</td>
<td>30,000</td>
</tr>
<tr>
<td>White paper in sunlight</td>
<td>25,000</td>
</tr>
<tr>
<td>Fluorescent lamp surface</td>
<td>10,000</td>
</tr>
<tr>
<td>Overcast sky</td>
<td>3,000</td>
</tr>
<tr>
<td>Moon’s disc</td>
<td>2,500</td>
</tr>
<tr>
<td>Blue sky</td>
<td>1,000</td>
</tr>
</tbody>
</table>

### Approximate Natural Illuminance Levels

<table>
<thead>
<tr>
<th>Source</th>
<th>Illuminance [lx]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct sunlight</td>
<td>100,000</td>
</tr>
<tr>
<td>Daylight excl. direct sunlight</td>
<td>10,000</td>
</tr>
<tr>
<td>Overcast sky</td>
<td>1,000</td>
</tr>
<tr>
<td>Heavy overcast</td>
<td>100</td>
</tr>
<tr>
<td>Twilight</td>
<td>10</td>
</tr>
<tr>
<td>Late twilight</td>
<td>1.0</td>
</tr>
<tr>
<td>Full moon</td>
<td>0.1</td>
</tr>
</tbody>
</table>

> A starry night has luminance levels of about 0.001 cd/m² – scopic vision kicks in between levels of 0.01 and 0.000001 cd/m²
ELECTROMAGNETIC WAVES

Energy emitted from oscillating charged particles takes the form of a self-propagating wave …

The wave consists of two separate components, each a sinusoidally varying field:
The electric field $E$, and the magnetic field $B$, are in phase, and orthogonal to one another and the direction of propagation.

Polarization describes the electromagnetic field's alignment.

… traveling at a characteristic speed in vacuum: the speed of light, $c$. 
Solids are made up of a compact lattice of atoms bound together with charge.

An incident wave exerts forces on the charged particles, causing them to oscillate, which in turn generates another wave.
LIGHT AND MATTER
LIGHT AND MATTER
LIGHT AND MATTER
LIGHT AND MATTER
LIGHT AND MATTER

Incident wave

Reflected wave

Solid

Wave propagating in material: Transmission
Absorption “Glass” Filters

Example: ‘red’ filter

Interference Filters

A spectral plot shows % transmission as a function of wavelength and represents the percentage of energy that gets through the filter -- what is not allowed to transmit is either absorbed or reflected. $T + A + R = 100\%$

Band Pass filter ET600-40x
- CWL: 600nm
- FWHM: 40nm
- >95% peak T
- OD5 abs 300-1100nm (out of pass band)
Metals and other inorganic and organic compounds are melted in with the glass upon manufacturing.

These materials absorb some wavelengths of light (convert light energy to heat), while transmitting others.

Many of these ‘dyes’ contain non-ROHS compliant substances, e.g. lead, cadmium.

Reflection occurs at the air interface because of the refractive index mismatch (n~1 for air, and n~1.5 for most glass, resulting in approx. 4% reflection per side) – antireflection (AR) coatings can reduce these to <1%R.

The glass filter depicted at left is impregnated with dyes that absorb shorter wavelength light, but allow longer (red) wavelengths to transmit.
A multi-layer stack of very thin coatings is deposited onto one or both sides of a glass substrate such that particular wavelengths of light interfere upon reflection at the multiple interfaces.

Stacks are typically alternating layers of two non-absorbing materials, one with a ‘high’ index of refraction, and one with a ‘low’ index of refraction.

The glass itself could be an absorption glass filter – this is common but becoming less popular due to the materials in the glass.

With high energy, high precision sputter deposited coatings, it is possible to get a durable “all-in-one” filter coating on a single side of relatively thin glass.

The filter depicted above has a multi-layer that reflects shorter wavelengths at >98%R on one side, and an anti-reflection (AR) coating on the opposite side.
Absorption “Glass” Filters

- The peak transmission is determined by how much the material absorbs as a function of wavelength, and how much material there is, i.e., the thickness of the glass.
- The spectral pass-band is generally broad, and not easily controlled because there are only so many materials with unique absorption characteristics that can be used.

