Practical Flatness

Tech Note

Understanding Laser Dichroic Performance

BrightLine® laser dichroic beamsplitters set a new standard for super-resolution microscopy with λ/5 P-V Reflected Wavefront Error (RWE) performance. We'll explain how dichroic beamsplitter flatness affects an optical wavefront in reflection, and how to calculate the practical impact of flatness specifications on your system.

The field of super-resolution microscopy (SRM) has exploded in recent years, giving rise to techniques like structured illumination microscopy (SIM), stimulated emission depletion (STED), selective plane illumination microscopy (SPIM), and methods utilizing TIRF like PALM (photoactivated localization microscopy) and STORM (stochastic optical reconstruction microscopy), among others. Many of these techniques require reflection of a beam from a dichroic beamsplitter at a crucial point in the system – either a laser beam for excitation, or an image beam for collection.

It is generally understood that dichroic beamsplitters used in SRM applications need to be very flat in order to minimize distortion of the wavefront upon reflection, but it is not always clear how the flatness specification from a manufacturer translates into performance, particularly for a beam diameter or operating wavelength that differs from what has been specified. Differences in terminology between manufacturers can also be confusing when comparing products (e.g., radius of curvature vs flatness, or flatness vs RWE and RWD). This tech note seeks to present the theory in practical terms, clarify the ambiguities, and simplify the math required to calculate changes in focal position and spot size with flatness.

Industry definitions of flatness explained

Focal plane shift in reflection quantified

Calculations for impact to spot size in reflection

Beam diameter limits by wavelength for BrightLine® laser dichroic beamsplitters

What is Flatness?

Flatness is defined as the deviation of an optic from a perfectly flat surface, and is typically specified in fractions of a wave of 632.8 nm light per inch of optical surface. (In this article, for simplicity of discussion we reference 632.8 nm as 633 nm.) It may be given as the RMS flatness, in which case it is calculated as the standard deviation of the optical surface from the ideal surface. This method tells the user something about the number of defects as well as their amplitude. Another method of calculation is peak to valley (P-V) flatness, which reports the absolute difference between the highest and lowest points on an optical surface relative to the ideal surface. The flatness of an optic is sometimes referred to as its surface figure.

For thick, planar optics, the primary deviation from flatness comes from defects and polishing artifacts on the surface. In the case of hard coated thin film optics on thin and/or large diameter substrates, the intrinsic stress of the coating can be different from that of the substrate, resulting in a slight bending or curvature of the dichroic. As dichroics tend to be coated on thin substrates (typically 1-3 mm), their flatness is generally dominated by spherical curvature. Peak to valley (P-V) is thus a more useful method

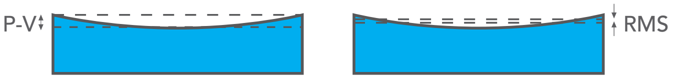


Figure 1. *Flatness as measured by the peak to valley method (P-V) vs RMS flatness.*

for specifying the flatness of dichroic beamsplitters for super-resolution microscopy applications than RMS error, as the degree of curvature of the dichroic is often key to performance of the system.

Radius of curvature is also a very useful metric to describe the flatness of dichroic beamsplitters, and is key to many calculations regarding optical wavefront distortion. Radius of curvature can be related directly to the P-V measure of flatness by the following equation:

$$\delta = \frac{D^2}{8R}$$

where δ is the flatness over the beam area, *D* is the diameter of the optical beam, and *R* is the radius of curvature of the dichroic beamsplitter. A factor of two increase in beam diameter thus degrades flatness by a factor of four. Conversely, a specification of $\lambda/10$ flatness per inch (i.e. 25 mm) is actually equivalent to better than $\lambda/60$ flatness for a smaller beam diameter of 10 mm. This underscores the importance of considering the beam diameter to be reflected from a dichroic when evaluating flatness needs, as illustrated in Figure 2 for Semrock BrightLine® laser dichroic beamsplitters.

