BrightLine® Laser Dichroic Beamsplitters

Tech Note

Steeper. Wider. Flatter.

Advancements in filter technology have enabled techniques like live cell imaging and multiphoton microscopy, allowing researchers to study the mechanics and structure of cells with greater clarity and specificity. Advanced microscopy applications, in turn, are demanding ever greater optical filter performance in their quest to improve signal to noise and sensitivity.

In recent years, techniques such as TIRF and super-resolution microscopy have spurred the development of laser dichroics with improved flatness to reduce aberrations and simplify system alignment. Even slight curvature of a dichroic beamsplitter in reflection can affect focusing within an optical system, shifting the focal plane and degrading image quality.

Semrock's newest line of BrightLine laser dichroic beamsplitters are flatter than ever before. Designed for the most demanding laser fluorescence microscopy and instrumentation applications, they offer the best image quality available, as well as allowing the use of larger illumination and imaging beams. Available with unprecedented 1λ RWE on a 1 mm substrate and λ/5 RWE on a 3 mm substrate (peak to valley), they offer the steepest edges and widest bands for higher throughput and signal collection.

Flattest dichroics in the industry

λ/5 RWE on 3 mm and 1λ RWE on 1 mm

The steepest edges for higher throughput

Wider reflection bands — into UV for photoactivation

Why is Flatness Important?

Dichroic beamsplitters are used for routing and splitting of light by wavelength in a microscope. In doing so they may affect not only the direction of propagation of an illumination or emission beam, but also its wavefront.

Many layers are applied to a substrate to create a dichroic with steep edges and broadband performance. The intrinsic stress of hard glass coatings can be different from that of the substrate, which results in a slight bending or curvature of the dichroic. While this is not an issue in traditional epifluorescence microscopes, it can negatively impact alignment and imaging in more advanced microscopy systems.

The consequences of a slightly curved dichroic are much greater in reflection than in transmission. For a beam transmitted through a dichroic oriented at 45°, curvature of the dichroic results only in a slight divergence of the beam, with negligible aberrations. This explains why dichroic flatness isn't a defining specification in epifluorescence microscopy, as the emission signal is transmitted through the dichroic beamsplitter.

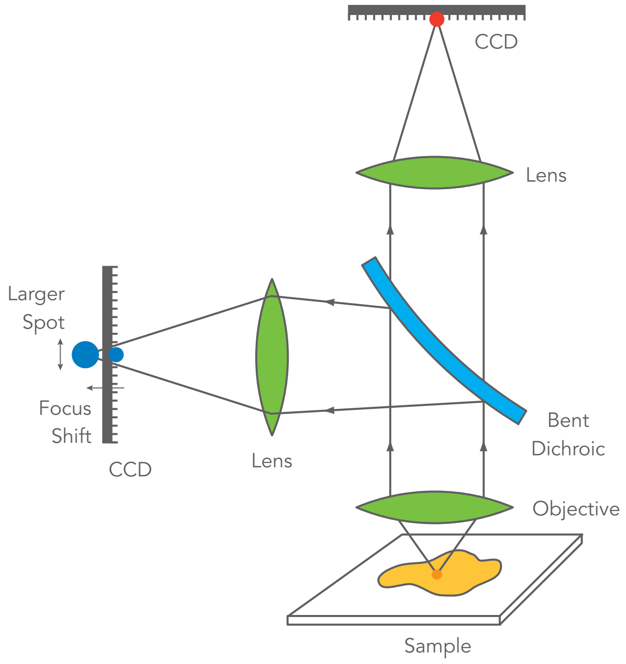

Figure 1. *A bent or curved dichroic shifts the focal plane of a beam in reflection, and affects spot size and shape.*

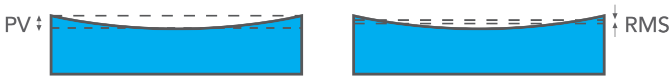

Figure 2. *Flatness as measured by the peak to valley method (P-V) vs RMS flatness.*

For a beam reflected from a dichroic beamsplitter oriented at 45°, however, any curvature of the dichroic has an additional focusing effect on the beam, shifting the position of the focal plane. The size or shape of the focused spot may also change. While the camera position can sometimes be adjusted to compensate for focal plane shift of an emission beam, both effects have the potential to significantly compromise image quality.