Interference Filters

- Can theoretically reach near 100% transmission in the pass-band.
- The number of layers and their individual thicknesses determine the spectral characteristics.
- The thicknesses of the layers are controlled to a very high precision, and in this way the band-shape can be engineered.
To effectively “block” unwanted wavelengths of light, an absorption filter must be thick (2-3mm); if two pieces are needed to define the edges of the pass-band (e.g., Red Bessel filter above) than the overall thickness of the filter can be several mm thick.

Overall coating thickness is typically < 20µm, and can be deposited onto glass substrates as thin as 0.5mm (3mm is typical) – and achieve an equivalent level of “blocking” as the 5mm thick absorption glass filter.
• The electric field is considerably more effective at exerting forces and doing work on charges than the magnetic field; can therefore think of electromagnetic waves as a simple single-component, 2-dimensional wave.

• Recall that many light sources are broad-band, or “white”, which means there are several wavelengths involved.
In order for waves to interfere, they must be of the same wavelength (monochromatic), and coherent (the waves must maintain their wave profile over the distance where interference is to occur).

A theoretical plane wave is infinitely coherent.

These waves are coherent even though the profile is obscured.

Here the profile changes as it propagates.
INTERFERENCE

In phase

Constructive Interference

Out of phase

Destructive Interference
For thick media, R1 and R2 cannot interfere – loss of coherence.

The reflected and transmitted intensity depends on the material’s optical properties, ex., refractive index.
For thin media, $R_1$ and $R_2$ can interfere. Reflected waves undergo a phase change going from a low index to a high index of refraction. $R_1$ can have a different phase than $R_2$ depending on the thickness of the film. Constructive interference results in reflection and $R_2$ – this is a basic anti-reflection coating. Thin film thicknesses that are multiples of $\frac{\lambda}{4}$ cause destructive interference of $R_1$.
Thin film thicknesses that are multiples of $\lambda/4$ cause destructive interference of $R_1$ and $R_2$ – this is a basic anti-reflection coating.
Incident Wave

Air

Thin Film

Glass

Transmission

Reflected Waves

Thin film thicknesses that are multiples of \( \lambda/4 \) cause destructive interference of \( R_1 \) and \( R_2 \) – this is a basic anti-reflection coating.
Multi-layer stacks use constructive interference of the reflected waves (at and off of the multiple interfaces) in order to “block” unwanted light.
INTRO TO SPUTTER COATING PROCESS

- Substrate is critically cleaned and loaded into a jig
- Vacuum chamber pumped to high vacuum (~ 10^-6 Torr)
- Dielectrics are made up of a metal target, and oxygen gas.
  - Metal is evaporated by magnetron sputtering
    - A negative high voltage is applied to the metal target and ionized Argon gas is accelerated toward the target thereby dislodging metal material.
    - The high energy of the sputtered material produces a much denser coating than what could be achieved using electron beam or thermal evaporation processes.
  - Oxygen gas is introduced and reacts with the metal during deposition, forming an oxide film on the substrate.
  - Ion-assist is used to deliver energy to the growing film in order to promote void-free growth and enhance film durability.

Dielectric, or metal-oxide deposition

![Diagram showing the sputtering process with substrates, targets, Ar+, O2, and an electric field.]
Quarter Wave Stack

- The basic stack is several alternating layers of high (H) and low (L) layers, each a quarter-wave of optical thickness.
- A quarter wave stack acts like a mirror, and is the basic building block for BPs, LPs, SPs, and other filter structures.
- The more stacks you add, the steeper the edges and the more reflection (“blocking”) you get.
Band-Pass Filter (Fabry-Perot)

- Several stacks, each with a series of alternating layers of high (H) and low (L) layers
- The basic stack is several quarter-waves of each material sandwiching a half-wave layer.
- The more stacks you add, the steeper the edges and more out of band blocking you get.
High Reflection, or “Blocking”

• To reject certain wavelengths, a series of ¼ wave stacks can be added along with a BP filter.