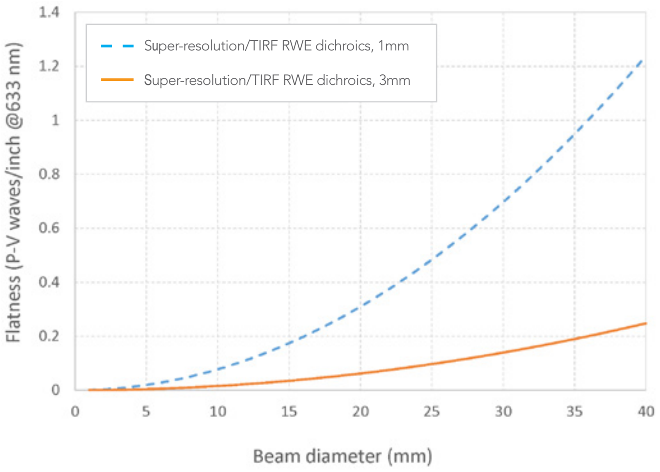


Figure 2. *Dichroic flatness in fractions of a wave as a function of beam diameter for BrightLine® laser dichroic beamsplitters.*

Reflection of Light by a Dichroic Beamsplitter

When light is reflected from a dichroic beamsplitter, the reflected wavefront acquires some curvature from the dichroic, the focusing effects of which must be added to the focusing due to other lenses within the system. The deviation of a wavefront reflected from an optic relative to a perfect wavefront reflected from an ideal plane surface is defined as the Reflected Wavefront Error (RWE) or Reflected Wavefront Distortion (RWD).

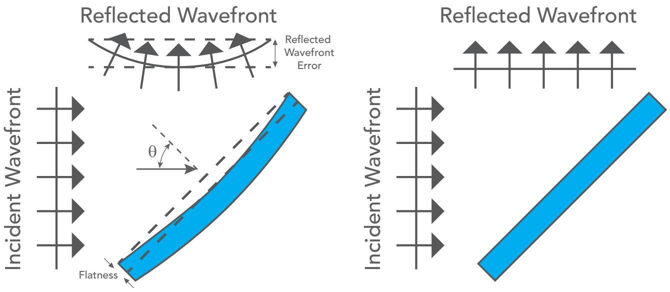


Figure 3. *Curvature of a dichroic beamsplitter has a focusing effect on the reflected beam.*

At normal incidence, the RWE is simply twice the flatness. At non-normal incidence, the relationship between flatness and RWE depends on the angle of incidence ***θ***.

$$RWE = 2\cos\theta \cdot Flatness$$
$$For\ \theta = 45°,\ RWE = 1.41 \cdot Flatness$$

Both the position and shape of the focus can be affected by curvature of a dichroic in reflection, an effect which diminishes with increasing radius of curvature. These effects were discussed in a previous tech note, BrightLine® Laser Dichroic Beamsplitters, primarily in qualitative terms. In the following sections, we will delve deeper into the mathematics of calculating focal shift and spot size for a given dichroic beamsplitter. As will be seen, radius of curvature is a more intuitively useful specification than

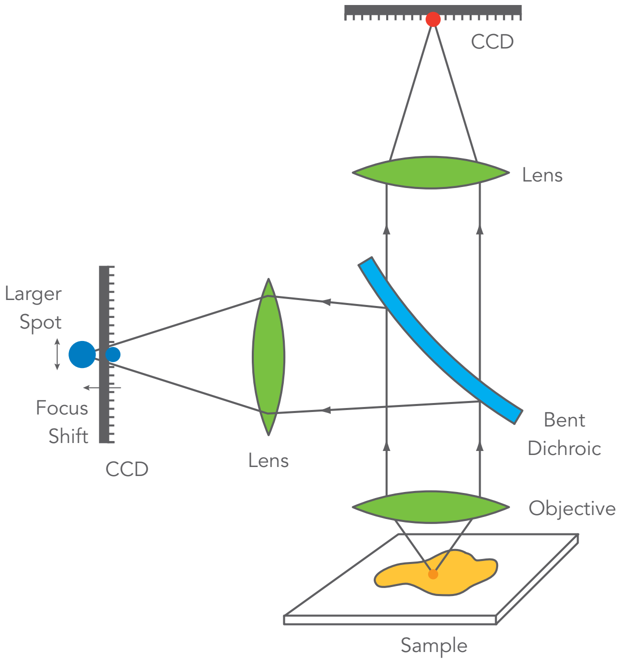


Figure 4. *A bent or curved dichroic shifts the focal plane of a beam in reflection, and affects spot size and shape.*

flatness in these calculations, as it relates more directly to focusing of the beam. Knowledge of the radius of curvature also allows these effects to be calculated for a given beam diameter or wavelength, which is particularly important when designing custom imaging systems.

