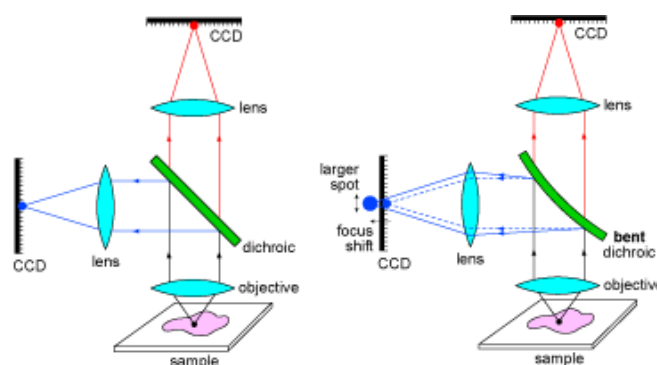




Flatness of Dichroic Beamsplitters Affects Focus and Image Quality

Optical filters are generally comprised of multi-layered thin-film coatings on plane, parallel glass substrates. All Semrock filters use a single substrate with coatings on one or both sides to maximize transmission and reliability and minimize artifacts associated with multiple interfaces. The glass substrate is not always perfectly flat, especially after it is coated, since the intrinsic stress of hard glass coatings can be different from that of the substrate thus causing slight bending of the substrate. Fortunately, this bending has no noticeable effect on light transmitted through an optical filter at or near normal incidence. For light incident at high angles of incidence, as is the case for a 45° dichroic beamsplitter, the only effect of a bent substrate on transmitted light is a slight divergence of the beam axis, similar to the effect of a small wedge in the substrate. The quality of a beam transmitted through a slightly bent substrate remains exceptional until the bending radius of curvature is very small (much less than 1 meter). Thus the focused spot size of a laser beam or the aberrations associated with an imaging beam transmitted through a bent filter are similar to their values with no filter present.

However, a bent filter substrate can have a significant impact on reflected light. This situation typically arises when an excitation beam is reflected off of a dichroic beamsplitter before impinging on a sample object, or when an imaging beam is split into two colors using a dichroic beamsplitter. Two main effects may occur: (1) the position of the focal plane shifts and (2) the size (or shape) of the focused spot changes or the quality of the image is compromised.



Focal plane shift

Often a small shift of the focal plane is not a problem, because a lens or camera adjustment can be made to compensate for the shift. For example, when an imaging beam is split by a dichroic onto two different cameras, a minor adjustment to the position of the camera associated with the reflected light path is generally possible. However, in some cases a focal shift cannot be tolerated. For example, when a laser beam is reflected off of a dichroic beamsplitter in a Total Internal Reflection Fluorescence (TIRF) microscope arranged in the epifluorescence configuration, the laser beam must be focused at the back focal plane of the objective, and too much focal shift caused by a bent dichroic can make it impossible to achieve this condition. Or, in a structured illumination microscope with broadband excitation, it might be impossible to image the grid onto the sample plane if the dichroic beamsplitter has too much bending.

When light is reflected off of a spherically curved surface with radius R , the light behaves as if it were transmitted through a lens of focal length $R/2$. If the light is subsequently focused by a different lens of focal length f , the reflection off of the curved surface causes the focus to shift by an amount Δf relative to the focus position resulting from reflection off of a perfectly flat surface. Based on a simple first-order optics approximation, the relative focal shift is $\Delta f/f \sim 2f/R$. Thus, to maintain a relative focal shift $\Delta f/f$ below a certain required value, the radius of curvature of the bent filter surface should exceed

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

For example, suppose a laser beam is reflected off of a dichroic beamsplitter before being focused to a spot at the sample plane by a 40X, 0.75 NA microscope objective, which has a focal length of $f = 5$ mm, and suppose we want to keep the focal shift below 5 mm, or the relative focal shift below 0.1%. Then the radius of curvature of the dichroic must be greater than about 10 meters.

When working with gaussian beams (often an excellent model for a laser beam), 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. Practically, the Rayleigh Range defines the depth of focus, because a beam (or image point) focused to a location within one Rayleigh Range of the waist appears to be in focus. If we use the criterion that the focal shift should be less than one Rayleigh Range, then the radius of curvature of a reflecting filter in front of a focusing lens should exceed

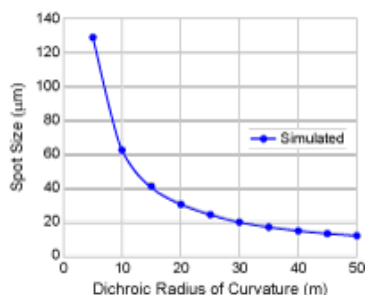
$$R > \frac{\pi D^2}{2\lambda}$$

where D is the diameter of the beam at the focusing lens and λ is the wavelength of light. Note that the required radius of curvature is independent of the focal length of the lens. As an example, for a 1 mm diameter laser beam the radius of curvature of the dichroic should be greater than about 3 meters. Semrock's laser-quality dichroic beamsplitters are generally specified with a maximum beam diameter, D, for which the focal shift will be less than one Rayleigh Range at the edge wavelength of the beamsplitter. For instance, the Di01-R488-25x36 is a BrightLine Laser Dichroic which will cause less than one Rayleigh Range of focal shift for a 488 nm laser beam as large as 2.5 mm in diameter. For a 1 mm diameter beam, the focal shift will be less than 1/6th of the Rayleigh Range.

As an imaging example, let's assume a 40X, 0.75 NA microscope objective, for which the beam diameter is about 11 mm. Thus the radius of the dichroic must exceed about 367 meters for green light, in order to observe no noticeable focal shift. Most dichroics are not nearly this flat, and thus in general there is a noticeable focal shift for light reflected off of a dichroic relative to a perfectly flat mirror. However, as pointed out above, this type of focal shift is generally easily accommodated.

Compromised image quality

When light incident at 45° is reflected off of a dichroic beamsplitter with a slight bend, then third- and higher-order aberrations (such as astigmatism) can degrade the quality of a focused spot size (for a collimated beam) after a focusing lens or of an image after an imaging lens. These effects can be accurately modeled using ray optics with standard optical modeling software. As an example, the graph below shows the spot size obtained at an image plane that results from a perfect point source object after reflecting off of a dichroic beamsplitter with various radii of curvature.



This plot is based on a typical epifluorescence microscope configuration, assuming the point source is at the sample location, and is imaged onto the image plane (e.g., CCD surface) by a 40X, 0.75 NA objective and a 200 mm focal length tube lens. The reflection off of the dichroic is assumed to occur mid-way between the objective and the tube lens. The field of view of the system is assumed to be limited by a 20 mm diameter field size at the camera plane. Similar results are achieved with a 10X, 0.3 NA objective, or a 100X, 1.3NA NA oil-immersion objective. For comparison, the diffraction-limited spot size that would result from perfect objective and tube lenses and a perfectly flat dichroic is approximately 12 µm.

Reflected image quality can be worse than the ideal diffraction-limited response for dichroics that are not perfectly flat, though it should be noted that the true spot size at the image plane will be appreciably larger than the diffraction-limited spot size due to aberrations from the objective and the tube lens. Nevertheless, care should be taken to select flatter dichroics, such as those designed for laser applications, when imaging reflected light.