Maximizing the Performance of Advanced Microscopes by Controlling Wavefront Error Using Optical Filters

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1. Abstract

A fluorescence imaging system includes a number of optical components in the light path. Any of these components may introduce distortions of the optical wavefront. These distortions can result in negative effects on image quality such as reduced contrast in standard microscopy, or compromised resolution in high performance microscopy. In a growing number of microscopy applications, reducing wavefront distortion is critical to even achieving the intended microscopy method. It is therefore becoming ever more important to know how to specify and select optical filters that minimize wavefront aberration in order to maximize or even to enable optical system performance. This is especially true since optical filters are the most frequently updated optical elements in a microscope after initial purchase, as well as a critical component in the design of new optical systems. This article (1) elucidates how to optimally select optical filters for different high performance microscopy applications, and (2) provides specific guidance on choosing filters from Semrock's extensive catalog that guarantee the wavefront distortion performance required for specific applications.

2. Background

2.1 Beam Distortions in Microscopes

A standard epifluorescence microscope can be cartooned as in Figure 1. Excitation and emission light traverses a series of optical elements in the microscope, including lenses and optical filters. Any of these elements can introduce wavefront aberrations, if the surfaces of the element depart from their respective ideal shapes, resulting in degraded image quality.

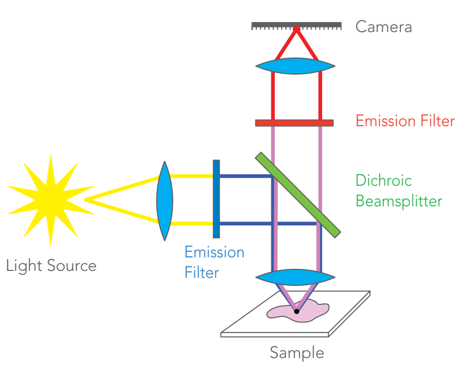


Figure 1: Schematic drawing of an epifluorescence microscope

Two types of beam distortions arise from non-flat surfaces of optical filters: Reflected Wavefront Error and Transmitted Wavefront Error. These wavefront errors are described in some detail in [3]; what follows is a summary. Both errors use the idea of a wavefront, defined as a surface along which the phase of the wave is constant; a wavefront is usually locally perpendicular to the local direction of propagation of light.

2.2 Flatness and RWE

The distance by which a reflecting surface deviates from perfect flatness, as shown in the Figure 2a, is called the Peak-to-Valley (PV or P-V) Flatness, and is measured in units of a reference wavelength (632.8 nm in the ANSI standard and 546.07 nm in the ISO standard) over a specified distance (often one inch, or 25.4 mm).

Figure 2a shows that a flat incident wavefront is deformed into a non-flat reflected wavefront by reflection from

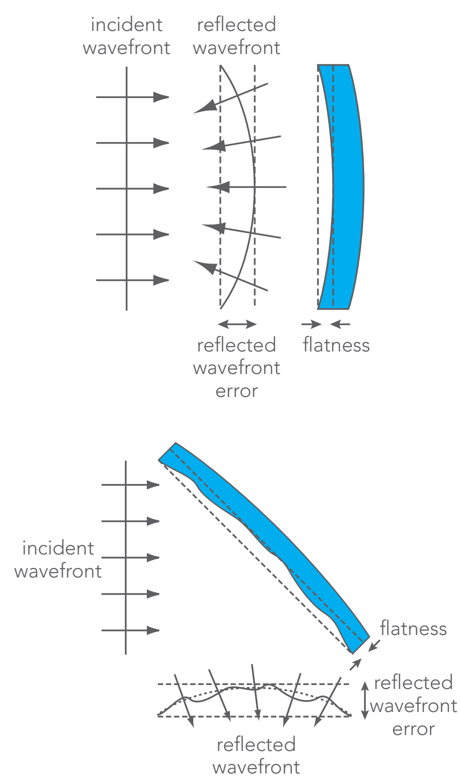


Figure 2: (a) Schematic effect of a non-flat reflecting surface on a wavefront incident at Angle of Incidence (AOI) = 0°. (b) Reflection at AOI = 45°, typical of a dichroic beamsplitter.

the non-flat surface. The deviation of the reflected wavefront from flat, called the Peak-to-Valley (PV) Reflected Wavefront Error (RWE), is twice the amount of surface deviation (i.e., 2 × PV Flatness). The "PV" specification is generally omitted in this paper, but Flatness and RWE should be understood here as PV.

The relation RWE = 2 × Flatness is true for zero Angle of Incidence (AOI = 0°). For AOI > 0° (Figure 2b), RWE = 2 × Flatness × cos (AOI). Like Flatness, RWE is usually specified in units of a reference wavelength over a specified distance, again usually one inch. RWE and Flatness can be specified with the same units, but RWE (unlike Flatness) must include specification of the associated AOI.

When a dichroic beamsplitter is mounted in a filter cube, its Flatness tends to undergo change due to nonzero mounting stress (See Appendix D). Flatness measured on the unmounted dichroic may therefore not provide a good basis for predicting wavefront error in the reflected beam once the dichroic is mounted in a cube. For component assemblies, such as the Semrock Super-resolution Microscopy Cubes, RWE is the preferred specification, as it better describes the performance of the cube in a system. Other ways to specify Flatness are given in Appendix A.

2.3 Impact of RWE

RWE in excitation and emission filters is not usually a source of significant image degradation in microscopy, because the reflected light is typically discarded and not used in creating the image of the sample.

In contrast, RWE due to dichroic beamsplitters can be a very significant source of image degradation. In microscopes in which a dichroic is used to *reflect* the excitation beam to the sample plane (see Figure 1), the entire reflected beam can acquire unwanted RWE from a non-flat surface, and this RWE will degrade the quality of the excitation beam. In some microscopy methods, such aberrations in the excitation beam may significantly compromise imaging, as is described in later Sections. Similarly, in systems where excitation light is *transmitted* through the dichroic beamsplitter to the sample plane, the emission signal is reflected by that dichroic and the emission beam can thereby acquire unwanted RWE from a non-flat dichroic surface, degrading image quality at the detector. More specific examples of deleterious effects of degraded dichroic beamsplitter RWE are found below.