The flatness of an optic is generally described in fractions of waves of 633 nm light per inch, and may be given as RMS or peak to valley (P-V). RMS flatness is calculated as the standard deviation of the optical surface from an ideal surface, yielding an indication of the number of defects as well as their amplitude. Peak to valley (P-V) flatness, in contrast, is the absolute difference between the highest and lowest points on an optical surface relative to the ideal surface.

P-V flatness is often a more useful metric for evaluating the flatness of dichroic beamsplitters than RMS flatness. Both in theory and in practice, the deviation from flatness of a dichroic beamsplitter is dominated by spherical curvature, which is captured well by measuring from peak to valley. Spherical curvature is responsible for any shift in the position of the focal plane after reflection from a dichroic.

When working with Gaussian beams, the depth of focus is quantified by the "Rayleigh range," which is the distance from the beam waist (smallest focused spot) to the point where the waist has increased by a factor of √2. A beam (or image point) focused to within one Rayleigh range of the waist still appears to be in focus, making this a useful metric for evaluating the impact of a dichroic's curvature. If the focal shift due to curvature is less than one Rayleigh Range, it can be considered negligible.

The primary aberration that results when light is reflected from a dichroic beamsplitter at 45° is astigmatism, an effect that results in two distinct and asymmetric foci at different depths (sagittal and tangential). Though the beam is never fully focused, a compromise can be found at the midpoint between the two foci that results in slight blurring of the image and a larger focal spot, a point called the "circle of least confusion."

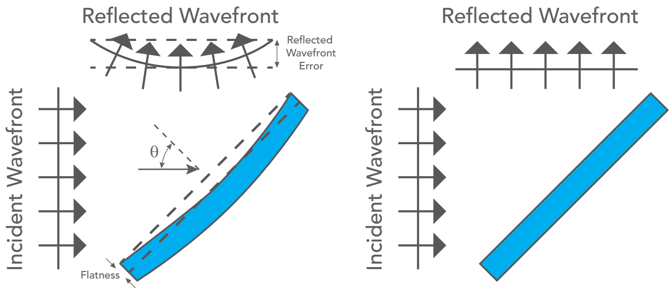

Figure 3. *Curvature of a dichroic beamsplitter has a focusing effect on the reflected beam.*

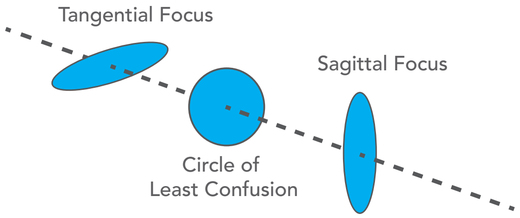

Figure 4. *Astigmatism due to dichroic curvature leads to two distinct foci in reflection, with an intermediate focus or "circle of least confusion" at the midpoint.*

Astigmatism becomes less of an issue as the radius of curvature of a dichroic beamsplitter increases. For one example experiment with an 11 mm diameter beam, the tangential and sagittal foci look very similar when using dichroics with radii of curvature larger than 150 m[1]. The geometric spot size for the circle of least confusion (D_C) can be estimated using the radius of curvature of the dichroic (*R*) and the focal length for the tube lens being used for imaging (f_{TL}). For very large values of *R*, the increase in beam diameter due to astigmatism becomes very small.

$$D_C \cong \frac{f_{TL}}{\sqrt{2}\,R} D$$

While astigmatism is the dominant aberration, third- and higher-order aberrations may also degrade the quality of a focused spot size for a collimated beam after a focusing or imaging lens.

