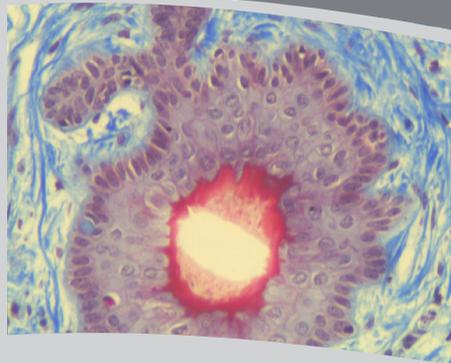


# Understanding Microscopy And Filtering Techniques

## A P P L I C A T I O N   N O T E S

- ▶ Digital Video Microscope Objective Setups
- ▶ Fluorophores And Filters For Fluorescence Microscopy
- ▶ Understanding Infinity-Corrected Objective Resolving Power And Magnification
- ▶ Optical Filters
- ▶ Fluorescence Microscopy: In-Line Illumination With Imaging Filters
- ▶ Understanding Microscopes And Objectives



## DIGITAL VIDEO MICROSCOPE OBJECTIVE SETUPS



Digital video microscopes utilize a camera to record and capture images. Traditional microscopes perform visual inspection with an eyepiece, but digital video microscopes achieve higher resolution and higher precision than what is typically seen with the human eye. There are numerous applications and setups for these microscopes, ranging from the use of standard DIN and JIS objectives to advanced infinity-corrected objectives. Infinity-corrected digital video microscopes can be quite complex to assemble; however, knowing which components to use and how they work together makes the process accessible for biological, industrial, and any other inspection applications.

To get started, consider the basic differences between finite-conjugate microscope objectives and their infinity-corrected counterparts. Finite-conjugate objectives, such as those found in traditional laboratory or university microscopes, focus light directly onto a sensor or eyepiece. Typically, these objectives

are achromatic and only correct for chromatic aberration at two wavelengths: red and blue. On the other hand, infinity-corrected objectives require an additional optical component, a tube lens, to focus light onto a sensor or eyepiece, but also allow access to the parallel optical plane. By accessing the optical plane, optics for attenuating or filtering light, beamsplitters for in-line illumination, or additional optical components can be incorporated into the setup. Typically, these optics are apochromatic and correct for spherical and chromatic aberration at three wavelengths (blue, yellow, and red), and also have a much higher numerical aperture for improved light levels and contrast.



### FOUR-COMPONENT DIGITAL VIDEO MICROSCOPE SYSTEM

When assembling a microscopy system with an infinity-corrected objective, there are two setups to incorporate a camera for live video feed and still frame capture. Depending on the simplicity of the setup, one can quickly and easily construct a

digital video microscope system with magnification as high as 200X. The most straightforward setup involves only four components: a camera, extension tube, tube lens, and microscope objective (Figure 1).

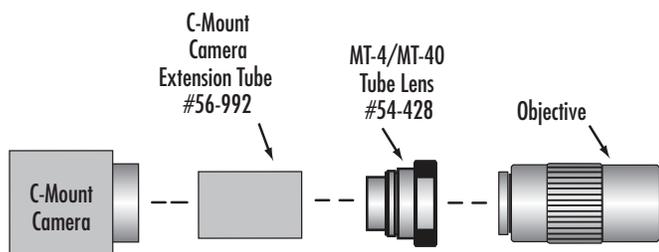


Figure 1: Simple Infinity-Corrected Digital Video Microscope System

Continue 

## FOUR-COMPONENT DIGITAL VIDEO MICROSCOPE SYSTEM

The four-component system enables the easy setup of a direct video microscope with high magnification and either high resolution or high frame rate. For example, one can achieve an extremely high resolution image with a pixel count of 3840 x 2748 (10.5MP) at a frame rate of 3 frames per second (fps), or an extremely fast image at 100 fps with a pixel count of 752 x 480 (0.36MP). It is important to remember that no matter the application requirements, the setup remains the same. As a great introductory system, consider using the stock numbers in

Figure 2 and listed in Table 1.

The sample components are threaded together and connected, with a full system length just under 267mm (10.5”) and optimized for 10X magnification. The digital video microscope system length will change slightly depending on the magnification power of the objective in place. A good rule of thumb is: The smaller the objective magnification, the shorter the system length.



Figure 2: #59-367 USB Camera + #53-838 Extension Tube + #54-428 Tube Lens + #59-877 Objective

Stock No.	Description
#59-367	EO-3112C 1/2" CMOS Co or USB Camera
#53-838	C-Mount Extension Tube (111.5mm Length)
#54-428	MT4 Accessory Tube Lens
#59-877	10X EO Plan Apo Long Working Distance Infinity-Corrected

Mounting the four-component digital video microscope system is as simple as assembling the components. Depending on the object under inspection, the configuration can be mounted in a vertical or horizontal fashion; cellular and biological samples typically require vertical orientation to ensure the sample or fluid does not run. Also, any length post or post holder can be used. As a great introductory system, consider using the stock numbers in Figure 3 and listed in Table 2.

Other mounting hardware can be used to hold the camera-objective system in place, such as the #85-008 Microscope Ob-

jective Nanopositioning System, a piezo driven, flexure guided focusing element system ideal for high precision and high resolution systems. #03-668 Three-Screw Adjustable Ring Mount or #03-669 Bar-Type Lens/ Filter Holder can also be used as simple mounting methods; two mounts are required to ensure maximum stability and grip of the digital video microscope system.

With only four primary components, one can create a digital video microscope system with magnification as high as 100 – 200X and resolution as low as 300nm.

Continue — — — — — ➔

## FOUR-COMPONENT DIGITAL VIDEO MICROSCOPE SYSTEM (CONT.)

**Table 2: Sample Stock No. for Mounting Four-Component Digital Video Microscope**

Stock No.	Description
#52-930	C-Mount Ring Mount
#39-355	90° Angle Mount
#52-220	3/4" Diameter x 9" Length Horizontal Arm
#39-351	3/4" Diameter x 12" Length Vertical Post
#54-262	3/4" Post Breadboard Adapter

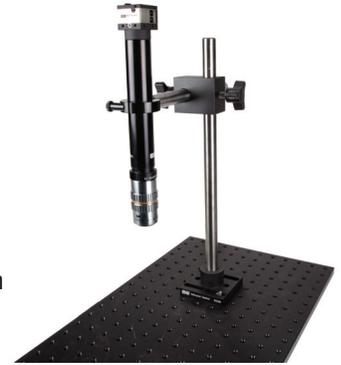


Figure 3: Simple Mounting Configuration for Four-Component Digital Video Microscope System

## SEVEN-COMPONENT DIGITAL VIDEO MICROSCOPE SYSTEM

Although the four-component infinity-corrected digital video microscope system is simple, it offers little adaptability. If adding additional optical components into the optical plane is necessary for an application, the seven-component system is the ideal choice. The extra optical elements include additional mounting hardware to accommodate for the 57mm of spacing, which enables the introduction of optical filters, beam-splitters, attenuation, or in-line illumination into the digital microscope. The seven-component system is typically used for fluorescence microscopy or applications that require maximum illumination and contrast. In-line illumination ensures there will be less noise in the system and minimizes glaring and ghosting.

The seven-component setup can be a little tricky, but the following explanation aims to make the process as simple as possible. To start, #58-329 MT-1/ MT-2 C-mount Adapter can be opened up and the MT-1 or MT-2 Tube Lenses fit inside. Since the tube lenses themselves have no threads, this adapter provides the necessary C-threads on both sides. Between this adapter and the C-mount camera, an additional 190mm of extension tubes is required. Since the tube lens does not attach directly to the objective, use #55-743 Mitutoyo to C-Mount

10mm Adapter to attach the objective (this adapter adds 10mm of length and adapts the M26 thread to a C-thread). An additional 76.5mm of space between the tube lens and objective is optimal, but it is common to only use about 56.5mm of space between #55-743 and #58-329 since each adapter adds about 10mm of space. With this 56.5mm of space, use extension tubes or add other optical components such as beam-splitters, optical filters, polarizers, etc.

It is important to note that 76.5mm is the recommended distance since these objectives are infinity corrected. However, if the distance is too short, the system may experience vignetting; if the distance is too long, the resultant image will be dim because of insufficient light. To summarize, refer to Figure 4 for an illustration of the seven-component digital video microscope system.

Digital video microscope systems are powerful optical tools for inspecting industrial and biological samples. They can be as simple or as complex as the user desires, ranging from four-component to seven-component systems. When it is necessary to include optical elements within the optical path of the camera-objective system, the seven-component infinity-corrected system is the ideal choice.

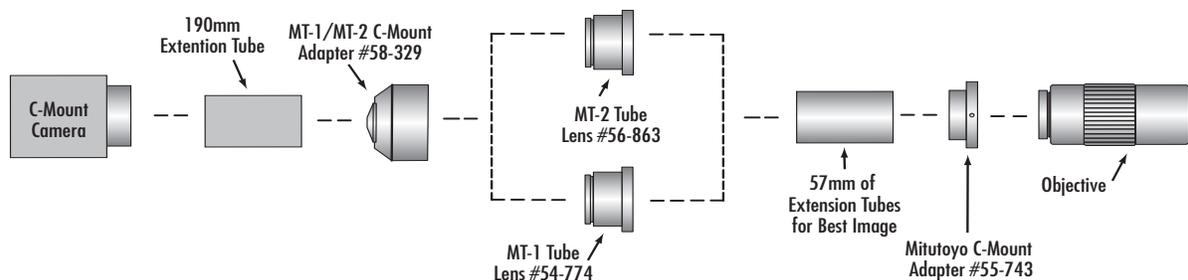


Figure 4: Seven-Component Infinity-Corrected Digital Video Microscope System

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# FLUOROPHORES AND FILTERS FOR FLUORESCENCE MICROSCOPY



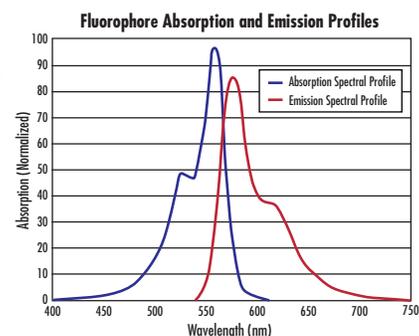
A fluorophore (or fluorochrome) is a type of fluorescent dye used to mark proteins, tissues, and cells with a fluorescent label for examination by fluorescence microscopy. A fluorophore works by absorbing energy of a specific wavelength region, commonly referred to as the Excitation Range, and re-emitting that energy in another specific wavelength region, commonly referred to as the Emission Range.