2.4 TWE

By analogy with RWE, PV Transmitted Wavefront Error (TWE) refers to the departure of the wavefront from perfect flatness due to passage *through* an optical element, as illustrated in Figure 3. Total TWE can include contributions due to Tilt (caused by transmission through a wedge), Power (caused by the lens-

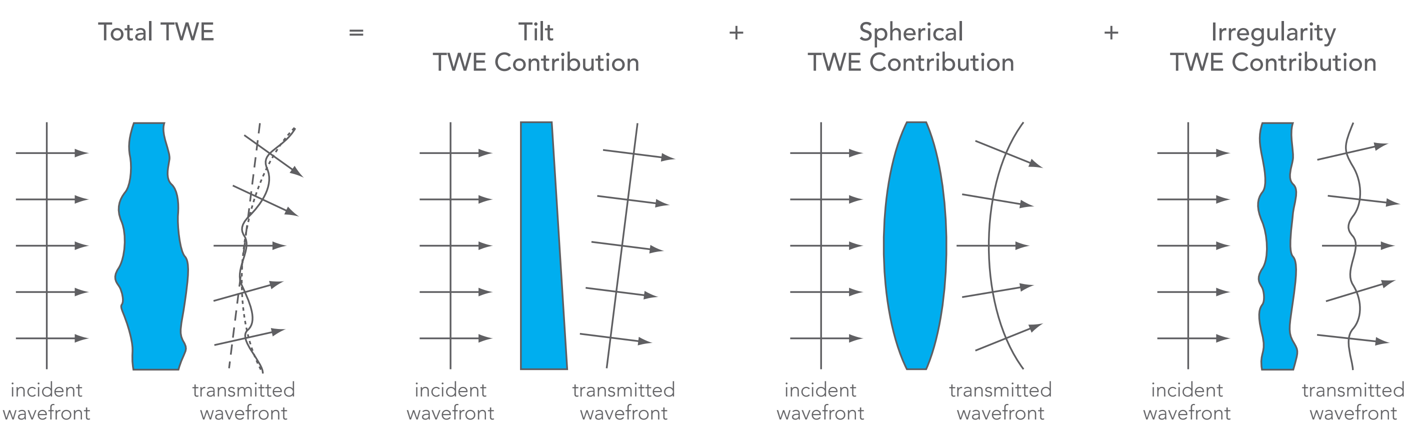


Figure 3: Schematic illustration of the effect of Transmitted Wavefront Error (TWE) on the wavefront of a transmitted beam.

like shape), and Irregularity (caused by uneven entrance and/or exit surfaces). In the case of optical filters, the dominant contributor to TWE is Irregularity. TWE is usually measured in units of a reference wavelength over a specified distance, again usually one inch. The specification "PV" is generally omitted in this paper, but TWE should be understood here to refer to the PV values.

2.5 Impact of TWE

Since microscope performance depends on transmission through many optical elements, excess TWE resulting from those elements can degrade both the excitation beam or image at the sample plane and the emission beam or image at the detector. For example, a laser beam to be focused to a diffraction-limited line or spot may not achieve this goal if TWE between source and destination is excessive.

3. Imaging and Non-imaging Light Beams

Any light beam in the excitation and emission paths of a fluorescence microscope can be broadly classified as an *Imaging Beam* or a *Non-imaging Beam*. Understanding this classification scheme is very useful when choosing optical elements for appropriate Flatness.

3.1 Imaging Beams

An Imaging Beam is a beam of light that must preserve two-dimensional image information through the optical system. For example, in a conventional widefield microscope, an Imaging Beam images the object from the sample plane to the eye or to a multi-pixel-based detector such as a CCD or sCMOS camera.

Correct two-dimensional image formation requires that the relative (x, y, z) locations of point image sources be precisely maintained between object and its image; if this is not the case, a blurred or distorted image results. Minimizing distortion of the spatial information in turn requires that wavefront error due to optical filters be minimized.

3.2 Non-imaging Beams

In contrast, a Non-imaging Beam need not preserve two-dimensional image information through the optical system. For example, the excitation beam in a conventional widefield microscope is a Non-imaging Beam, since it does not contain two-dimensional information. However, not all microscopes use Non-imaging Beams for excitation; for example, structured illumination systems convey patterned excitation light to the sample plane, and are examples of Imaging Beams used in the excitation light path.

The next Sections provide guidance on Flatness requirements for Imaging and Non-imaging Beams.

4. Applications

Many of a growing number of high performance fluorescence microscopy techniques are listed in Table 1. This article does not cover the full range of technologies; readers are referred to excellent overviews of some of these techniques [1, 2].

Table 1 indicates for each type of microscope system if Imaging and/or Non-imaging Beams are used. Note that an excitation beam can be either an Imaging or a Non-imaging Beam, and similarly for an emission beam.

The color coding of each cell border in the Table indicates the criterion to be used when choosing the RWE for a dichroic beamsplitter used for reflection; these criteria (green for Rayleigh Range, yellow for Airy Disk) are discussed in Section 6.

4.1 Excitation

Fluorescence excitation sources in earlier microscope systems were either broadband gas discharge lamps or monochromatic lasers with small diameter (< 5 mm) beams of circular profile. Incoherent broadband sources such as arc lamps are now being replaced by light engine based arrays of semi-monochromatic light emitting diodes (LEDs). Single-point scanning approaches continue to use coherent laser light sources, but now with much higher average light intensities at the sample (e.g. multiphoton and STED). Irrespective of the microscopy technique, excitation light can be categorized as an Imaging or a Non-imaging Beam (Table 1).

Demand for higher microscope resolution has resulted in increases in the Numerical Aperture (NA) of the

Microscopy Technique	Excitation Path		Emission Path	
	Light Source	Beam Type	Detector	Beam Type
Widefield Fluorescence Microscopy	Broadband / Laser	Non-imaging	Pixel array	Imaging
Widefield Fluorescence Microscopy with Deconvolution	Broadband / Laser	Non-imaging	Pixel array	Imaging
Total Internal Reflection Fluorescence (TIRF) Microscopy	Laser	Non-imaging	Pixel array	Imaging
Photoactivatable Localization Microscopy (PALM)	Laser	Non-imaging	Pixel array	Imaging
Stochastic Optical Reconstruction Microscopy (STORM)	Laser	Non-imaging	Pixel array	Imaging
Structured Illumination Microscopy (SIM)	Broadband / Laser	Imaging	Pixel array	Imaging
Patterned Illumination Microscopy	Broadband / Laser	Imaging	Pixel array	Imaging
Programmable Array Microscopy	Broadband / Laser	Imaging	Pixel array	Imaging
Confocal Single-point Scanning Microscopy	Laser	Non-imaging	Point	Non-imaging
Confocal Multi-point Scanning Microscopy	Broadband / Laser	Non-imaging	Pixel array	Imaging
Stimulated Emission Depletion (STED) – Pulsed Microscopy	Laser	Non-imaging	Point	Non-imaging
Stimulated Emission Depletion (STED) – Continuous Wave Microscopy	Laser	Non-imaging	Point	Non-imaging
Multiphoton Fluorescence Microscopy	Laser	Non-imaging	Point	Non-imaging
Multi-spot Multiphoton Fluorescence Microscopy	Laser	Non-imaging	Pixel array	Imaging
Coherent Anti-Stokes Raman Spectroscopy (CARS)	Laser	Non-imaging	Point	Non-imaging
Stimulated Raman Scattering (SRS)	Laser	Non-imaging	Point	Non-imaging
Second Harmonic Generation (SHG)	Laser	Non-imaging	Point	Non-imaging
Third Harmonic Generation (THG)	Laser	Non-imaging	Point	Non-imaging
Combining multiple laser beams	Laser	Non-imaging	N.A.	N.A.