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[1] *Flatness of Dichroic Beamsplitters Affects Focus and Image Quality, Semrock white paper*

Impact of Flatness on Applications

Dichroic beamsplitter flatness impacts the quality of the illumination light beam in TIRF microscopy, where the dichroic is used to reflect the illumination or excitation light into the objective for routing to the sample. A flatter dichroic provides a more uniform illumination beam at the sample plane, reducing background noise. If TIRF illumination beams are small, i.e., a few millimeters in diameter or less, the effect of dichroic curvature on the size and shape of the best focus spot is not as critical as it is for beams approaching 10 mm. Location of focus is a different matter. When a laser beam is reflected from a dichroic beamsplitter in a TIRF microscope, the beam must be focused at the back focal plane of the objective. A curved dichroic shifts this focal plane, so if the microscope has limited ability to adjust the collimation of the laser beam, it can be difficult to achieve successful TIRF excitation. TIRF forms the basis of many super-resolution imaging techniques such as PALM and STORM.

Compensating for focal plane shift can also be an issue in structured-illumination microscopy (SIM) with broadband or laser excitation, as the mask must be imaged in the excitation light path onto the sample. If the dichroic beamsplitter does not have sufficient flatness, it may prove impossible to image the grid onto the sample plane, or to construct a good quality super-resolution image from the emitted fluorescence signal, due to degradation of illumination imaging beam.

Regardless of the application, beam size plays a large role in determining the required flatness, as both focal plane shift and degree of astigmatism increase with beam diameter. A dichroic beamsplitter that is adequate for one researcher may not offer sufficient flatness for another researcher working with a larger beam within the same microscopy technique.

Two Well-focused Solutions

Given the trend toward larger beam diameters in TIRF and super-resolution microscopy and the prevalence of users developing their own super-performance microscope systems, dichroic beamsplitter flatness is becoming more critical than ever. Semrock's new and improved line of BrightLine laser dichroics with "Super-resolution/TIRF" grade of Flatness/RWE (see Flatness Classification table) offers two industry-leading solutions for these applications, coated on 1 or 3 mm substrates with the same spectral performance. Complete with the steep edges and broadband performance for which Semrock is known, these dichroics maintain low polarization splitting and reduced sensitivity to AOI. They also offer reflectivity down to 350 nm for photoactivation and super-resolution techniques, and extended transmission into the IR (1200 nm or 1600 nm) for maximum fluorophore compatibility and signal collection.

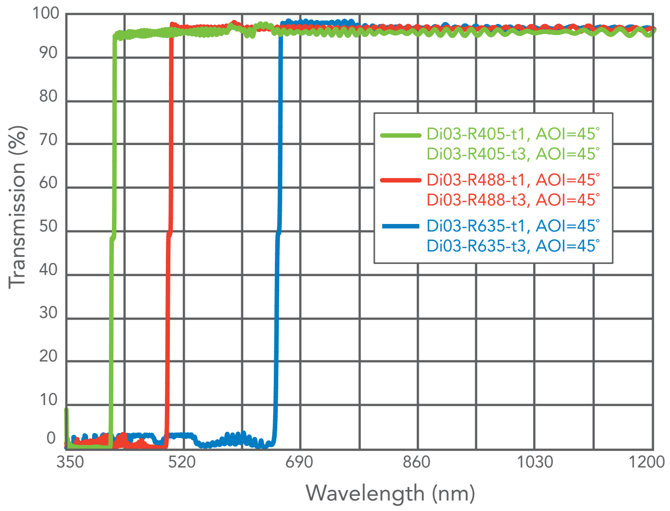


Figure 5. *Transmission spectra for three redesigned BrightLine single-edge laser dichroics. Note that spectral performance is identical for the Di03-Rxxx-t1 series on 1 mm substrates and Di03-Rxxx-t3 series on 3 mm substrates, including the steep edges and extended reflection and transmission bands.*