In general, a fluorophore will be excited (or absorb energy) by high frequency illumination (wavelengths in the ultraviolet, violet, or blue region of the spectrum), and emit energy at slightly lower frequencies (wavelengths in the green, red, or NIR region of the spectrum). Each fluorophore has a wavelength at which it absorbs energy most efficiently, referred to on our interactive filter table as the Peak Excitation  $\lambda$  (nm), and a corresponding wavelength at which the maximum amount of absorbed energy is re-emitted, referred to on our interactive

filter table as the Peak Emission  $\lambda$  (nm). Selecting individual optical filters with the maximum amount of transmission at each of those wavelengths will ensure brilliant fluorescent images.

A typical fluorescence microscope setup used in fluorescence microscopy applications consists of three filters. Refer to Figure 2 for a typical setup.

Figure 1: A generalized fluorophore spectral curve



## FILTER #1: EXCITATION FILTER

The excitation filter is placed within the illumination path of a fluorescence microscope. Its purpose is to filter out all wavelengths of the light source, except for the excitation range of the fluorophore under inspection. The Minimum Transmission % of the filter will dictate the brightness and brilliance of images. Edmund Optics recommends a minimum of 40% transmission for any excitation filter, with an ideal transmission being >85%. The Bandwidth of the excitation filter should fall entirely within the excitation range of the fluorophore, with the ideal filter having a Center Wavelength (CWL) as close as possible to

the peak excitation of the fluorophore in question. The Optical Density (OD) of the excitation filter will dictate the darkness of the background image, as optical density is a measure of how well a filter blocks wavelengths outside of its bandwidth. Edmund Optics recommends a minimum optical density of 3.0, with an ideal optical density of 6.0. An optical filters with the ideal combination of Center Wavelength, Minimum Transmission %, and Optical Density will provide the brightest possible images, with the deepest possible blocking, ensuring detection of the faintest emission signals.

Continue

## FILTER #2: EMISSION FILTER

The emission filter is placed within the imaging path of a fluorescence microscope. Its purpose is to filter out the entire excitation range of the fluorophore under inspection, and to transmit the emission range of that fluorophore. The same recommendations for Minimum Transmission, Bandwidth, Optical Density, and Center Wavelength for Excitation Filters holds

true for Emission Filters. Again, an emission filter with the ideal combination of Center Wavelength, Minimum Transmission %, and Optical Density will provide the brightest possible images, with the deepest possible blocking, ensuring detection of the faintest emission signals.

## FILTER #3: DICHOIC FILTER OR BEAMSPLITTER

The dichroic filter or beamsplitter is placed in between the excitation filter and emission filter, at a 45° angle. Its purpose is to reflect the excitation signal towards the fluorophore under inspection, and to transmit the emission signal towards the detector. An ideal dichroic filter or beamsplitter will have a sharp transition between maximum reflection and maximum transmission with a reflection % >95% for the bandwidth of the excitation filter, and a transmission of >90% for the bandwidth of the emission filter. The filter should be selected with the Intersection Wavelength ( $\lambda$ ) of the fluorophore in mind, to ensure minimal stray-light and a maximum signal-to-noise ratio in the fluorescent image. Although selection of an appropriate dichroic filter or beamsplitter is tricky, let Edmund Optics help you select the appropriate optical filter for your application. To discuss your requirements, please contact us.

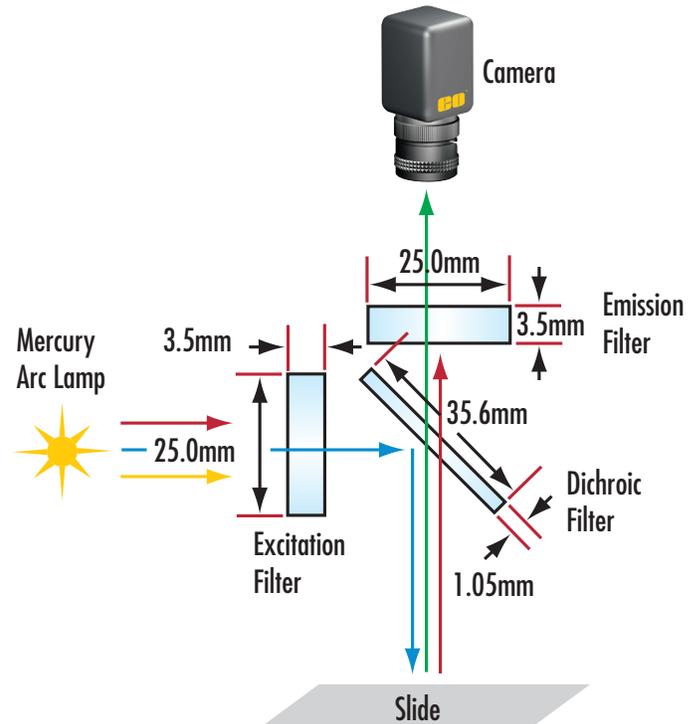


Figure 2: A typical fluorescence microscope setup

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## UNDERSTANDING INFINITY-CORRECTED OBJECTIVE RESOLVING POWER AND MAGNIFICATION



In the optics industry, microscopes are used for both machine vision and life science, or biological, applications. Machine vision applications support semiconductor, electronics, assembly, and manufacturing markets, to name a few. Life science applications look at cellular or biological samples at various objective magnifications. To understand the interplay between resolving power, magnification, and other common objective specifications for life science, consider how an infinity-corrected objective images skin culture at 5X, 10X, 20X, and 50X magnifications.

The four application examples detailed in the following sections utilize Edmund Optics infinity-corrected objectives. Mitutoyo objectives can be substituted as well. Although Mitutoyo objectives are synonymous with machine vision and industrial inspection, they perform extremely well in low light conditions and applications that focus on cellular and micro inspection.

### KEY OBJECTIVE TERMINOLOGY

Infinity-corrected objectives image well, no matter the magnification. They are ideal for applications requiring high precision and the modularity of adding optical filters, polarizers, beam splitters, and in-line illumination components into the optical path. However, there are tradeoffs for using infinity-corrected objectives:

- 1)** Higher magnifications yield higher numerical apertures, but shorter working distances and smaller fields of view.
- 2)** Lower magnifications yield lower numerical apertures, but longer working distances and larger fields of view.
- 3)** Resolution is dictated by magnification, and both increase proportionally to each other.

Magnification, numerical aperture, working distance, and resolution are all related for infinity-corrected objectives. Magnification is calculated by dividing the focal length of the tube lens by the focal length of the objective. Numerical aperture (NA) is a function of the focal length of the entrance pupil diameter; NA affects the amount of light entering the infinity-corrected system. Working distance (WD) is determined by the parfocal distance of the objective's optical path; WD is specified as the distance from the front optical element to the object under inspection. Resolving power is one of the trickiest specifications to properly explain. Since it is difficult to visualize what an actual object under inspection will look like at a particular magnification and how to quantify resolving power, it is best to learn by studying the application examples in the following sections.

Continue — — — — — →

## APPLICATION EXAMPLES

There are a large number of applications for infinity-corrected objective systems. In terms of biological applications, the most common is fluorescence microscopy, ranging from the most basic fluorophore detection systems to the more elaborate confocal and multiphoton fluorescence systems. The most complex of these systems involve high magnifications, precision

mechanics, high-quality optical filters, and powerful illumination sources, such as lasers. In contrast, the simpler systems involve a standard broadband light source, basic filtering, simple mechanics, and low to high magnifications depending on the samples under inspection.

## LOW MAGNIFICATION IMAGING OF SKIN CULTURE

Depending on the sample, there are a few rules of thumb to help with the selection of an infinity-corrected objective. A typical cell has a size of  $10\mu\text{m}$ ; a low magnification and low resolution objective is suitable for imaging a grouping of cells. If one needs to differentiate cellular membranes or intracellular components such as mitochondria, ribosomes, or a nucleus, then resolution on the order of  $1\mu\text{m}$  or less is best.

In Figures 1 – 4, the samples for inspection are 3D Skin Culture Models with a Trichrome Stain, which was cultured and prepared by Zen-Bio Incorporated located in Research Triangle Park, North Carolina, US. In Figure 1, it is clear to see cellular components surrounded by the extracellular matrix (ECM), which holds the tissue together. Within the ECM are the interstitial matrix and basement membrane, where polysaccharides and fibrous proteins reside and act as a compression buffer

against external stress. Within the basement membrane reside a number of sheets that are stacked upon one another with epithelial cells resting between. To clearly see the polysaccharide gel and epithelial cells, it is best to use a high magnification infinity-corrected objective. The tissue matrix is the surrounding blue stained material, the cells and cellular membrane are marked by the purple stains, and within each cell is a smaller white and partially red stained region that marks denser intracellular material, such as mitochondria and the nucleus.

Figure 1 was captured using #59-876 5X M Plan Apo Objective. This infinity-corrected objective has a numerical aperture of 0.14, field of view on a  $\frac{1}{2}$ " sensor of  $1.28\text{mm} \times 0.96\text{mm}$ , and resolving power of  $2\mu\text{m}$ . Since typical human cells are roughly  $10\mu\text{m}$  in size, the objective specifications for #59-876 make it the ideal choice.