Table 1: A list of popular standard and advanced microscopy methods, categorized as to Imaging or Non-imaging Beam status. Pixel array detectors include CCD, EMCCD and sCMOS cameras; Point detectors include PMTs, APDs, SiPMs and SPADs. The color coding of a cell border gives the criterion for choosing RWE for a dichroic beamsplitter — green for Rayleigh Range and yellow for Airy Disk; see the text.

objective lens, resulting in increased diameter of objective lenses to beyond the standard Royal Microscopical Society value of 20.32 mm. The back aperture (exit pupil) of the lens must be completely filled (or slightly overfilled) by the excitation light for best use of the high NA. New OEM instruments are being designed to image bigger fields of view using much larger diameter objective lenses and cameras with larger sensors. All this has meant that larger excitation beam diameters are reflected (or transmitted, in some microscopes) towards the objective lens by dichroic beamsplitters. This in turn requires recognition of the importance of dichroic RWE when reflecting large diameter beams (e.g. Figure 2 in [3]).

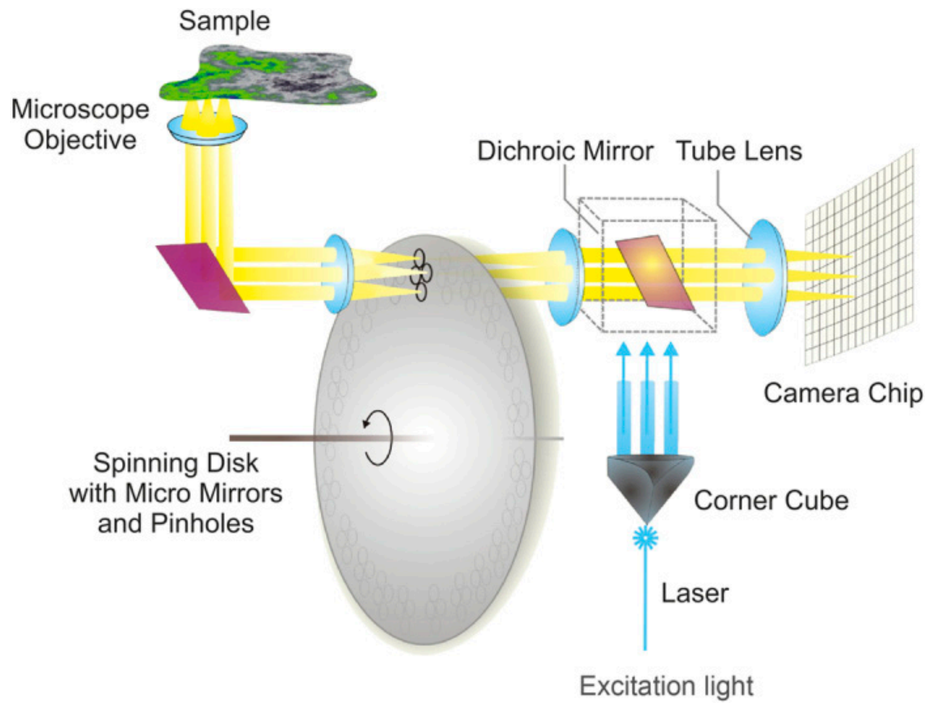


Figure 5: Layout of a typical spinning disk (multipoint) confocal microscope system. The blue laser excitation light is reflected towards the sample by the same dichroic beamsplitter that transmits the yellow emission light to the detector, and must be treated as an Imaging Beam since the positional array information of excitation light must be maintained after passing through the pinholes. Figure is reprinted unmodified from [5] under the Creative Commons Attribution License (CC BY) (http://creativecommons.org/licenses/by/4.0/).

4.2 Emission

On the emission side, dichroic beamsplitters in standard widefield fluorescence microscopes can transmit a ~20 mm diameter Imaging Beam that represents the real optical image (i.e. the imaged field of view, or FOV, of the specimen) to an image detector, and in these cases minimizing TWE in both dichroics and emission filters is required for best performance. In general, RWE is always critical when the emission signal of an Imaging Beam is reflected by a dichroic beamsplitter (or by a mirror) for imaging on a pixel array detector (see Table 1). Some widefield system designs – such as some spinning disk or multi-point confocal microscope configurations, and emission image splitters (e.g. dual camera systems) – reflect rather than transmit Imaging Beam information on the emission side, and in these systems ensuring low RWE is critical to prevent loss of focus and resolution in the system.

In contrast to standard widefield microscope systems, dichroic beamsplitters in single point scanning microscopy methods typically transmit discrete point sources of emitted fluorescence that are generated by raster scanning the excitation beam over the sample one point at a time. Each point source of fluorescence is transmitted to a "point" detector such as a photomultiplier tube (PMT), and the final digital image is formed one point (pixel) at a time based on the number of photons collected by the detector for each given point (pixel) in the final image. These are Non-imaging Beams, because the detectors are simply point detectors and no optical image is formed. In such systems, which use point detectors (see Table 1), there is little difference whether these Non-imaging Beams are reflected or transmitted by a dichroic beamsplitter, except perhaps to slightly decrease the efficiency of photon collection if significant aberrations arise from dichroic TWE or RWE.

Multipoint scanning confocal and multipoint multiphoton fluorescence microscopy methods such as the Yokogawa Spinning Disc confocal (Figure 5) require additional discussion. This method very rapidly scans an array of separate beamlets over the sample, and collects the resultant fluorescence emission, point by point, on a pixel array detector. In that sense, these are still "pixel by pixel" approaches, where no optical image is formed or transmitted simultaneously to a detector, and with each *individual* emitted fluorescence point source behaving as a Non-imaging Beam. Additionally, since multiple laser beams are either focused on a pinhole array in the excitation path, or on a pinhole array imaged onto a detector in the emission path, the dichroic beamsplitter thickness must have tight dimensional tolerances in order to avoid defocus for spatially distinct pinholes. For such applications, it is required that the dichroic not only maintain the required RWE over the diameter of individual beamlets, but also reduce wavefront error over the entire beam diameter spanned by the beamlets. Overall, because spatial relationships between all emitted point sources must be maintained in order to accurately reconstruct the final image, and because all emitted point sources are transmitted practically simultaneously to a pixel array detector, RWE and TWE criteria must be carefully evaluated utilizing optical systems design principles.