• The more blocking required, the more stacks you need for a particular range of wavelengths.

• The larger the range of wavelengths needed to be blocked/rejected, the more sets of stacks you need.
AOI = Ψ = 0°
Cone Angle = θ = 0° (collimated)
AOI = $\Psi$

Cone Angle = $\theta = 0^\circ$ (collimated)

AOI = angle of incidence: defines the angle between the surface normal and the incoming light beam’s axis;
CONE ANGLE VS. AOI

Acceptance Angle (range of AOI): 0° to Ψ
Cone Angle = θ = 0° (collimated)

Ψ = 45°  Ψ = 22°  Ψ = 0°
AOI = 0°
Cone Angle = θ
Semi-Cone Angle = θ/2

* The filter "sees" the integrated angles from 0 to θ/2°

* The filter doesn’t treat positive and negative angles differently, so the half-cone angle, NOT the full-cone angle, is the important factor

This sliver of light is incident at 0°
This sliver of light is incident at θ/2°
AOI = 0°
Cone Angle = θ
Semi-Cone Angle = θ/2

Diverging beam
Original Design without cone-angle consideration
Original Design without cone-angle consideration
New Design with cone-angle consideration
Filters can be designed for minimal shift due to cone-angle or acceptance angle.
LIGHT BALANCING FILTERS

Tungsten light source

![Sun Icon]

LBF

![Arrow] Bluer light

Graph showing the transmission of light against wavelength (nm). The transmission is high at 550 nm and decreases at shorter wavelengths.
## FILTER TRADEOFFS

<table>
<thead>
<tr>
<th>Feature</th>
<th>Low</th>
<th>Med</th>
<th>High</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blocking Depth (near-band OD)</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Blocking Range (esp. NIR)</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Blocking Range AND Depth</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Steep Edges</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blocking Range AND Depth AND Steep Edges</td>
<td></td>
<td></td>
<td>XXX</td>
<td></td>
</tr>
<tr>
<td>High Transmission</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size - small</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size - large</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Size - small w/ high volume (handling)</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Size - large w/ high volume</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Flatness (dichroics &amp; mirrors)</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Surface Quality better than 60/40</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Surface Quality better than 40/20</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Surface Quality better than 20/10</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Surface Quality better than 10/5</td>
<td></td>
<td></td>
<td>XX</td>
<td></td>
</tr>
</tbody>
</table>
TRANSMISSION + REFLECTION + ABSORPTION = 1
ANGLE OF INCIDENCE

Wavelength (nm)

%T

0.0 5.0 10.0 15.0 20.0 25.0 30.0 35.0 40.0 45.0 50.0 55.0 60.0 65.0 70.0 75.0 80.0 85.0 90.0 95.0 100.0

460 470 480 490 500 510 520 530

T495lp xr

55° 45° 35°
SURFACE QUALITY

<table>
<thead>
<tr>
<th>Optical Quality of Beam splitter</th>
<th>RWD (waves/inch) (p-v)</th>
<th>TWD (waves/inch) (p-v)</th>
<th>Wedge (arc-sec)</th>
<th>S/D</th>
<th>Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Field</td>
<td>10</td>
<td>1</td>
<td>60</td>
<td>40/40</td>
<td>1</td>
</tr>
<tr>
<td>Laser</td>
<td>2</td>
<td>0.25</td>
<td>5</td>
<td>40/20</td>
<td>1</td>
</tr>
<tr>
<td>TIRF</td>
<td>0.5</td>
<td>0.1-0.25</td>
<td>5</td>
<td>40/20</td>
<td>2-3</td>
</tr>
<tr>
<td>STED/SI</td>
<td>0.25</td>
<td>0.1</td>
<td>5</td>
<td>40/20</td>
<td>3-5</td>
</tr>
</tbody>
</table>