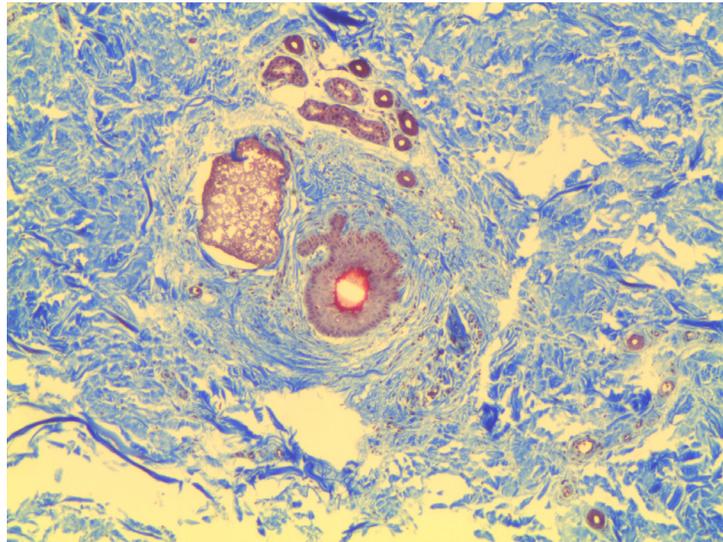


Figure 1: Trichrome Stain of Dermal Tissue Samples at 5X Magnification Using #59-876 Objective

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## LOW MAGNIFICATION IMAGING OF SKIN CULTURE

Figure 2 was captured using #59-877 10X M Plan Apo Objective. It has a numerical aperture of 0.28, field of view on a 1/2" sensor of 0.64mm x 0.48mm, and a resolving power of 1 $\mu$ m. In this image, it is clear to see the stacking and weaving of the ECM and interstitial structures. Additionally, the cellular

membrane is very pronounced and it is evident that there are a number of intracellular structures such as ribosomes, mitochondria, and a large nucleus present at the central position.

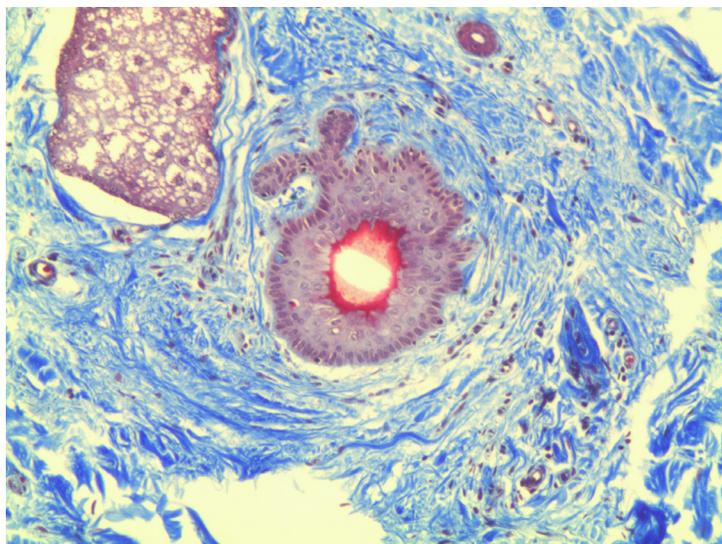


Figure 2: Trichrome Stain of Dermal Tissue Samples at 5X Magnification Using #59-876 Objective

## HIGH MAGNIFICATION IMAGING OF SKIN CULTURE

Figure 3 was captured using #59-878 20X M Plan Apo Objective. This infinity-corrected objective has a numerical aperture of 0.42, field of view on a 1/2" sensor of 0.32mm x 0.24mm, and a resolving power of 0.7 $\mu$ m. Figure 3 displays the entire

cell within the given field of view; the surrounding extracellular matrix is sectioned in greater detail, and the intracellular molecules are much larger and more visible than in either Figure 1 or Figure 2.

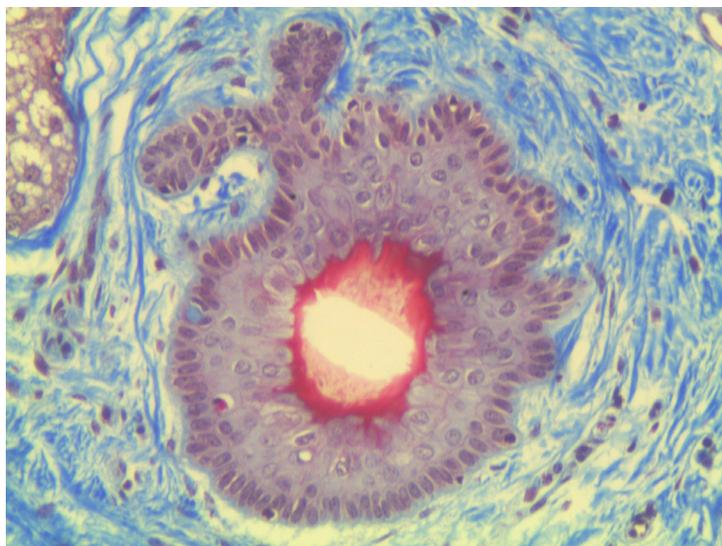


Figure 3: Trichrome Stain of Dermal Tissue Samples at 20X Magnification Using #59-878 Objective

Continue — — — — — →

## HIGH MAGNIFICATION IMAGING OF SKIN CULTURE (CONT.)

Figure 4 demonstrates 50X magnification - the highest magnification easily achieved without mechanical stages or piezo actuators stabilizing the infinity-corrected objective and image plane. At this magnification, slight vibrations from an illuminator or computer's fan can cause the video feed to dramatically shake and jump out of focus. Figure 4 was captured using #59-879 50X M Plan Apo Objective. It has a numerical aperture of 0.55, field of view on a 1/2" sensor of 0.128mm x 0.096mm, and a resolving power of 0.5 $\mu$ m. The depth of focus for this particular objective is only 0.9 $\mu$ m, making focusing a tedious process if the appropriate mechanics are not utilized. In Figure 4, the cellular membrane and intracellular components are very clear

and vibrant compared to the previous 5X, 10X, and 20X images. Also, the size and shape of the cellular constituents become truly evident. When comparing Figure 1 (5X magnification) to Figure 4 (50X magnification), the increase in magnification is immediately apparent. The resolving power increases by four and the field of view is minimized by a factor of twenty. When imaging at 50X magnification, high light intensity and contrast is required to increase illumination and to digitally adjust shutter speed and gain. The digital settings can be set to automatically compensate for darkness or frame rate - excellent for constructing a digital video microscope for the first time.

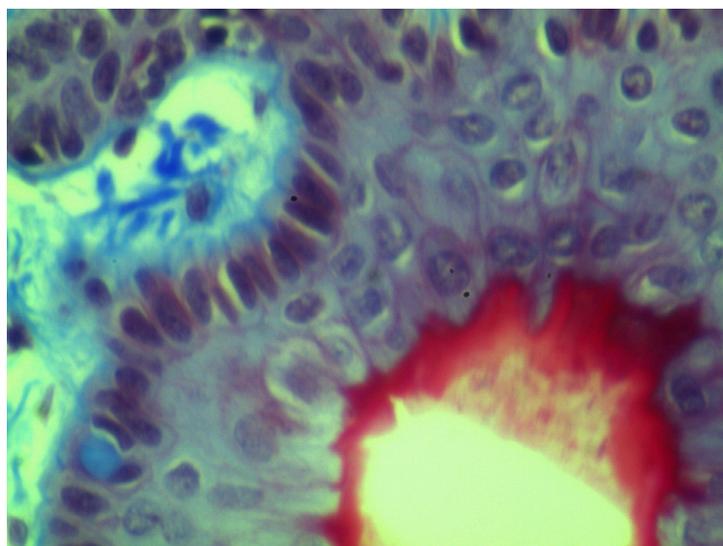


Figure 4: Trichrome Stain of Dermal Tissue Samples at 50X Magnification Using #59-879 Objective

Infinity-corrected objectives are ideal for machine vision inspection and life science applications. When imaging biological samples such as dermal tissue, it is important to understand what can be achieved with different objective magnifications.

5X and 10X objectives are ideal for seeing groups of cells and slight structures in the extracellular matrix. 20X and 50X objectives provide greater resolution and are capable of seeing intracellular molecules.



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# OPTICAL FILTERS



Optical filters can be used to attenuate or enhance an image, transmit or reflect specific wavelengths, and/or split an image into two identical images with controlled brightness levels relative to each other. To understand the importance of choos-

ing the correct optical filter for any application, consider key terminology, fabrication techniques, and the various types of filters available today.

## KEY OPTICAL FILTER TERMINOLOGY

Before delving into fabrication techniques and the types of optical filters available in the industry today, it is first important to review key terminology associated with them. Since all fil-

ters, independent of how they are manufactured, pass, absorb, and/or reflect some portion of incident light, they share common optical parameters.

### CENTRAL WAVELENGTH

Center Wavelength (CWL) is the midpoint between the wavelengths where transmittance is 50% of the specified minimum transmission, referred to as the Full Width at Half Maximum

(FWHM). For interference filters, the peak is typically not at the midpoint wavelength. Refer to Figure 1 for an illustration of CWL and FWHM.

### BANDWIDTH + BLOCKING RANGE

Bandwidth is a wavelength range used to denote a specific part of the spectrum that passes incident energy through a filter. Bandwidth is also referred to as FWHM (Figure 1).

Blocking Range is a wavelength interval used to denote a spectral region of energy that is attenuated by the filter (Figure 2). The degree of its blocking is typically specified in terms of optical density.

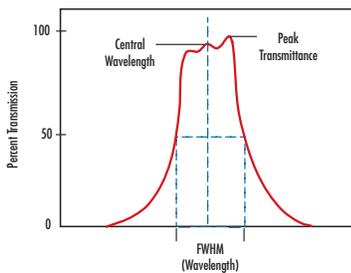


Figure 1: Illustration of Center Wavelength and Full Width at Half Maximum

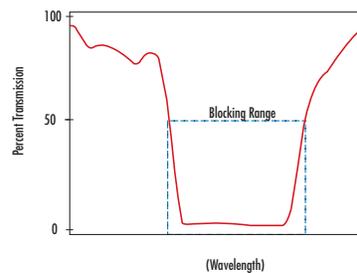


Figure 2: Illustration of Blocking Range

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## OPTICAL DENSITY

Optical Density (OD) describes the blocking specification of a filter and is related to the amount of energy transmitted through it (Equations 1 – 2). A high optical density value indicates very low transmission, and low optical density indicates high transmission. Figure 3 depicts three different optical densities:

$$(1) \quad T (\text{Percent Transmission}) = 10^{-OD} \times 100$$

$$(2) \quad OD = -\log\left(\frac{T\%}{100}\right)$$

OD 1.0, OD 1.3 and OD 1.5 that show the higher the OD value, the lower the transmission.