Note that whether a particular microscopy method uses an Imaging or Non-imaging Beam on the emission side depends on the design of that system. That is, a given green or yellow bordered method in Table 1 may either reflect or transmit an Imaging Beam, or sometimes both; see Section 6.

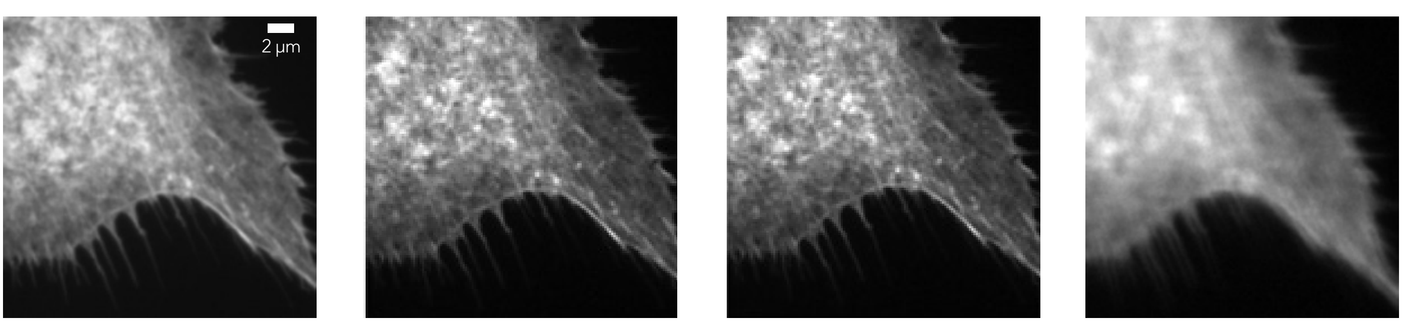
4.3 Imaging Beams

Generally speaking, in any of these microscope systems whose entries are bordered yellow in Table 1, excess of either RWE and/or TWE in the system's Imaging Beams may result in the final image having altered focus or blur, i.e. with diminished system resolution, and will often reduce the system's actual resolving power from the diffraction-limited ideal. As an example, Figure 4 demonstrates the effects on focus and resolution of using an insufficiently flat dichroic beamsplitter to reflect an Imaging Beam from the specimen image [6].

For super-resolution microscopy approaches aimed at breaking the diffraction barrier, the intended gain in resolution with these systems may not actually be realized, unless key optical elements are used to minimize RWE and TWE so as not to compromise maximum achievable resolution [4].

If we think of Imaging Beams as not just images of the specimen, but rather any two dimensional image information for which relative (x, y, z) localization of point sources must be maintained over some area in order to preserve system performance and resolution, then it can be understood that in some cases the *excitation* path may also contain an Imaging Beam. The yellow-bordered entries in Table 1 in the Excitation columns are such cases. Examples of such methods include structured illumination methods (Figure 6B) where faithful representation in x, y and z of spatial grid patterns projected into the sample plane is required for expected system performance.

Next we consider STED (STimulated Emission Depletion) microscopy. STED microscopy uses one laser to excite the fluorophore; this is a Non-imaging Beam. A second (depletion) laser is used to quench only the periphery of the resultant point spread function (PSF) using a doughnut-shaped (annular) beam



Radius of Curvature (m) 3414 158 84 7

Figure 4: Images of F-actin in bovine pulmonary artery endothelial cells (FluoCells® Prepared Slide #1, ThermoFisher Scientific, Waltham, MA, USA) after reflection by dichroic beamsplitters with varying radii of curvature on an BX41 microscope (Olympus Corporation of the Americas, Center Valley, PA, USA) with a 40×, 0.75 NA objective and Retiga camera (QImaging, Surrey, BC, Canada).

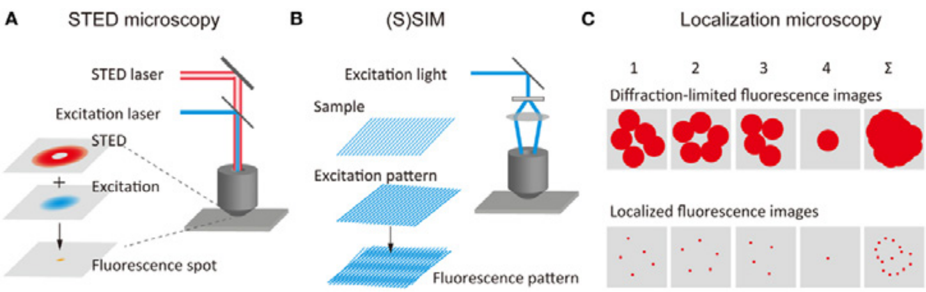


Figure 6: Schematic layouts of key principles of STED microscopy, (saturated) structured illumination microscopy ((S)SIM), and localization microscopy methods such as fluorescence photoactivation localization microscopy (FPALM) and stochastic optical reconstruction microscopy (STORM). Note that preserving the shape and/or pattern of the STED depletion laser (red annular beam in A) and the grid patterns in B is critical to system performance. However, the illumination beam in STED is a Non-imaging Beam, whereas the SIM excitation beam is an Imaging Beam. The Figure is reprinted unmodified from [9] under the Creative Commons Attribution License (CC BY) (http://creativecommons.org/licenses/by/4.0/).

profile (Figure 6A), thereby increasing resolution by effectively reducing the size of the initially excited PSF to below the diffraction limit. This depletion beam is also a Non-imaging Beam. STED "is extremely sensitive to optical aberrations that alter the expected size, shape, or regularity of the excitation and depletion beams, particularly the 'donut-shaped' depletion beam" [4]. Astigmatism is the dominant optical aberration caused by reflection from insufficiently flat beamsplitters [6], and some have found that the annular profile depletion beam required for STED is so sensitive to the effects of astigmatism that "in the presence of astigmatism, the focal fields are already so distorted that these two patterns probably would be useless for super-resolution optical microscopy" [7]. Preserving the annular profile (i.e. spatial representation) of the depletion beam is therefore critical to achieving super-resolution performance of the STED system. In order to achieve the best resolution, the STED beam typically utilizes a high powered laser, and the application demands the highest possible Flatness optics appropriate for typically expanded beam diameter in order to achieve highest resolution (Table 1) [8]. Both the STED and illumination beams are Non-imaging Beams. Section 6 further shows that Non-imaging Beams require lower RWE (higher Flatness) dichroic beamsplitters for a specified beam diameter.