- Flatness is the most important parameter of a dichroic - application dependent
- The flatness and wedge specs can be expended into exciters and emitters
- In addition to surface quality, set up of the system is also an important consideration for experimental success, e.g. FRET
POLARIZATION-FRESNEL EQUATIONS

\[ R_s = \left( \frac{n_1 \cos \alpha - n_2 \cos \beta}{n_1 \cos \alpha + n_2 \cos \beta} \right)^2 \]

\[ R_p = \left( \frac{n_2 \cos \alpha - n_1 \cos \beta}{n_2 \cos \alpha + n_1 \cos \beta} \right)^2 \]

\( R_s \) and \( R_p \) . . . . . . reflectivity for s- and p-pol

\( \alpha \) . . . . . . . . . . . . angle of incidence (AOI)

\( \beta \) . . . . . . . . . . . . angle of reflection (AOR)

\[ R = \left( \frac{n_2 - n_1}{n_2 + n_1} \right)^2 \]

For normal incidence

\( (\alpha = \beta = 0) \)
BREWSTER'S ANGLE

$n_1 = 1, \quad n_2 = 1.5$

Reflection coefficient (%)

<table>
<thead>
<tr>
<th>Angle of incidence $\theta_i$ (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>30</td>
</tr>
<tr>
<td>40</td>
</tr>
<tr>
<td>50</td>
</tr>
<tr>
<td>60</td>
</tr>
<tr>
<td>70</td>
</tr>
<tr>
<td>80</td>
</tr>
<tr>
<td>90</td>
</tr>
</tbody>
</table>

$R_S$  $R_P$

Brewster's angle

Incident ray (unpolarised)

Reflected ray (polarised)

Reflected ray (slightly polarised)
# SPUTTER MATERIALS

<table>
<thead>
<tr>
<th>Material</th>
<th>Common Name</th>
<th>Material Type</th>
<th>Approx. Index of Refraction (in useful range)</th>
<th>Useful Range (spectrally has low absorption)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>aluminum</td>
<td>metal</td>
<td>n/a</td>
<td>200nm-IR</td>
</tr>
<tr>
<td>Ag</td>
<td>silver</td>
<td>metal</td>
<td>n/a</td>
<td>400nm-IR</td>
</tr>
<tr>
<td>SiO₂</td>
<td>silicon dioxide or &quot;silica&quot;</td>
<td>dielectric</td>
<td>1.4-1.5</td>
<td>200nm-IR</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>aluminum oxide or &quot;alumina&quot;</td>
<td>dielectric</td>
<td>1.7-1.9</td>
<td>200nm-IR</td>
</tr>
<tr>
<td>HfO₂*</td>
<td>hafnium oxide or &quot;hafnia&quot;</td>
<td>dielectric</td>
<td>2.0-2.5</td>
<td>250nm-IR</td>
</tr>
<tr>
<td>Ta₂O₅</td>
<td>tantalum pentoxide or &quot;tantala&quot;</td>
<td>dielectric</td>
<td>2.1-2.7</td>
<td>300nm-IR</td>
</tr>
<tr>
<td>Nb₂O₅</td>
<td>niobium pentoxide or &quot;niobia&quot;</td>
<td>dielectric</td>
<td>2.3-2.8</td>
<td>350nm-IR</td>
</tr>
<tr>
<td>a-Si**</td>
<td>amorphous silicon or just silicon</td>
<td>semi-conductor</td>
<td>3.6-3.8</td>
<td>1100nm-IR</td>
</tr>
</tbody>
</table>
**SPUTTER MATERIALS**

**Physical Thickness of a $\lambda/4$**

The graph shows the physical thickness (nm) of a $\lambda/4$ layer as a function of wavelength ($\lambda$) for different refractive indices ($n$):

- Blue line: $n=1.47$
- Red line: $n=1.7$
- Green line: $n=2.5$
- Purple line: $n=3.7$

The thickness increases linearly with wavelength for each material.
CONTACT

Jeff Carmichael
Technical and Product Marketing

John Atkinson
Optical Engineer

Chroma Technology Corp
Bellows Falls, Vermont
+1 (800) 824-7662
jcarmichael@chroma.com
jatkinson@chroma.com