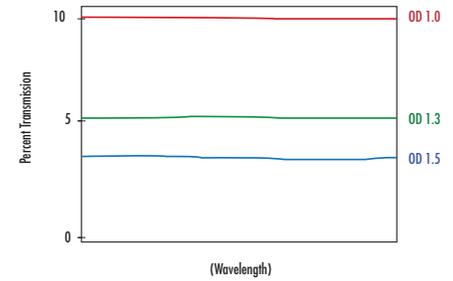


Figure 3: Illustration of Optical Density

## DICHROIC FILTER

A Dichroic Filter is a type of filter used to transmit or reflect light, depending on the wavelength; light of a specific wavelength range is transmitted, while light of a different range is

reflected or absorbed (Figure 4). Dichroic filters are commonly used for longpass and shortpass applications.

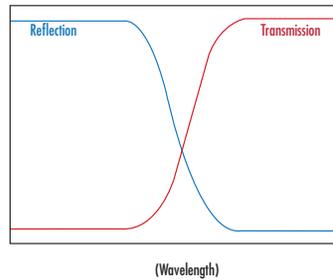


Figure 4: Illustration of a Dichroic Filter Coating

## CUT-ON AND CUT-OFF WAVELENGTH

Cut-On Wavelength is a term used to denote the wavelength at which the transmission increases to 50% throughput in a longpass filter. Cut-on wavelength is indicated by  $\lambda_{\text{cut-on}}$  in Figure 5.

Cut-Off Wavelength is a term used to denote the wavelength at which the transmission decreases to 50% throughput in a shortpass filter. Cut-off wavelength is indicated by  $\lambda_{\text{cut-off}}$  in Figure 6.

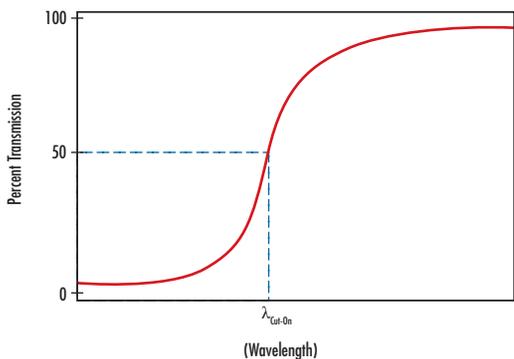


Figure 5: Illustration of Cut-On Wavelength

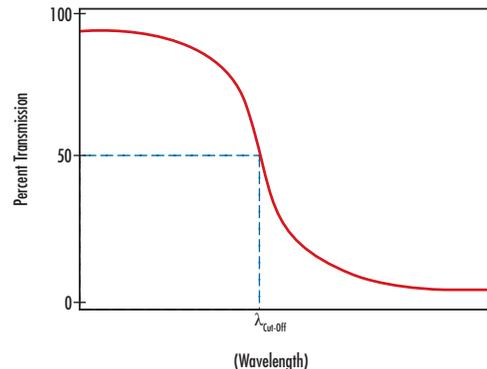


Figure 6: Illustration of Cut-Off Wavelength

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## ABSORPTIVE AND DICHROIC FILTERS

The wide range of optical filters can be broken into two main categories: absorptive and dichroic. The difference between the two does not lie in what they filter, but how they filter. In an absorptive filter, light is blocked based on the absorption properties of the glass substrate used. In other words, light that is blocked does not reflect off the filter; rather, it is absorbed and contained within the filter. In applications where noise in a system from unwanted light is an issue, an absorptive filter is ideal. Absorptive filters also have the added bonus of not being very angle sensitive; light can be incident upon the filter from a wide range of angles and the filter will maintain its transmission and absorption properties.

Conversely, a dichroic filter works by reflecting unwanted wavelengths, while transmitting the desired portion of the spectrum. In some applications, this is a desirable effect because light can be separated by wavelength into two sources.

This is achieved by adding a layer, or multiple layers, of material of varying indexes of refraction to exploit the interference nature of light waves. In interference filters, light traveling from a lower index material will reflect off a higher index material; only light of a certain angle and wavelength will constructively interfere with the incoming beam and pass through the material, while all other light will destructively interfere and reflect off the material (Figure 7).

Unlike absorptive filters, dichroic filters are extremely angle sensitive. When used for any angle(s) outside of their intended design, dichroic filters cannot meet the transmission and wavelength specifications originally indicated. As a rule of thumb, increasing the angle of incidence through a dichroic filter will shift it towards shorter wavelengths (i.e. towards bluer wavelengths); and decreasing the angle will shift it towards longer wavelengths (i.e. towards redder wavelengths).

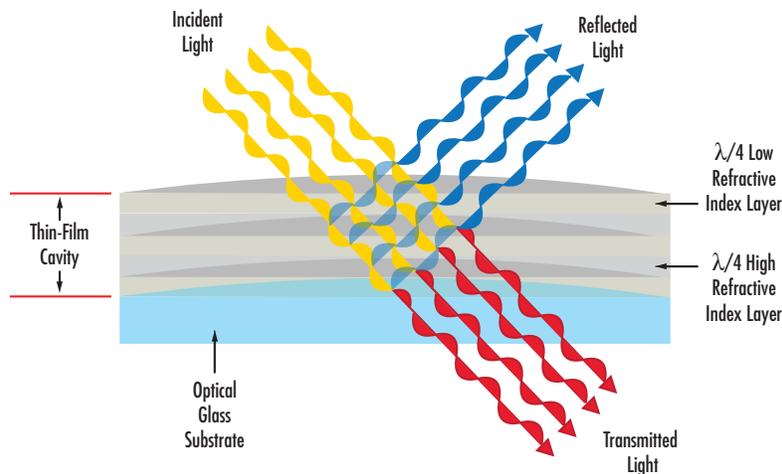


Figure 7: Deposition of Multiple Layers of Alternating High and Low Index Materials onto a Glass Substrate



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## EXPLORING DICHROIC BANDPASS FILTERS

Bandpass filters are used in a wide range of industries and can be either dichroic or color substrate. Dichroic bandpass filters are manufactured by two different techniques: traditional and hard sputtered, or hard coated. Both techniques achieve their unique transmission and reflection properties by a deposition of numerous layers of alternating high and low index of refraction materials onto glass substrates. In fact, depending upon the application, there can be more than 100 layers of material deposited per face of a given substrate.

The difference between traditional-coated filters and hard-sputtered filters is the number of substrate layers. In tradition-

al-coated bandpass filters, layers of varying index materials are deposited onto multiple substrates which are then sandwiched together. For example, imagine the illustration in Figure 7 repeated up to and even more than 100 times. This technique leads to a thick filter with reduced transmission. This reduction in transmission is caused by incident light traveling through and being absorbed and/or reflected by numerous substrate layers. Conversely, in hard-sputtered bandpass filters, materials of varying indices are deposited onto only a single substrate (Figure 8). This technique leads to thin filters with high transmission.

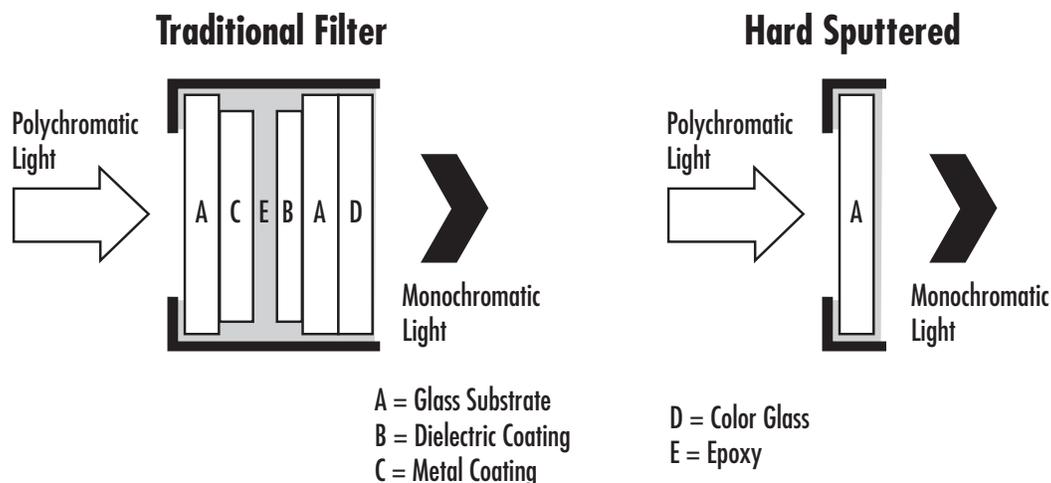


Figure 8: Traditional Filter (Left) and Hard-Sputtered Filter (Right)

## TYPES OF OPTICAL FILTERS

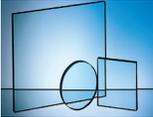
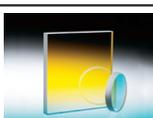
To aid in understanding the similarities and differences between the large variety of optical filters available today, consider ten of the most popular types. The following selection

guide contains a brief description, as well as sample product images and performance curves for easy comparison.



Continue — — — — — →

## TYPES OF OPTICAL FILTERS

Optical Filter Selection Guide	
Sample Image	Optical Filter Type
	<p><b>Bandpass Filters</b></p> <p>Bandpass filters have extremely narrow band (&lt;2nm to 10nm) or broadband (50nm and 80nm) transmittance across the substrate. They are particularly angle sensitive, so care should be taken when mounting and placing them within an optical setup. Hard sputtered filters should be chosen to maximize the transmission of selected wavelengths.</p>
	<p><b>Longpass Filters</b></p> <p>Longpass filters transmit all wavelengths longer than the specified cut on wavelength. Longpass filters include cold mirrors, colored glass filters, and Thermoset ADS (optical cast plastic) filters.</p>
	<p><b>Shortpass Filters</b></p> <p>Shortpass filters transmit all wavelengths shorter than the specified cut off wavelength. Shortpass filters include IR cutoff filters, hot mirrors, and heat absorbing glass.</p>
	<p><b>Heat Absorbing Glasses</b></p> <p>Heat absorbing glasses will transmit visible light and absorb infrared radiation. The absorbed energy is then dissipates as heat into the air around the glass. Forced air cooling is typically recommended to remove the excess heat. Heat absorbing glass can also be used as shortpass filters.</p>
	<p><b>Cold Mirrors</b></p> <p>Cold mirrors are specific types of dichroic filters designed to have high reflectivity in the visible spectrum while maintaining high transmission in the infrared. Cold mirrors are designed for use in any application where heat build-up can cause damage or adverse effects.</p>
	<p><b>Hot Mirrors</b></p> <p>Hot mirrors are specific types of dichroic filters designed to have high reflectivity in the infrared spectrum and high transmission in the visible. Hot mirrors are used primarily in projection and illumination systems.</p>
	<p><b>Notch Filters</b></p> <p>Notch filters are designed to block a pre-selected bandwidth while transmitting all other wavelengths within the design range of the filter. Notch filters are used to remove a single laser wavelength, or narrow band, from an optical system.</p>
	<p><b>Color Substrate Filters</b></p> <p>Color substrate filters are manufactured from substrates with inherently different absorption and transmission properties across a specific spectral region. Color substrate filters are often used as longpass and bandpass filters. The boundary between transmission and blocking is less sharp compared to some coating based filters.</p>
	<p><b>Dichroic Filters</b></p> <p>Dichroic filters are coated with thin-films to achieve a desired transmission and reflection percentage across a given spectrum. They are often used as color filters (both additive and subtractive). Dichroic filters are slightly angle sensitive but are much more forgiving than interference filters.</p>
	<p><b>Neutral Density (ND) Filters</b></p> <p>Neutral density (ND) filters are designed to reduce transmission evenly across a portion of a certain spectrum, ultraviolet and visible, visible, or infrared. There are two types of ND filters: absorptive and reflective. The absorptive type absorbs light that is not transmitted through the filter, while the reflective type reflects it back toward the direction from which it was incident. Special care should be taken when using the former type in order to ensure that any reflected light does not interfere with the application setup. ND Filters are often used to prevent blooming or overexposure of cameras and other detectors.</p>