In summary, while the effects of insufficiently flat dichroic beamsplitters and/or excessive RWE or TWE may be most noticeable when they impact the Imaging Beam of the specimen itself in the emission path (e.g. Figure 4), in the other examples above we can see that they may also similarly degrade system resolution and performance when the affected Imaging Beam is in the excitation path.

4.4 Non-imaging Beams

Effects of excess RWE and/or TWE in Non-imaging Beams on system performance are usually more subtle, particularly in widefield fluorescence applications. The excitation path in TIRF microscopy constitutes a not uncommonly experienced exception to this general rule; a practical example is given in the next Section.

More subtle performance deficits can occur, resulting from effects of TWE and/or RWE on excitation path Non-imaging Beams. A significant defocus error caused by excessive RWE will shift and expand the size of the expected excitation focal volume [6]. This will at minimum cause a deviation in the precise (x, y, z) position of the focal volume from the intended location. Applications requiring high power density such as multiphoton excitation may therefore experience a lower rate of multiphoton events due to excessive wavefront distortion over the beam diameter. Importantly, the ubiquitous epifluorescence based systems provide limited opportunity to adjust for defocus error of the excitation light, because the same objective must both focus excitation light on the sample, and focus light emitted from the sample on the detector. Dichroic beamsplitter RWE is of increased importance in such applications, particularly where focused spot size and location are critical to instrument performance.

Effects of excess TWE or RWE on performance of microscopes using Non-imaging Beams in the emission path are likely to be relatively minor, with the most drastic effects being possibly a slightly decreased efficiency of photon collection and decreased signal to background (contrast) ratio.

5. Practical Examples of Suboptimal Filter Choices

5.1 TIRF

TIRF microscopy is an unusual case in that the excitation path is Non-imaging, but there is a considerable negative impact of a suboptimal filter choice. If a non-flat dichroic beamsplitter is used in a TIRF system to reflect the excitation beam towards the sample, the curvature of the dichroic causes a shift in the focal position of the beam, which then may not achieve the intended focus at the back focal plane of the TIRF objective lens. This may make it difficult to achieve TIRF, as the excitation beam photons will have a much wider range of angles, and any small resulting TIRF signal may be masked by a much larger non-TIRF signal from deeper ranges of the sample. Figure 7 shows the excitation beam of a TIRF system with insufficiently and sufficiently flat dichroic beamsplitters.

5.2 Emission Image Splitting

Figure 8 illustrates the microscope configuration in an example of two emission channel Imaging Beams. The dichroic beamsplitter in the emission path reflects part of the spectrum towards one pixel array detector, and transmits a different part of the spectrum towards another. The two array detectors are co-registered, i.e. pixel-aligned, and mathematical combination of the two images yields maps of quantities relevant to biology, e.g. Ca^{++} concentrations and FRET values. The image splitting dichroic must be sufficiently flat to assure no distortion of the waveform in the reflected channel. If the RWE is too high, the reflected image is distorted, and image co-registration is impossible. Figure 8 illustrates this issue, for a telescope application rather for a microscope, though there is little difference in concept between the two systems in this regard. The upper panel shows aberration-free images of point objects, unaffected by transmission through an insufficiently flat dichroic beamsplitter; the left panel shows very significantly aberrated images of those same point objects, now reflected from that same non-flat dichroic.

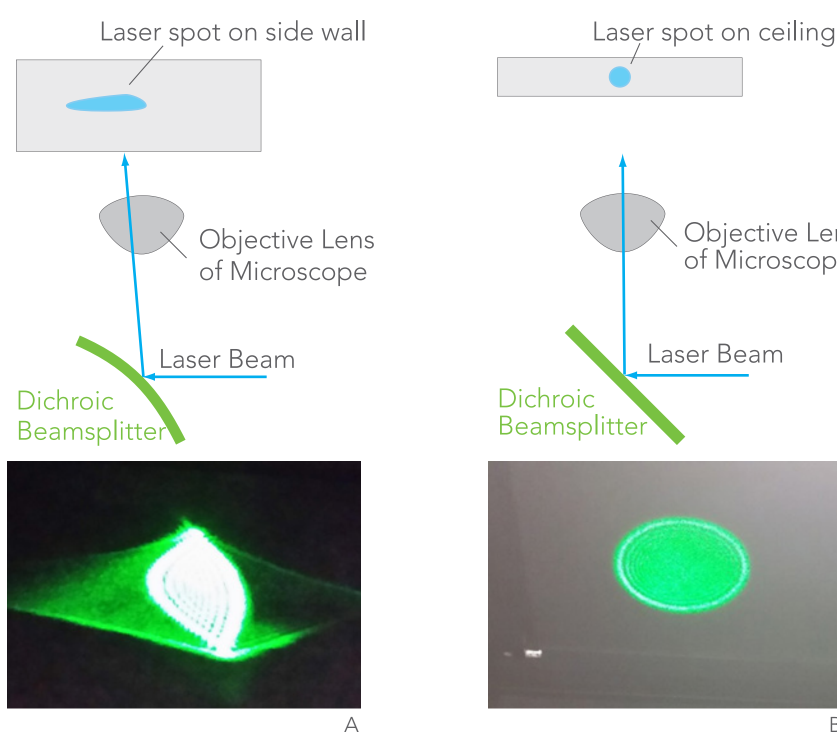


Figure 7: Effect of excessive RWE of a reflecting dichroic beamsplitter on a TIRF system excitation beam. The green areas show the laser beam shape beyond the microscope objective when reflected by (A) an insufficiently flat dichroic and (B) a suitably flat dichroic. Laser spot images on side wall or ceiling were captured using a mobile phone camera.