Focal Plane Shift in Reflection

If light is reflected from a curved dichroic of radius R, it experiences a focusing effect as if it were transmitted through a lens of focal length $R/2$. This causes the position of the focal plane to shift, an effect that can often be compensated for by translating the detector or camera. In TIRF, however, the reflected beam is that of the excitation laser, so if the microscope is unable to adjust for the focal shift, successful generation of an evanescent wave to achieve fluorescence excitation can be difficult.

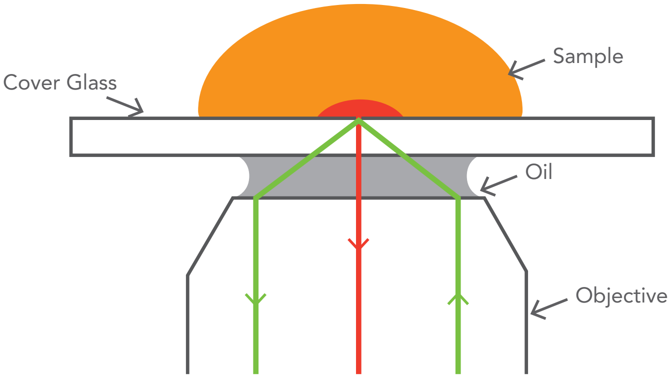


Figure 5. *Transmission of the illumination beam (green) through an objective in a microscope configured for TIRF. Emission signal is shown in red.*

In a system where light reflected from a dichroic beamsplitter is then focused by a lens of much shorter focal length f, the additional focusing effect of the dichroic (or relative focal shift Δf) can be approximated as $\Delta f/f \approx 2f/R$. Thus, the minimum focal shift that will occur can be expressed as:

$$\Delta f > \frac{2f^2}{R}$$

The larger the radius of curvature of the dichroic, the less focal shift will be experienced. Another approach is to look at the radius of curvature for which the focal shift will remain within a Rayleigh range of its undeviated position, i.e., within a distance that the beam will still appear to be focused. Using this method, it is not necessary to know the focal length of the lens being used in the system, but it is important to consider the beam diameter, D. In fact, to remain within the Rayleigh range when reflecting from a dichroic beamsplitter of radius of curvature R, the requirement for maximum beam diameter can be expressed as:

$$D < \sqrt{\frac{2\lambda R}{\pi}}$$

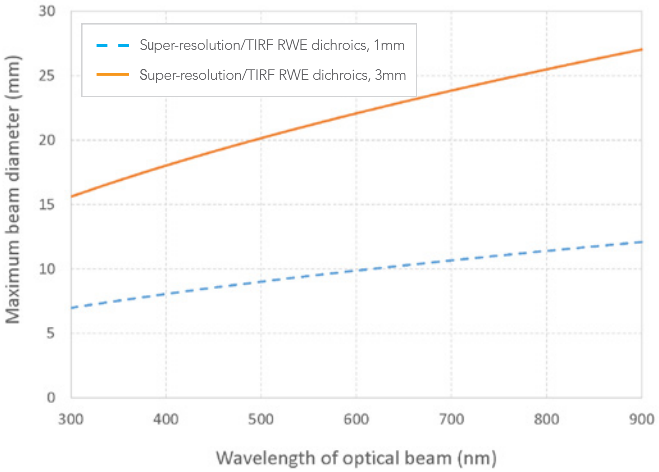


Figure 6. *Maximum beam diameter allowed to keep focal shift within a Rayleigh range as a function of operating wavelength for BrightLine® laser dichroic beamsplitters.*

It is important to note that the focal shift in this case has some dependence on the operating wavelength of the beam being reflected from the dichroic beamsplitter, i.e., the TIRF excitation wavelength or emission wavelength of the fluorophore in the imaging beam. The magnitude of this effect is demonstrated in Figure 6 for Semrock BrightLine® laser dichroic beamsplitters. As can be seen, larger beam diameters can be used with flatter dichroic beamsplitters due to their excellent flatness.