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EXAMPLE 1: COLOR MATCH IMAGING

Monochrome cameras cannot inherently differentiate different colors. However, the addition of a color filter greatly increases the contrast between objects. A good rule of thumb is that a given color filter will lighten objects of the same color, while darkening objects of opposing colors. Consider an example where two red and two green pills are imaged with a monochrome camera. Figures 9a - 9d show actual images of a sample under inspection and various images using color filters. It is clear to see that with no filter (Figure 9b), the mono-

chrome camera cannot distinguish between red and green. It would be impossible to inspect these pills on a factory floor. On the other hand, when a red filter is used (Figure 9c), objects of its opposing color (i.e. the green pills) appear gray due to increased image contrast and can be easily discerned from the red pills. Conversely, when a green filter is used (Figure 9d), the red pills appear gray.

SAMPLE UNDER INSPECTION

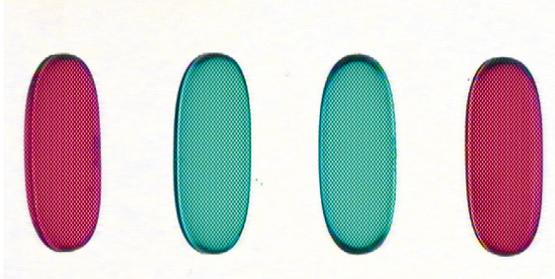


Figure 9a: Contrast Enhancement: Sample under Inspection

NO FILTER

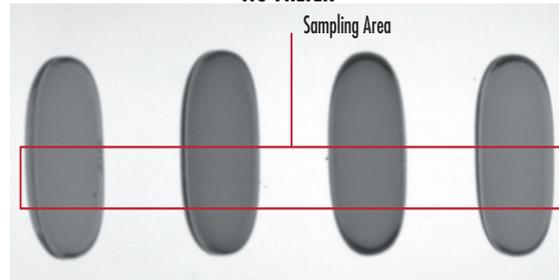


Figure 9b: Contrast Enhancement: No Filter

RED FILTER

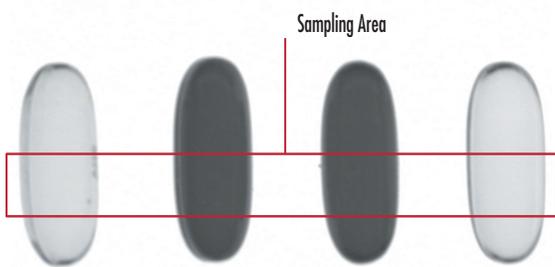


Figure 9c: Contrast Enhancement: Red Filter

GREEN FILTER

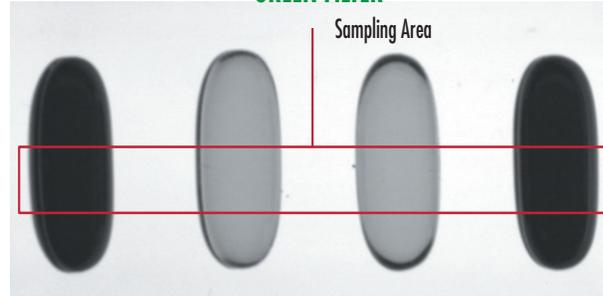


Figure 9d: Contrast Enhancement: Green Filter



## FLUORESCENCE MICROSCOPY: IN-LINE ILLUMINATION WITH IMAGING FILTERS



Advancements in camera technology have revolutionized microscopy in biological and industrial applications. Biologists or engineers no longer need to spend hours tediously looking through eyepieces, constantly adjusting focus. Today, simplifying the recording and analysis of data is done with digital video microscope systems.

To really see the benefit of a digital video microscope system, consider a configuration utilizing in-line illumination and filtering for optimal contrast and emission quantification. For a more complex digital video microscope configuration, please view Advanced Optical Filtering in Fluorescence Microscopy.

## THE BASICS OF FLUORESCENCE MICROSCOPY

Fluorescence microscopy is ideal for measuring and analyzing the absorption and excitation of various wavelengths of light. An in-line fluorescence microscopy setup utilizes a plate beamsplitter to redirect light from an illuminator into the parallel optical path. Mechanically, this setup is less complex than some other digital video microscope systems, and follows closely with Figure 1. Like most optical systems, this system begins with a sensor, an optical component, and an object under inspection. For the purposes of this discussion, #59-367

EO-3112C 1/2" CMOS Color USB Camera is used for the sensor in addition to #59-877 10X EO M Plan Apo Infinity-Corrected Objective and #54-774 MT-1 Accessory Tube Lens for the optical components. #54-774 is a type of accessory lens required to form an image from the infinity-corrected objective. The object under inspection can include such items as biological samples, plants or insects, glass or metal materials for inspection, and targets.

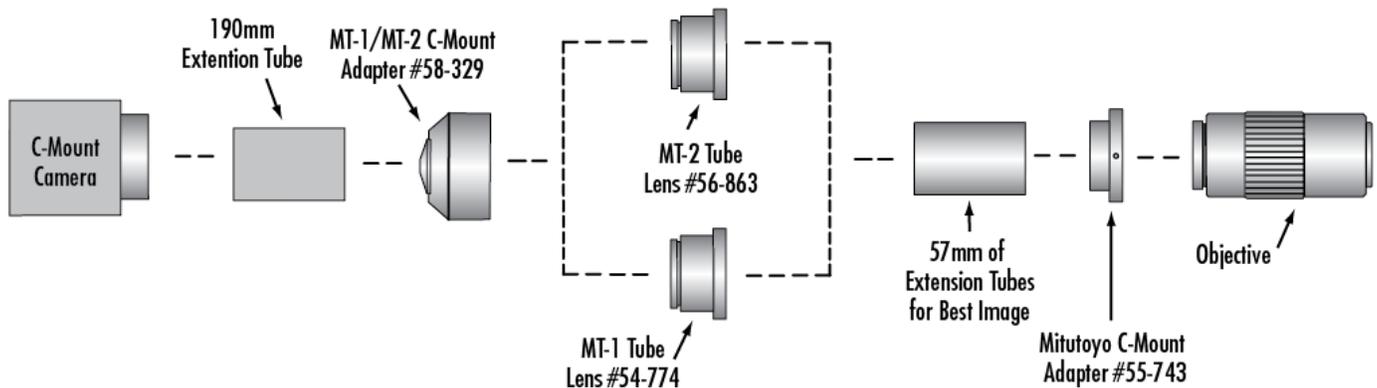


Figure 1: Seven-Component Infinity-Corrected Digital Video Microscope System for Fluorescence Microscopy



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## THE BASICS OF FLUORESCENCE MICROSCOPY (CONT.)

The choice of optical filters is important when selecting the excitation and emission wavelengths in the digital video microscope system from Figure 1. With the addition of optical filters, this type of setup is more commonly referred to as a fluorescence microscope (Figure 2), which is the backbone for more advanced techniques such as confocal, multiphoton, and Coherent anti-Stokes Raman scattering microscopy.

Blocking certain wavelengths allows one to bombard a sample with an excessive amount of light – samples typically imaged under a fluorescence microscope require a large amount of excitation energy to emit a quantifiable amount of light. Hard-

coated filters with high optical densities and dichroic filters block certain wavelengths quickly and easily. The dichroic and emission filters are the two most crucial filters in the system. The dichroic filter reflects shorter wavelengths that typically excite fluorophores, and transmits longer wavelengths that are emitted. Together these optical filters prevent non-emission energy and stray light from reaching the sensor. The important parameters of a filter include the center wavelength (CWL), minimum transmission percentage, optical density (OD), and bandwidth, which at times can also be referred to as the full width at half maximum (FWHM).

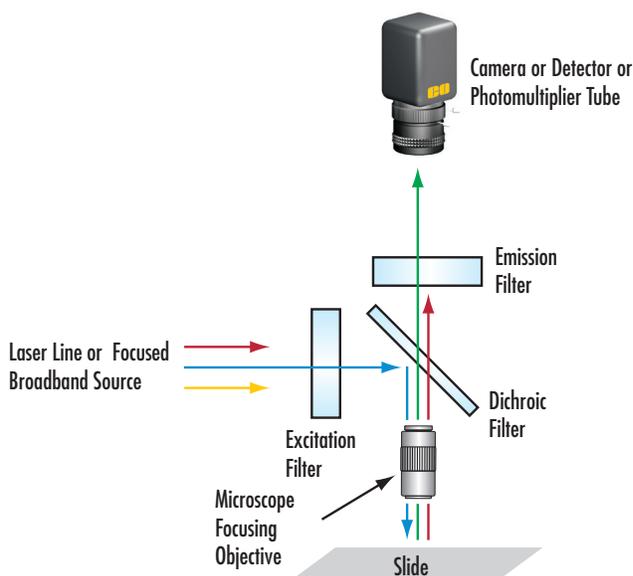


Figure 2: Basic Optical Filtering Arrangement for Fluorescence Microscopy

## OPTICAL AND MECHANICAL COMPONENTS

There is a variety of optical, imaging, and positioning components needed to create a precision fluorescence microscopy setup. To make the selection process as easy as possible, Table 1 includes a complete component list (known as a bill of materials) detailing suggested products, their stock numbers, and their quantities.