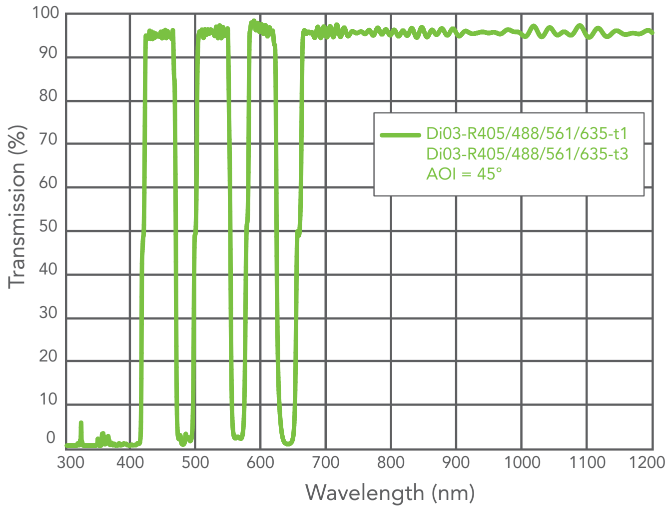


Figure 6. *Transmission spectra for a redesigned BrightLine quad-band laser dichroic. Note that spectral performance is identical for the Di03-Rxxx-t1 dichroic on 1 mm substrate and Di03-Rxxx-t3 dichroic on 3 mm substrate, including optimized transmission and reflection bands and extended IR transmission.*

On 1 mm substrates, Semrock's laser dichroics have been redesigned to considerably improve performance to 1λ P-V RWE for a finished part. This assures a focal shift of less than one Rayleigh range upon reflection for beams up to 10 mm in diameter. Availability of ultra-flat 1 mm dichroics for the first time ever allows use of standard microscope cubes. It also simplifies switching between cubes and minimizes beam shift in transmission, thus avoiding the re-alignment typically required when transitioning between 1 mm and 2 mm dichroics.

It is important to note that when working with dichroic beamsplitters, the mounting method itself can add considerable external stress, degrading flatness of the mounted dichroic. Therefore, in order to maintain high flatness, mounting of dichroics requires careful considerations.

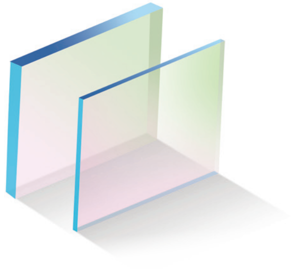
On 3 mm substrates, Semrock now offers unparalleled λ/5 RWE for a finished part. This allows beams of up to 22.5 mm to be used in reflection with less than one Rayleigh of shift in focus. These 3 mm dichroics are ideal for researchers designing their own ultra-high performance benchtop systems for super-resolution microscopy, as they allow use of larger beams with best image quality.

A comparison of Semrock's standard and laser dichroic offerings is summarized below, with radii of curvature to facilitate spot size calculations and specifications for predicted shift in focal plane.

Flatness / RWE Classification	Example Applications	Nominal Radius of Curvature	Maximum Reflected Beam Diameter, mm	Reflected Wave-front Error at 632.8 nm, PV	Dichroic Family, and Example Part Numbers
Super-resolution / TIRF	TIRF, PALM, STORM, STED	~ 1275 meters	22.5	<0.2λ	BrightLine Laser (Di03-R405-t3-)
		~ 255 meters	10	<1λ	BrightLine Laser (Di03-R405-t1-)
Laser	Confocal, combining/splitting laser beams	~ 30 meters	2.5	<6λ	BrightLine Laser (Di02-R405-) RazorEdge® (LPD01-488RU-) LaserMUX™ (LM01-503-)

Conclusion

With multiple options for flatness and thickness, Semrock offers more standard catalog solutions for laser dichroics with steeper edges and wider bands than any other optical filter provider. Whether for advanced imaging techniques like super-resolution microscopy and TIRF or confocal instruments, researchers now have access to the flattest dichroics available to optimize image quality and enable the use of larger beams.



For ordering, technical support, and contact information please visit www.semrock.com