Astigmatism: Focus Spot Size Increase in Reflection

Curvature of a dichroic beamsplitter used in reflection also results in aberration, dominated by astigmatism. The optical system then develops two distinct, asymmetric foci at different depths. The "circle of least confusion" found at their midpoint is the point of both best symmetry and smallest spot size, though it results in some blurring of the image.

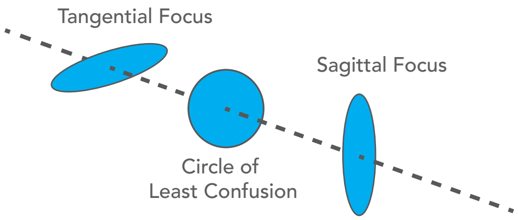


Figure 7. *Astigmatism due to dichroic curvature leads to two distinct foci in reflection, with an intermediate focus or "circle of least confusion" at the midpoint.*

The diameter of the circle of least confusion can be defined in terms of the original beam diameter, the radius of curvature of the dichroic beamsplitter, and the focal length of the tube lens f_{TL} being used, as shown in the equation below. While third and higher-order aberrations can also affect spot size, this approximation yields a worst-case scenario.

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

Though this calculation can reasonably estimate spot size for larger beams, smaller beams are limited by diffraction, and can only be focused so tightly. This tends to be the case for the values of *R* found in super-resolution imaging applications, and thus the Airy disk is considered a more useful measure. The Airy disk represents the diffraction-limited focus, and thus so long as the circle of least confusion remains below this value, the spot size will not appear to be affected by astigmatism. This will be true for beam diameters which meet the conditions of the following equation:

$$D < \sqrt{1.22\sqrt{2}\,R\lambda}$$

As with focal shift, the condition for negligible spot size change is dependent on the wavelength of the reflected light, as illustrated below for Semrock BrightLine® laser dichroic beamsplitters.

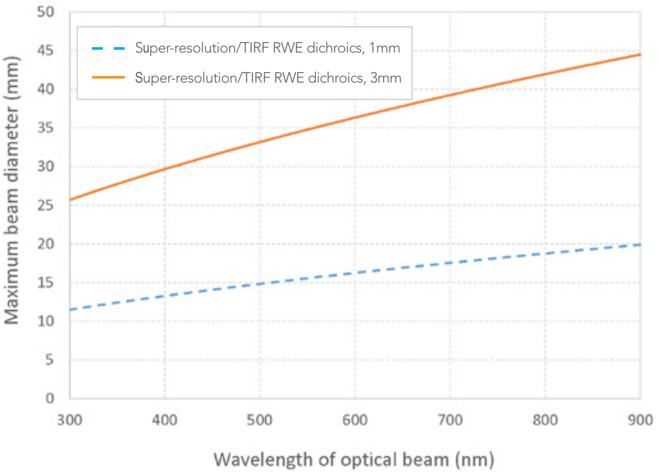


Figure 8. *Maximum beam diameter allowed to ensure spot size is not increased by astigmatism, shown as a function of operating wavelength for BrightLine® laser dichroic beamsplitters.*

How Flat is Flat?

Traditional epifluorescence microscopy does not place stringent flatness requirements on the dichroic beamsplitters used, as the illumination beam does not have stringent requirements on reflected beam quality in order to achieve widefield illumination.

Laser dichroic beamsplitters for super-resolution microscopy techniques, however, are extremely sensitive to optical wavefront distortion, and require extremely high flatness. When a thicker dichroic or smaller beam diameter cannot be used, this must be achieved through careful design and manufacture to minimize coating-induced stress that can lead to curvature. Our BrightLine® laser dichroic beamsplitters are offered in two high flatness options with the steepest edges, highest transmission, and widest transmission and reflection bands in the industry for the brightest, clearest images possible.

On 3 mm substrates, our dichroic beamsplitters offer λ/5 P-V RWE performance. This allows the use of larger beam diameters than ever before in TIRF methods like PALM and STORM with minimal impact to the reflected beam – up to 22.5 mm for reflected laser beams and up to 37 mm for reflected imaging beams at 633 nm. This thicker substrate also facilitates mounting in custom-designed tabletop systems.