Figure 3 illustrates a real-world setup with products from Table 1. From top to bottom, the assembly starts with #59-367 EO-3112C ½" CMOS Color USB Camera, and continues with #58-329 and #55-743 C-mount adapters which connect the #54-774 MT-1 Accessory Tube Lens. The space after the tube

lens and prior to the objective is the parallel optical path, which is the benefit of an infinity-corrected system. This optical path allows for the introduction of optical components without distortion or aberrations. In Figure 3, #56-658 6 Position Filter Wheel Assembly holds a number of bandpass emission filters, and a dichroic beamsplitter which reflects white light downward for in-line illumination and cleans up the unwanted light being transmitted back through the objective to the sensor. Lastly, #59-877 10X EO M Plan Apo Infinity-Corrected Objective along with the series of mechanical components bring the sample into best focus. Figure 4 provides a closer look at the filter wheel assembly and placement after the tube lens.

Continue — — — — — ➔

## OPTICAL AND MECHANICAL COMPONENTS (CONT.)

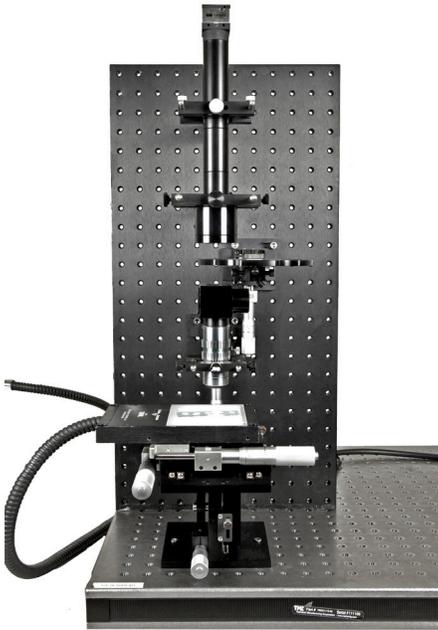


Figure 3: Sample Fluorescence Microscope Setup

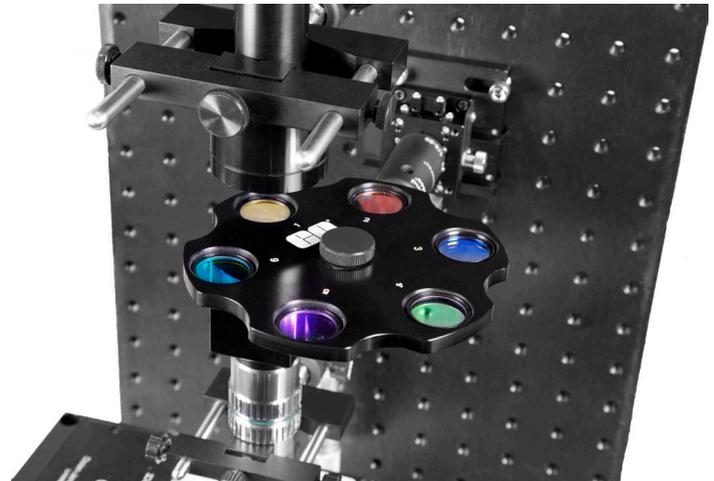


Figure 4: Close-Up of Filter Wheel Assembly and Bandpass Filters in Parallel Optical Path

**Table 1: List of Required Components for Setup**

Description	Stock No.	Quantity
VIS Plate Beamsplitter 35mm x 35mm, 50R/50T	#49-754	1
10X EO M Plan Apo Infinity-Corrected Objective	#59-877	1
6 Position Filter Wheel Assembly for 1" Diameter Filters	#56-658	1
EO-3112C 1/2" CMOS Color USB Camera	#59-367	1
EO USB CAM 1/4-20 Mounting Plate	#59-473	1
MI-150 Illuminator without Iris or Filter	#59-235 (115V) #59-236 (220V)	1
MT-1 Accessory Tube Lens	#54-774	1
Mitutoyo MT-1/MT-2 C-Mount Adapter	#58-329	1
Mitutoyo to C-Mount 10mm Adapter	#55-743	1
Bench Plate 24" x 12"	#03-640	1
Bench Plate 18" x 12"	#56-934	1
C-Mount Cube for 35mm Square x 3mm Thick Plate	#56-265	1

Digital video microscope objective setups are powerful optical tools for inspecting industrial and biological samples. They can be as simple or complex as the user desires, ranging from four-components to greater than twelve components. Systems utilizing more than twelve components typically have intricate filtering methods and mechanics required to place the optical

filters in the optical path. When it is necessary to include optical elements within the optical path of the camera-objective system, the seven-component infinity-corrected system is the ideal choice, especially for fluorescence microscopy applications.



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## UNDERSTANDING MICROSCOPES AND OBJECTIVES



A microscope is an optical device used to image an object onto the human eye or a video device. The earliest microscopes, consisting of two elements, simply produced a larger image of an object under inspection than what the human eye could observe. The design has evolved over the microscope's history to now incorporate multiple lenses, filters, polarizers, beam-splitters, sensors, illumination sources, and a host of other

components. To understand these complex optical devices, consider a microscope's components, key concepts and specifications, and applications.



## COMPONENTS OF MICROSCOPES

A compound microscope is one that contains multiple lens elements. It works similar to a simple magnifier which utilizes a single lens to magnify a small object in order for the human eye to discern its details. With a simple magnifier, the object is placed within the focal length of the single lens. This produces a magnified, virtual image. With a microscope, a relay lens system replaces the single lens; an objective and an eyepiece work in tandem to project the image of the object onto the eye, or a sensor – depending upon the application. There are two parts to a microscope that increase the overall system magnification: the objective and the eyepiece. The objective, located closest to the object, relays a real image of the object to the eyepiece. This part of the microscope is needed to produce the base magnification. The eyepiece, located closest to

the eye or sensor, projects and magnifies this real image and yields a virtual image of the object. Eyepieces typically produce an additional 10X magnification, but this can vary from 1X – 30X. Figure 1 illustrates the components of a compound microscope. Additionally, Equation 1 demonstrates how to calculate the overall system magnification.

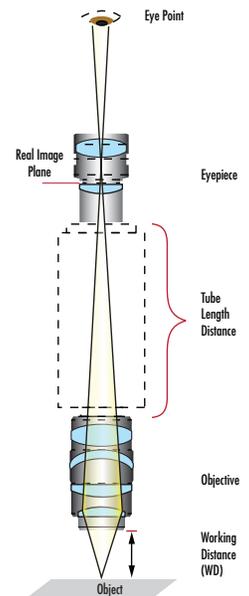


Figure 1: Components of a Compound Microscope

$$\text{Magnification}_{\text{System}} = \text{Magnification}_{\text{Objective}} \times \text{Magnification}_{\text{Eyepiece}}$$

(1)



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## EYEPIECES

When microscopes were first invented, eyepieces played a major role in their design since they were the only means to actually see the object under inspection. Today, analog or digital cameras are used to project an image of the object onto a monitor or a screen. Microscope eyepieces generally consist of a field lens and an eye lens, though multiple designs exist that each creates a larger field of view than a single element design.



## ILLUMINATION

Illumination within a microscope is just as important as selecting the proper eyepiece or objective. It is crucial to choose the correct illumination in order to obtain the most conclusive results. Before deciding on the type of illumination setup to work with, consider the application setup, object under inspection, and desired results.

Many microscopes utilize backlight illumination compared to traditional direct light illumination because the latter usually over-saturates the object under inspection. A specific type of backlight illumination used in microscopy applications is

Koehler illumination. In Koehler illumination, incident light from an illumination source, such as a light bulb, floods the object under inspection with light from behind (Figure 2). It employs two convex lenses: the collector lens and the condenser lens. It is designed to provide bright and even illumination on the object plane and on the image plane where the image produced from the objective is then reimaged through the eyepiece. This is important because it ensures the user is not imaging the filament of the light bulb. Since backlight illumination floods the object with light from behind, it is also referred to as brightfield illumination.

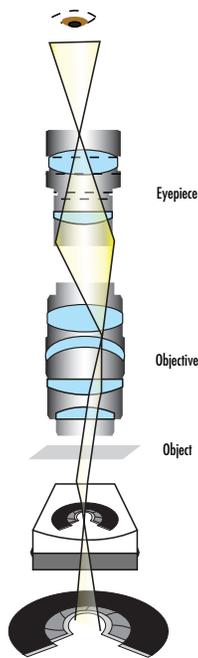


Figure 2: Koehler Illumination Setup

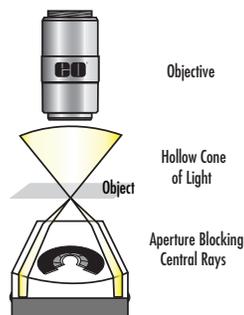


Figure 3: Darkfield Illumination Setup

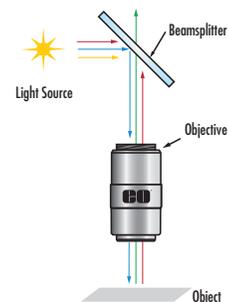


Figure 4: Epi-Illumination Setup

Continue 

## ILLUMINATION

Brightfield illumination requires a change in opacity throughout the object. Without this change, the illumination creates a dark blur around the object. The end result is an image of relative contrast between parts of the object and the light source. In most cases, unless the object is extremely transparent, the resulting image allows the user to see each part of the object with some clarity or resolution. In cases where an object's transparency makes it difficult to distinguish features using brightfield illumination, darkfield illumination can be used.

With darkfield illumination, direct rays of light are not sent into the objective but instead strike the object at an oblique angle. It is important to keep in mind that these rays still illuminate the object in the object plane. The resultant darkfield illumination image produces high contrast between the transparent object and the light source. When used in a microscopy setup, darkfield illumination produces a light source that forms an inverted cone of light blocking the central rays of light but still allowing the oblique rays to light the object. Figure 3 illustrates a sample darkfield illumination setup where the hollow cone of light is the numerical aperture of the objective. By comparison, no rays are blocked in a brightfield illumination setup. The design of darkfield illumination forces the light to illuminate the object under inspection, but not to enter the optical system, making it better for a transparent object.