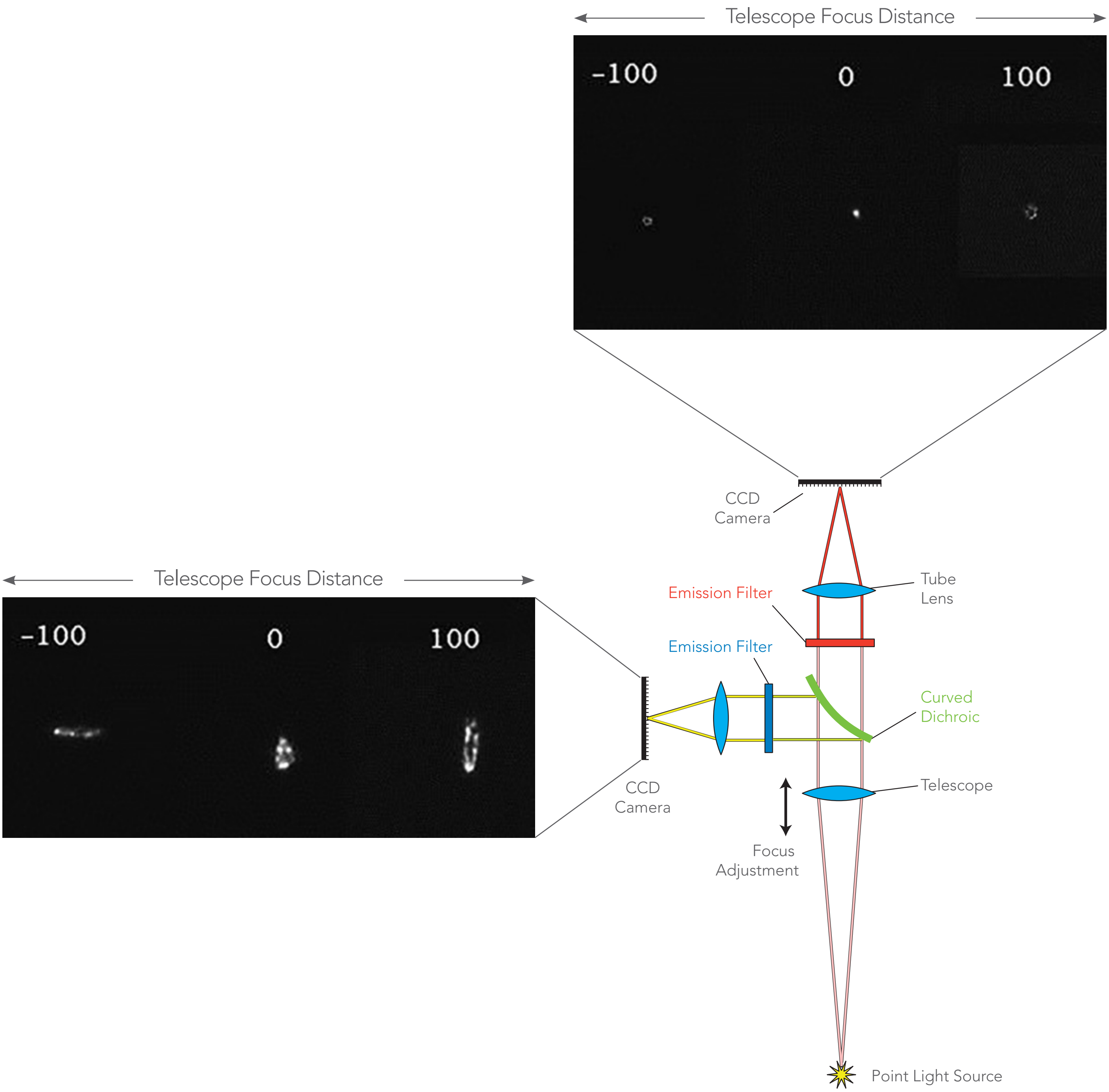


Figure 8: The image of a point light source was captured in an "Image Splitting" configuration, whose principle of operation is captured in the schematic. "Significant astigmatism" was observed in the left (reflected) image due to excessive RWE of the dichroic beamsplitter in the reflected beam. Such aberration is not observed in the light beam transmitted by the dichroic. The images were collected with the NESSI (NN-EXPLORE Exoplanet and Stellar Speckle Imager) instrument at the WIYN telescope on Kitts Peak, AZ, USA. This setup utilizes lenses of 150 mm focal length with beam diameter of ~1", and images are captured on two identical Andor iXon Ultra EMCCD cameras. Focus is achieved by shifting the telescope optics; eight steps of shift equal one µm of physical lens travel. The displacement of any powered optic along the optical axis affects the focal plane position, as a function of the optical power. The telescope focus distance is in steps. Images are courtesy of Nicholas J. Scott, NASA, Ames Research Center, Mountain View, CA, USA.

6. How to Determine Wavefront Requirements for Optical Systems

As indicated in the discussion above, dichroic beamsplitters are the components most likely to introduce excessive wavefront aberration to a reflected light beam. This Section details how to select dichroic beamsplitters with appropriate RWE specifications, and goes on to discuss requirements on RWE for non-dichroics, and on TWE for filters in general.

6.1 RWE for Dichroic Beamsplitters

The steps in selecting appropriate RWE dichroic beamsplitters are as follows:

1. Determine if it is critical to limit the RWE in the imaging system. Not all imaging systems are equally sensitive to RWE. Sections 5 and 6 provide examples of applications in which wavefront aberrations play a critical role.

2. Determine the beam type (Imaging or Non-imaging; see Section 3 for definitions) and beam diameter at the dichroic beamsplitter surface.

3. Use one of the two following graph-and-equation sets to determine the required RWE specification. Note that the graph and data apply only for AOI = 45°; use the equation for the general case.

   a. For Non-imaging Beams, use Figure 9 or Equation 1 to calculate the maximum RWE.

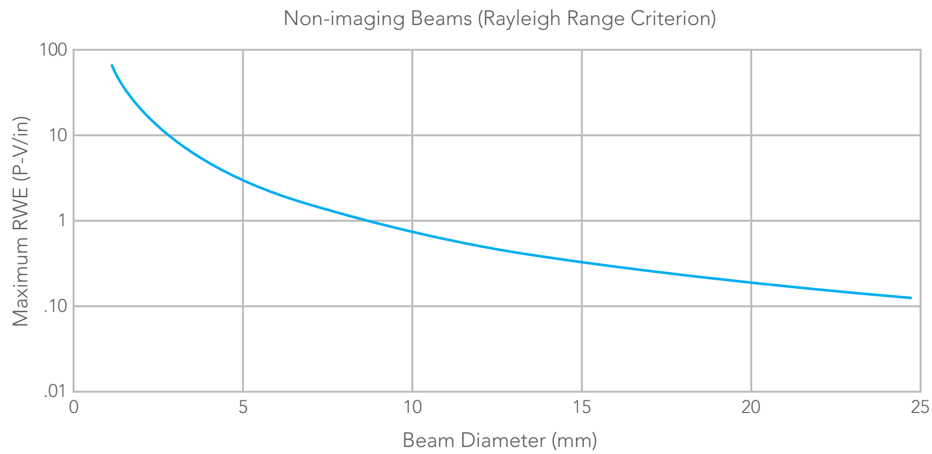


Figure 9: Maximum RWE (in waves PV/inch at 632.8 nm) for a Non-imaging Beam (Rayleigh Range Criterion) as a function of beam diameter. The graph on the left corresponds to the data on the right. The graph and data apply only for AOI = 45°.