On 1 mm substrates, our dichroic beamsplitters offer 1λ P-V RWE performance. These dichroics are ideal for use in SIM, SPIM, laser-based confocal microscopy and all methods utilizing TIRF. Use of a standard dichroic substrate thickness allows these dichroic beamsplitters to be mounted in industry standard cubes. They minimize beam shift in transmission and require no realignment when switching between cubes. Up to 10 mm diameter laser beams can be used in reflection, and up to 16.7 mm imaging beams can be reflected without compromising imaging performance. (See chart on page 6)

Measuring Dichroic Beamsplitter Flatness

High flatness dichroic beamsplitters require careful measurement to ensure they meet the requirements for a given SRM application, as identifying the source of wavefront aberrations once installed in a system can be challenging at these low levels.

Semrock tests the flatness of individual dichroic beamsplitters using a Fizeau laser interferometer such as the Zygo "Mark" or "Fiz" series, at normal incidence in reflection. Measurement of unmounted flatness is not sufficient for dichroics to be installed in cubes, as the mechanical mounting process can induce significant stress and distortion, even reducing performance to multiple waves per inch. This is why we test our 1 mm thick mounted dichroic beamsplitters after cube mounting to ensure optimal flatness performance from our *Super-resolution Microscopy Cubes.* These cubes are designed to maximize SNR and minimize artifacts in TIRF, Confocal, PALM, STORM, SIM, and other super-resolution techniques.

The images below capture representative wavefront measurements corresponding to dichroic beamsplitters of different flatness.

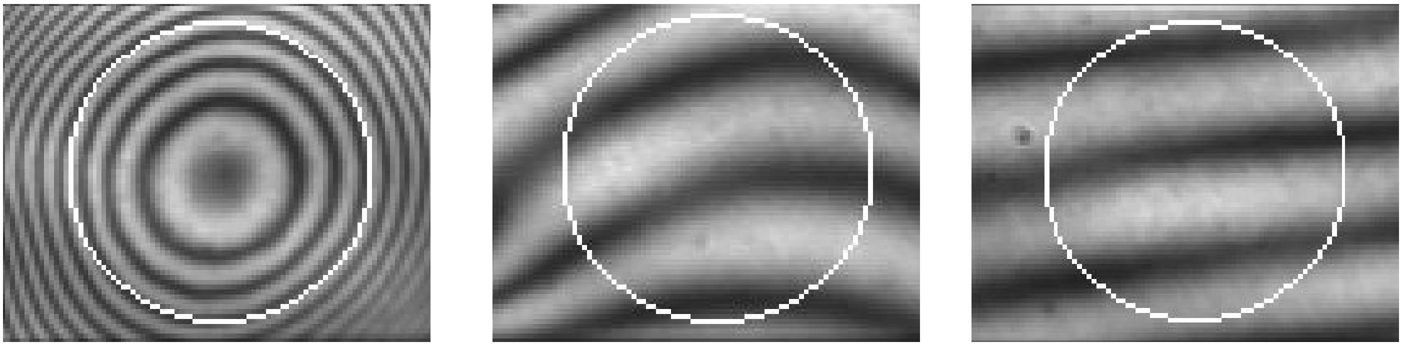
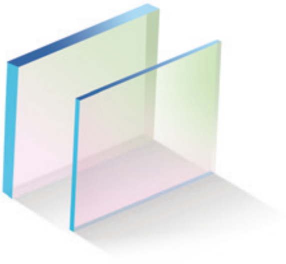


Figure 9. *Interferometer fringe patterns for dichroic mirrors of different flatness (left to right: 3λ, 1λ, λ/5 P-V RWE @ 633). Final flatness is a combination of coating film stress, substrate irregularity, and stress from mechanical mounting.*

Conclusion



Many terms are used to describe the flatness of dichroic beamsplitters for super-resolution microscopy, from surface figure to TWE and RWE and radius of curvature. By understanding the theory behind reflected wavefront distortion, it is easy to translate radius of curvature of your particular dichroic into system performance and its limitations for a given beam diameter and wavelength. With more solutions for high flatness laser dichroic beamsplitters with better performance than any other optical filter provider, Semrock sets a new standard for signal and SNR ratio in super-resolution microscopy.

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-)

Related Articles

- Product Overview - BrightLine Laser Dichroic Beamsplitters
- Tech Note - BrightLine Laser Dichroic Beamsplitters
- White Paper - Flatness of Dichroic Beamsplitters Affects Focus and Image Quality
- Presentation - Optical Filters: Flatness

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