A third type of illumination used in microscopy is epi-illumination. Epi-illumination produces light above the objective. As a result, the objective and epi-illumination source substitute for a Koehler illumination setup. Using the objective for a large section of the illumination makes epi-illumination very compact – a major benefit of this design. Figure 4 illustrates an epi-illumination setup which is used frequently in fluorescence applications.

## OBJECTIVES

Objectives allow microscopes to provide magnified, real images and are, perhaps, the most complex component in a microscope system because of their multi-element design. Objectives are available with magnifications ranging from 2X – 200X. They are classified into two main categories: the traditional refractive type and reflective. Each category is further divided into types: finite conjugate and infinite conjugate (infinity corrected). In order to choose the correct objective, it is important to know the benefits of one category and type from another.



## OBJECTIVES: REFRACTIVE

The most commonly used category of objectives is refractive. In a refractive design light passing through the system is refracted, or bent, by the optical elements. Each optical element is typically anti-reflection coated to reduce back reflections and improve overall light throughput. Refractive objectives are often used in machine vision applications that require resolution of extremely fine details. There are multiple refractive ob-

jective designs each utilizing different optical configurations. The designs can range from two elements in basic achromatic objectives (an achromatic lens and a meniscus lens) to fifteen elements in plan-apochromatic objectives (Figure 5). Plan-apochromatic objectives are the most complex, high-end objective design with chromatic and flat field correction done within the objective itself.

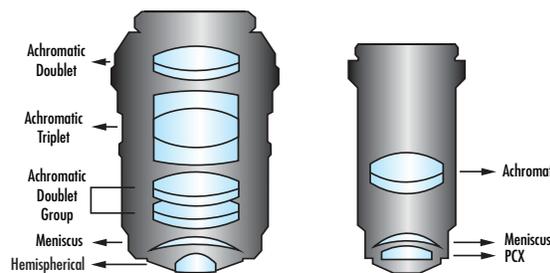


Figure 5: Apochromatic (Left) vs. Achromatic (Right) Objective Design

Continue — — — — — →

## OBJECTIVES: REFLECTIVE

Reflective objectives utilize a reflective, or mirror-based design. They are often overlooked in comparison to their refractive counterparts, though they can correct for many issues present in the latter. Reflective objectives consist of a primary and secondary mirror system (Figure 6) to magnify and relay the image of the object under inspection. Edmund Optics® utilizes the popular Schwarzschild design, though other designs are available. Since light is reflected by metallic surfaces and not refracted by glass surfaces, reflective objectives do not suffer from the same aberrations as refractive objectives

and, thus, do not need the additional designs to compensate for these aberrations. Reflective objectives can produce higher light efficiency as well as better resolving power for fine detail imaging because the system is primarily dependent upon the mirror coating instead of upon the glass substrate being used. Another benefit of reflective objectives is the possibility of working deeper into either the ultra-violet (UV) or infrared (IR) spectral regions due to the use of mirrors compared to conventional refractive optics.

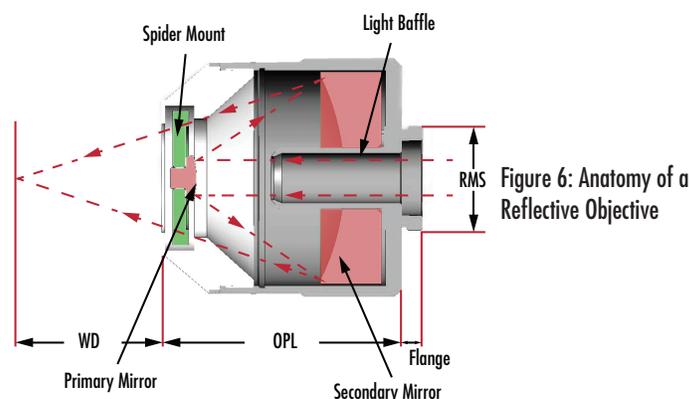
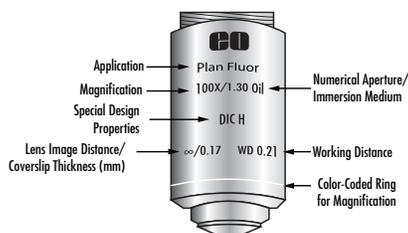


Figure 6: Anatomy of a Reflective Objective

## KEY CONCEPTS AND SPECIFICATIONS

Most microscope objective specifications are listed on the body of the objective itself: the objective design/standard, magnification, numerical aperture, working distance, lens to image distance, and cover slip thickness correction. Figure 7 shows how to read microscope objective specifications. Since the specifications are located directly on the body of the objective, it is easy to know exactly what one has, a very important fact when incorporating multiple objectives into an application. Any remaining specifications, such as focal length, field of view, and design wavelength, can easily be calculated or found in the specifications provided by the vendor or manufacturer.



Magnification	1X	2X	3X	4X	10X	20X	40X	60X	100X
Color Code	Black	Gray	Red	Yellow	Green	Light Blue	Light Blue	Dark Blue	White

Figure 7: Typical Transmissive Microscope Objective

## THE OBJECTIVE STANDARD

If the objective follows a simple microscope standard (such as DIN or JIS) then it is listed on the body to show what required specifications must be present within the system. Most compound microscopes employ the Deutsche Industrie Norm, or DIN, standard. The DIN standard has a 160mm distance from the objective flange to the eyepiece flange (Figure 8). The other available standard is the Japanese Industrial Standard, or JIS. The JIS standard has a 170mm distance from objec-

tive flange to eyepiece flange (Figure 9). Paying attention to these two distances is necessary when choosing the proper objective and eyepiece in order to make sure that the image projected from the former is properly imaged through the latter. Though the image distances are different for DIN and JIS, there is no difference in optical performance; they are equal in quality. Similarly, each standard utilizes the same RMS mounting thread of 0.7965" x 36TPI.

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## THE OBJECTIVE STANDARD (CONT.)

DIN and JIS have historically been used when considering a classic compound microscope. Some microscope manufacturers prefer to list the tube lens length by the optical properties instead of the mechanical. For a DIN standard objective, this changes the tube lens length to 150mm because the eyepiece is imaging the intermediate image plane (Figure 8). Lastly, there

is a dimension typically listed for objectives to allow the user to consistently know what length it is: the parfocal distance (PD). The parfocal distance is the distance from the flange of the objective to the object under inspection. For DIN objectives this distance is a standard 45mm and for JIS it is 36mm (Figures 8 and 9).

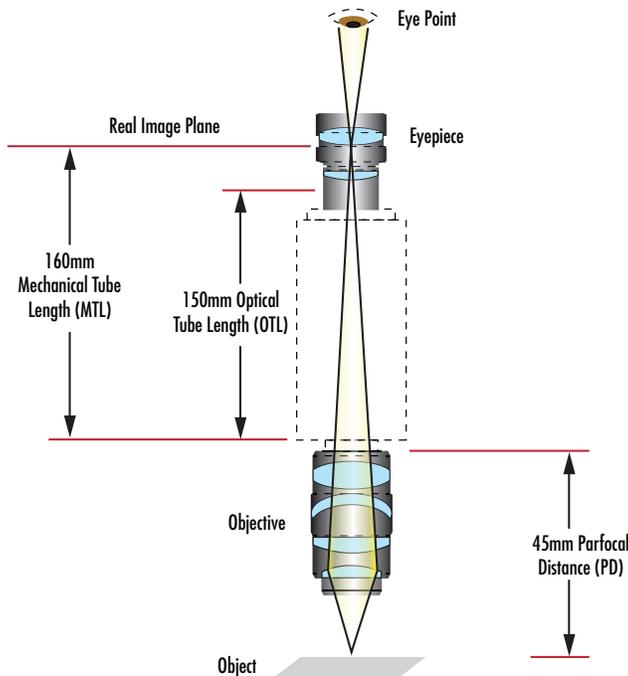


Figure 8: DIN Standard

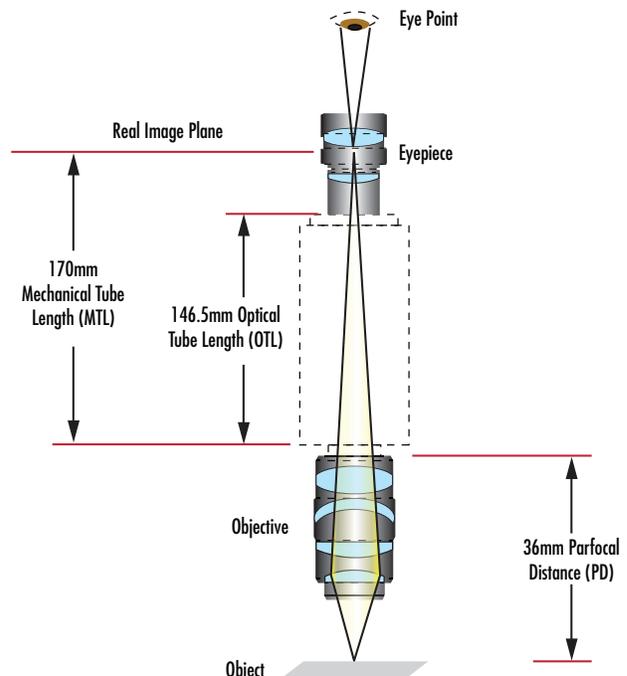


Figure 9: JIS Standard

## MAGNIFICATION

Eyepieces and objectives both have magnification that each contribute to the overall system magnification. Magnification is usually denoted by an X next to a numeric value. Most objec-

tives contain a colored band around the entire circumference of the body that indicates their magnification (Figure 7). For example, a yellow band denotes a 10X magnification.

## NUMERICAL APERTURE

The Numerical Aperture (NA) of an objective is a function of the focal length and the entrance pupil diameter. Large NA objectives sometimes require the use of immersion oils between the object under inspection and the front of the objective. This is because the highest NA that can be achieved within air is an NA of 1 (corresponding to 90° angle of light). To get a larger

angle and increase the amount of light entering the objective (Equation 2), it is necessary to use immersion oil (index of refraction typically = 1.5) to change the refractive index between the object and the objective. High NA objectives in conjunction with immersion oil are a simple alternative to changing objectives, a move that may be costly.