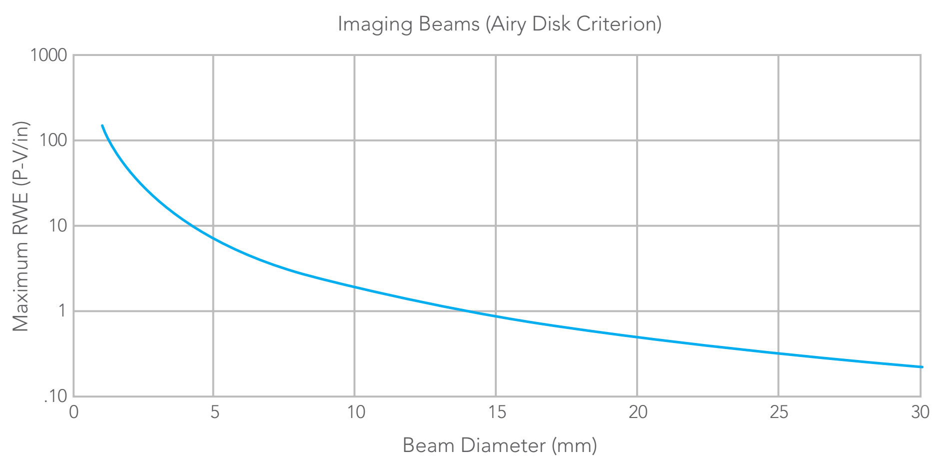


Figure 10: Maximum RWE (PV/inch @ 632.8 nm) for an Imaging Beam (Airy Disk Criterion) as a function of beam diameter. The graph on the left corresponds to the data on the right. The graph and data apply only for AOI = 45°.

$$RWE_{max} = \frac{1}{2\pi}\left(\frac{25.4}{d}\right)^2 \cos\theta \qquad \text{(Equation 1)}$$

where RWE_{max} is the maximum RWE (in waves PV), θ is the AOI, and d is the beam diameter (in mm).

   b. For Imaging Beams, use Figure 10 or Equation 2 to calculate the maximum RWE. The graph and data apply only for AOI = 45°; use the equation for the general case.

$$RWE_{max} = \frac{1.22}{2\sqrt{2}}\left(\frac{25.4}{d}\right)^2 \cos\theta \qquad \text{(Equation 2)}$$

where RWE_{max} is the maximum RWE (in waves PV), θ is the AOI, and d is the beam diameter (in mm).

4. Select either standard catalog filters (discussed in the next Section), or specify custom filters to meet the RWE needs.

Utilizing the above approach, for example when using a 10 mm diameter Non-imaging Beam, it is acceptable to use a dichroic beamsplitter with less than 0.73 waves PV per inch RWE and, when using a 10 mm diameter Imaging Beam, it is acceptable to use a dichroic with less than 1.97 waves PV per inch RWE. It is interesting that, for a given beam diameter, the RWE requirements are more demanding for a Non-imaging Beam than for an Imaging Beam.

In general, if there is any question as to whether system performance might be compromised, it is always better to use a lower RWE dichroic beamsplitter over one with higher RWE. This will help minimize possible or unknown compromises in system performance.

6.2 Rationale for Dichroic Beamsplitter RWE Criteria

The listed RWE criteria for Imaging and Non-imaging Beams are described in detail in [6]. Briefly, the Rayleigh Range Criterion is used to determine the wavefront error requirements for Non-imaging Beams. This criterion prescribes that the focal shift due to RWE should be less than one Rayleigh Range for the beam diameter at the dichroic beamsplitter. Similarly, the Airy Disk Criterion is used to determine the wavefront error requirements for

Beam Type	Criterion	Details
Non-imaging Beam	Rayleigh Range	The focal shift due to RWE should be less than one Rayleigh Range for the beam diameter
Imaging Beam	Airy Disk	The increase in image size due to RWE should be no more than 1.5 times Airy disk diameter for the beam diameter

Table 2: Rules for the relationship between Beam type and the criteria used to assign maximum permissible deviation from filter RWE.

Imaging Beams. This criterion specifies that the increase in spot size should be no more than 1.5 times the Airy Disk diameter for the beam diameter at the dichroic. This is summarized in Table 2.

Table 1 provides guidance on selecting the appropriate RWE so as not to impair performance of the corresponding optical system. Green bordered cells in this Table require use of the Rayleigh Range Criterion, and yellow bordered cells require the Airy Disk Criterion.

6.3 RWE for Non-dichroic Filters

As stated above, the performance of optical systems does not depend significantly on the RWE of excitation and emission filters because wavelengths reflected by the excitation or emission filters represent unwanted light that must be blocked (by reflection, assuming there is little absorption by the filters).

6.4 TWE for Filters in General

Unlike the case with RWE, there are no rules of thumb for assessing under what conditions TWE will give rise to excess aberration in a beam. Designers of optical systems rely on geometrical ray tracing to determine what amount of TWE can be allowed in a system, and how much of that budget can be allocated to filters as opposed to lenses and mirrors.

Note however that beams transmitted through parallel-plane glass plates plates (such as dichroic beamsplitters) can suffer optical aberrations; two examples are described in Appendices B and C.

It should be noted that appropriate wavefront error (RWE and TWE) is only one of the several design considerations for dichroic beamsplitters [11]. Choosing filters with suitable spectral designs is also of critical importance and is further discussed in the next Section. Example numeric values of RWE for Semrock dichroic beamsplitters are given in Tables 3 to 5 in Section 7.

7. Selecting Semrock Catalog Filters for Specific RWE Needs

Semrock offers an extensive and industry-leading range of catalog filters for a variety of applications with specific wavefront needs, including RWE. For an example of additional filter design requirements see the Semrock white paper [11]. This Section details how to select catalog filters for application-specific RWE needs.

Before choosing a catalog filter, use the steps in Section 6 to define the RWE requirements. Then select appropriate catalog product families using one of the following tables.

All product families in Table 3 are designed to work with popular laser sources in life science applications. It should be noted that BrightLine Laser Dichroic Beamsplitters are also configured as a part of various Laser Fluorescence Filter Sets, which include exciters, emitters and dichroics – all of which are designed to maximize overall imaging system performance. Follow this link to see all available Laser Filter Sets. Note also that conventional mechanical spring-loaded mounting of filters can significantly alter the RWE of dichroic beamsplitters (Appendix D); Semrock's catalog Super-resolution Microscopy cubes provide a mounted-filter solution that guarantees RWE.