(2)

$$NA = n \sin \theta$$

Continue — — — — — →

## FIELD OF VIEW

Field of View is the area of the object that is imaged by a microscope system. The size of the field of view is determined by the objective magnification. When using an eyepiece-objective system, the field of view from the objective is magnified by the eyepiece for viewing. In a camera-objective system, that field of view is relayed onto a camera sensor. The sensor on a camera is rectangular and therefore can only image a portion

$$(3) \quad \text{Field of View}_{\text{Camera-Objective}} = \frac{\text{Camera Sensor Size}}{\text{Magnification}_{\text{Objective}}}$$

of the full circular field of view from the objective. In contrast, the retina in your eye can image a circular area and captures the full field of view. This is why the field of view produced by a camera-microscope system is typically slightly smaller than that of an eyepiece-microscope system. Equations 3 and 4 can be used to calculate the field of view in the aforementioned systems.

$$(4) \quad \text{Field of View}_{\text{Eyepiece-Objective}} = \frac{\text{Field Stop}_{\text{Eyepiece}}}{\text{Magnification}_{\text{Objective}}}$$

## COVER SLIP THICKNESS

When viewing fluid materials such as bacteria, cell cultures, blood, etc, it is necessary to use a cover slip in order to protect the object under inspection and microscope components from contamination. A cover slip, or glass microscope slide, changes the way light refracts from the object into the objective. As a result, the objective needs to make proper optical corrections to

produce the best quality image. This is why objectives denote a range of cover slip thicknesses for which they are optimized. Typically, this is listed after the infinity symbol (which denotes that an objective is an infinite conjugate, or infinity-corrected design) and ranges from zero (no cover slip correction) to 0.17mm.

## QUALITY CORRECTION

The quality of an objective and eyepiece determine how well the system performs. In addition to choosing the magnification and complexity of the design, understanding correct quality correction is extremely important when deciding on the type of objective to use. Quality correction (i.e. achromatic, apochromatic, plan, semi-plan) is denoted on the objective itself to allow the user to easily see the design of the objective in question. There are typically two levels of chromatic aberration correction: achromatic and apochromatic. Achromatic objectives are among the simplest and least expensive of objectives. They are designed to correct for chromatic aberration in the red and blue wavelengths, in addition to being corrected for spherical aberration in a green wavelength. Limited correction for chromatic aberration and lack of a flat field of view reduce objective performance. Apochromatic objectives, by contrast, provide higher precision and are chromatically corrected for red, blue, and yellow. They also provide spherical aberration correction for two to three wavelengths and generally have a high numerical aperture (NA) and long working distance.

Apochromatic objectives are ideal for white light applications, whereas achromatic objectives are best suited for monochromatic. Both objective designs, however, suffer significantly from distortion and field curvature, which worsen as objective magnification increases. Therefore, it is always important to focus on the complete system performance, rather than just objective performance alone.

Plan, also known as planar, semi-plan, semi-planar, or micro-plan, objectives correct for field curvature. Field curvature is a type of aberration present when the off-axis image cannot be brought to focus in a flat image plane, resulting in a blurred image as it deviates from the optical axis. Figure 10 illustrates field flatness measured radially from the center in achromatic, semi-plan, and plan objective designs. Achromatic objectives have a flat field in the center 65% of the image. Plan objectives correct best overall and display better than 90% of the field flat and in focus. Semi-plan objectives are intermediate to the other two types with 80% of the field appearing flat.

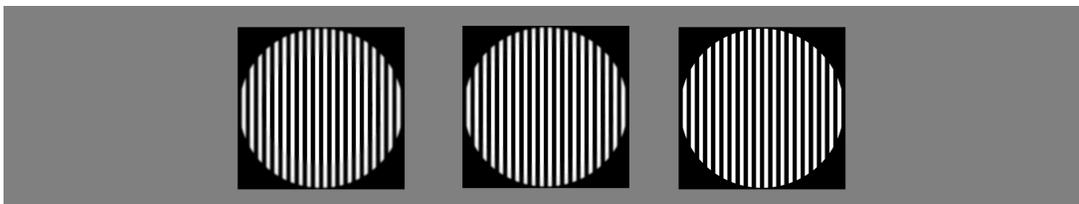


Figure 10: Flat Field Correction: Achromatic 65% (Left) vs. Semiplan 80% (Center) vs. Plan 90% (Right)

Continue

## FINITE CONJUGATE

In a finite conjugate optical design, light from a source (not at infinity) is focused down to a spot (Figure 11). In the case of a microscope, the image of the object under inspection is magnified and projected onto the eyepiece, or sensor if using a camera. The particular distance through the system is characterized by either the DIN or JIS standard; all finite conjugate microscopes are either one of these two standards. These types of objectives account for the majority of basic microscopes. Finite conjugate designs are used in applications where cost and ease of design are major concerns.



Figure 11: Simplified Finite Conjugate Microscope Design

## INFINITE CONJUGATE (INFINITY-CORRECTED)

In an infinite conjugate, or infinity-corrected, optical design, light from a source placed at infinity is focused down to a small spot. In an objective, the spot is the object under inspection and infinity points toward the eyepiece, or sensor if using a camera (Figure 12). This type of modern design utilizes an additional tube lens between the object and eyepiece in order to produce an image. Though this design is much more complicated than its finite conjugate counterpart, it allows for the introduction of optical components such as filters, polarizers, and beamplitters into the optical path. As a result, additional image analysis and extrapolation can be performed in complex systems. For example, adding a filter between the objective and the tube lens allows one to view specific wavelengths of light or to block unwanted wavelengths that would otherwise interfere with the setup. Fluorescence microscopy applications utilize this type of design. Another benefit of using an infinite conjugate design is the ability to vary magnification according to specific application needs. Since the objective magnification is the ratio of the tube lens focal length to the objective focal

length (Equation 5), increasing or decreasing the tube lens focal length changes the objective magnification. Typically, the tube lens is an achromatic lens with a focal length of 200mm, but other focal lengths can be substituted as well, thereby customizing a microscope system's total magnification. If an objective is infinite conjugate, there will be an infinity symbol located on the body of the objective.

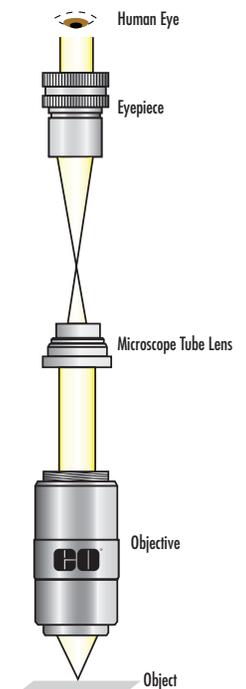


Figure 12: Simplified Infinite Conjugate (Infinity-Corrected) Microscope Design

$$(5) \quad \text{Magnification}_{\text{Objective}} = \frac{\text{Focal Length}_{\text{Tube Lens}}}{\text{Focal Length}_{\text{Objective}}}$$

Continue

## OPTICAL MICROSCOPY APPLICATION EXAMPLES

In order to understand how the components of a microscope can be integrated with various optical, imaging, and photonics products, consider the following optical microscopy applications:

fluorescence microscopy and laser ablation. Each utilizes its own unique setup in order to work with components from a microscope.

### FLUORESCENCE MICROSCOPY

A fluorophore (or fluorescent dye) is used to mark proteins, tissues, and cells for examination or study. Fluorophores can absorb light of one wavelength and emit (fluoresce) light of another wavelength. In a typical fluorescence microscopy setup three filters are used: an excitation filter, an emission filter and a dichroic filter. Each fluorophore has a specific absorption or excitation wavelength band, the excitation filter will transmit only that specific range of wavelengths. The fluorophore, once excited, will emit a different range of wavelengths. The emission filter transmits only the emission wavelengths. A dichroic filter that is specifically designed to reflect the emission wavelengths and transmit the excitation wavelengths is used

to separate the excitation and emission channels. Figure 13 illustrates a typical fluorescence imaging setup.

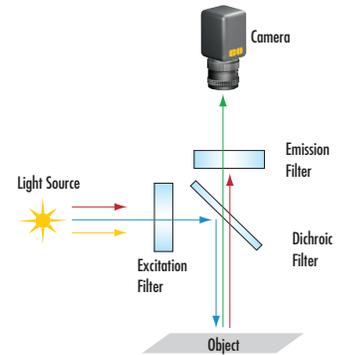


Figure 13: Typical Fluorescence Microscope Setup

### LASER ABLATION

Two common uses of lasers are to (1) heat material onto a base or (2) to ablate material off of a base. Laser ablation systems require microscope components because of the precision beam manipulation (i.e. focusing, bending, scattering reduction, etc) required. A laser ablation setup typically contains custom optics, rather than off-the-shelf components, with the laser precisely designed into the system (Figure 14). The laser is oriented in an epi-illumination design to utilize the microscope objective's ability to focus light at the object plane, and to produce extremely small spot sizes with minimal aberrations. Also, an eyepiece allows the user to see where the laser is located and to make sure everything is working properly. Filters are necessary to block the laser from causing damage to the user's eye. Laser ablation setups are used in medical and biological applications because they offer higher precision than conventional surgical methods.

Microscope and objectives are complex optical systems with many uses. They are no longer used solely for biological setups (e.g. looking at cheek cells in an introductory biology class);

rather, they can be used to study the emission wavelength of a fluorophore, to analyze a 5 $\mu$ m defect on a machined part, to oversee the ablation of material off a base, and within a host of other applications in the optics, imaging, and photonics industries. Understanding the importance of each constituent part of a microscope and their specifications enables any user to choose the best system and achieve the best results.

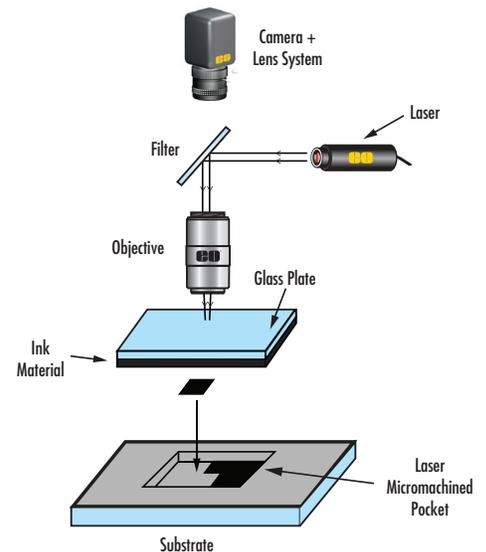


Figure 14: Typical Laser Ablation Setup

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