BrightLine Laser Dichroic Beamplitters can also be used with LED and other broadband sources, when better RWE is needed. However, spectral edge compatibility of the LED sources should be carefully evaluated when using these beamsplitters. Semrock's popular SearchLight spectral plotting and analysis tool can be used for such evaluations.

When fluorescence emission beams are to be reflected by a dichroic beamsplitter, choose from the product families listed in Table 4, depending upon the beam diameter.

All Semrock catalog dichroic beamsplitter families and their Flatness / RWE classifications are summarized in Table 5 for reference.

Maximum Diameter of Non-imaging Beam, mm	Substrate Thickness, mm	Dichroic Beamsplitter Family and Example Part Numbers	Nominal Radius of Curvature (ROC), m	Reflected Wavefront Error at 632.8 nm (PV)	Flatness / RWE Classification
22.5	3	BrightLine Laser (Di03-R405-t3-)	~1275	0.2λ	Super-resolution / TIRF
10.0	1	BrightLine Laser (Di03-R405-t1-)	~275	< 1λ	
2.5	1	BrightLine Laser (Di02-R405-) RazorEdge® (LPD01-488RU-) LaserMUX™ (LM01-503-)	~30	< 6λ	Laser

Table 3: Semrock catalog product families and information appropriate to Non-imaging Beams, e.g. laser Illumination. See Section 3 and Table 1 for more information.

Maximum Diameter of Imaging Beam, mm	Substrate Thickness, mm	Dichroic Beamsplitter Family and Example Part Numbers	Nominal Radius of Curvature (ROC), m	Reflected Wavefront Error at 632.8 nm	Flatness / RWE Classification
37.0	3	BrightLine Image-splitting (FF509-FDi02-t3-)	~1275	< 0.2λ	Image-splitting
10.0	1	BrightLine Image-splitting (FF509-FDi01-)	~100	< 2λ	

Table 4: Semrock catalog product families and information appropriate to Imaging Beams, e.g. fluorescence emission. See Section 3 and Table 1 for more information.

Flatness / RWE Classification	Dichroic Family, and Example Part Numbers	Substrate Thickness, mm	Nominal Radius of Curvature (ROC), m	Reflected Wavefront Error at 632.8 nm, PV	Maximum Reflected Non-imaging Beam Diameter, mm	Maximum Reflected Imaging Beam Diameter, mm
Super-resolution / TIRF	BrightLine® Laser (Di03-R405-t3-)	3	~1275	< 0.2λ	22.5	37.0
	BrightLine Laser (Di03-R405-t1-)	1	~255	< 1λ	10.0	16.7
Image-splitting	BrightLine Image-splitting (FF509-FDi02-t3-)	3	~1275	< 0.2λ	22.5	37.0
	BrightLine Image-splitting (FF509-Di01-)	1	~100	< 2λ	6.3	10.0
Laser	BrightLine Laser (Di02-R405-) RazorEdge Dichroic™ (LPD02-488RU-) LaserMUX™ (LM01-503-) StopLine® Notch Dichroic (NFD01-488-)	1	~30	< 6λ	2.5	6.0
Standard Epi-fluorescence	BrightLine (FF495-Di03-)	1	~6	>> 6λ	Not applicable	Not applicable

Table 5. Semrock catalog dichroic beamsplitter RWE classification system with examples. The Maximum Reflected Non-imaging and Imaging Beam diameter values are calculated using the Rayleigh Range Criterion and Airy Disk Criterion, respectively, described briefly in Section 6 and with more detail in [6].

8. Summary

Any imaging instrument has a limited tolerance for wavefront error – and every optical element in the light path (including dichroic beamsplitters) contributes to wavefront error, thereby limiting overall imaging system performance. When aiming to achieve diffraction-limited imaging performance or better, it is important to consider wavefront errors from all optical elements in order to calculate the total instrument wavefront error, and to compare it to the maximum allowable value. This paper assumes that entire allowable wavefront aberration is allocated to dichroic beamsplitters, but in reality other optical elements in the light path such as lenses also introduce wavefront aberrations. For best results, select the lowest RWE dichroic beamsplitters to help maximize instrument performance.

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Appendix A: Alternative Specifications for RWE

This article uses PV values of RWE (see Section 2); alternative specifications can include the following:

- Radius of Curvature (ROC), in meters. ROC is an easily understood measure for comparing RWE of various optical filters. ROC values for the several Semrock product lines are listed in Table 5. Users of high performance optics are more likely to use RWE or Flatness for these specifications.
- Peak-to-Valley (PV) values of Flatness, in waves, which denotes the absolute distance between the highest and lowest points on an optical surface relative to the ideal.
- Root-Mean-Squared (RMS) values of Flatness, in waves. This is the square root of the average squared distance between the optical surface and the ideal. This measure may be preferred when it is critical to specify irregularity or surface imperfection.

Appendix B: Wavefront Error in Uncollimated Beams

If an uncollimated beam passes through a glass plate placed at an angle to the beam path, the transmitted beam will be degraded by the addition of coma, astigmatism and spherical aberration [11]. Thus if the usual flat plate dichroic beamsplitter is employed in a divergent or convergent beam, the transmitted beam can be expected to show this extra aberration. The degree of aberration is proportional to the thickness of the plate, so an uncollimated beam traversing a thicker dichroic may be more likely to give rise to noticeable aberration in high performance microscopy.

Appendix C: Tilt Aberration

If a beam, whether collimated or uncollimated, passes through a curved glass plate, the beam is deviated from its original path [13]. This situation is equivalent to deviation caused by passing through wedged glass, i.e. a glass plate with nonzero wedge angle, and is therefore designated as Tilt Aberration (Figure 3).

Appendix D: Dichroic Beamsplitter Mounting Considerations

It is important to consider the potential effects of filter mounting on RWE and TWE. Fastening a dichroic beamsplitter into a housing is not expected to increase the filter TWE, as differential change in optical path length is small, but can introduce RWE that may exceed what is recommended for certain applications, some of which are discussed here. This is because the standard microscope dichroic beamsplitter is mounted in a filter cube, and most off-the-shelf cubes and mounting methods result in significant changes to the dichroic Flatness and therefore to RWE. For this reason special mounting techniques must be used to preserve dichroic Flatness if RWE is critical to microscope performance. Descriptions of stress-free mounting of lenses can be found in the literature, e.g. [12]. Semrock has developed proprietary techniques for mounting dichroic beamsplitters in a cube so that 1 λ RWE is preserved over the Clear Aperture of standard thickness dichroics; see Section 7.

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