Microscopy Training & Overview

Product Marketing
October 2011

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OVERVIEW AND PRESENTATION FLOW

- Glossary and Important Terms
- Introduction
  - Timeline
  - Innovation and Advancement
  - Primary Components
- Edmund Optics Product Offering
- Illumination Techniques
- Microscopy Techniques
  - Brightfield
  - Darkfield
  - Phase Contrast
- Microscope Objectives
  - Finite Conjugate
  - Infinity Corrected

GLOSSARY AND IMPORTANT TERMS

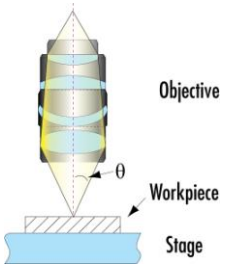
- Numerical Aperture (NA)

Function of the focal length and entrance pupil diameter. NA determines resolving power, depth of field, and contrast of the image. The higher the NA, the greater the resolving power and smaller the depth of field. NA can be calculated using Equation 1.

$$NA = n*Sin\theta$$

Equation 1

Where n is the index of refraction of the medium in which the lens is working (n=1.0 for air) and θ is the half-angle of the maximum cone of light that can enter or exit the lens.



- Resolving Power (R)

Minimum distance between points or lines that are just distinguishable as separate entities. The resolving power of a system is determined by N.A. and wavelength of light (λ), as shown in Equation 2.

$$R = 0.61*\lambda / N.A.$$

Equation 2

- Working Distance (WD)

Distance between the surface of the specimen and the front face of the objective when in focus

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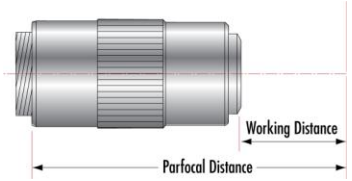
GLOSSARY AND IMPORTANT TERMS

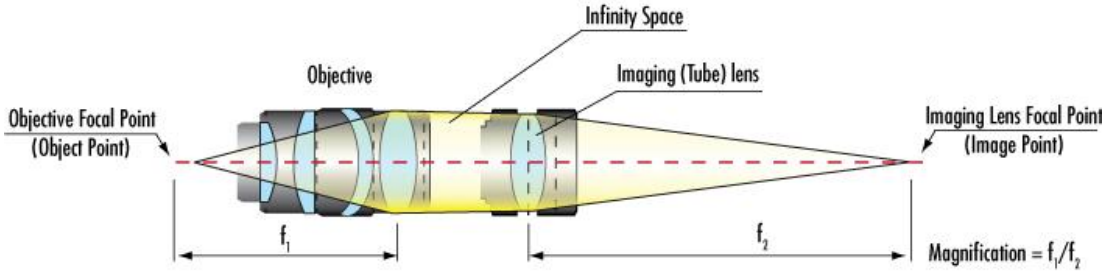
- Parfocal Length

Distance between the surface of the specimen and the objective mounting position when in focus.



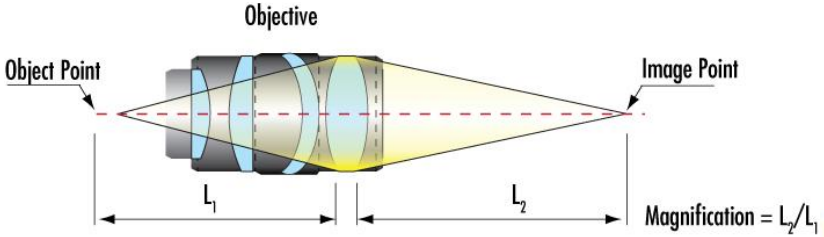
- Infinity Corrected Optical System

An optical system in which the image is formed by an objective and a tube lens with an *Infinity Space* between them, into which optical accessories can be inserted.



- Finite Conjugate Optical System

An optical system in which the image is formed only by an objective.



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GLOSSARY AND IMPORTANT TERMS

- Focal length (F)

Distance between a principal point and a focal point. F1 is the focal length of an objective. F2 is the focal length of a tube lens. For infinity-corrected systems, magnification is determined by the ratio of the focal length of the tube lens to that of the objective.

Magnification of Objective = Focal length of tube lens / Focal length of objective

- Field Number and Field of View (FOV)

The field number of an eyepiece (expressed in mm) is determined by the field stop diameter of the eyepiece.

FOV is the area of the specimen that is observable, and is determined by the field number of the eyepiece and magnification of the objective.

FOV = Field number of eyepiece / magnification of objective

- Depth of Field (DOF)

Vertical distance in the specimen, measured from above and below the exact plane of focus, which still yields an acceptable image. The larger the NA, the smaller the depth of field.

± DOF = λ / (2*(NA)^2) - ***standard wavelength of 550nm***

- Aperture Diaphragm

Adjusts the amount of light passing through, and is related to the brightness and resolving power of an optical system. This diaphragm is especially useful in width dimension measurement of cylindrical objects with contour illumination, and provides the highest degree of correct measurement/observation by suppressing diffraction in an optimal aperture.

GLOSSARY AND IMPORTANT TERMS

- Field Stop

Used for blocking out unwanted light and preventing it from degrading the image

- Vignetting

This unwanted effect is the reduction of an image's brightness or saturation at the periphery compared to the image center. May be caused by external (lens hood) or internal features (dimensions of a multi-element objective).

- Double Image

An image degrading phenomenon in which an image appears as if it is a double image due to redundant light projection and optical interference within the optical system.

- Flare

Lens flare is typically seen as several starbursts, rings, or circles in a row across the image or view, and is caused by unwanted image formation mechanisms, such as internal reflection and scattering of light.

INNOVATION AND ADVANCEMENT IN OPTICAL MICROSCOPY

- **1590** - Approximate microscopy conception date by lens grinders in Middleburg, Netherlands
- **1625** - Term *microscope* is coined from Galileo's design
- **1644** - First living tissue is examined: insect eyes
- **1660** - Extensive research begins in Italy, Holland, and England
- **1667** - Robert Hooke's *Micrographia* is microscopy's first big release
- **1676** - Antoni van Leeuwenhoek discovers micro-organisms and analyzes red-blood cells and bacteria
- **1830** - Joseph Jackson Lister combines weak lenses, forming doublets and triplets to eliminate spherical aberration
- **1878** - Ernst Abbe formulates the relationship between wavelength and resolution
- **1893** - August Kohler develops innovative illumination technique that is thought to be central to modern day optical microscopy
- **1953 – 1955** - Fritz Zernike and George Nomarski advance illumination with phase contrast and Differential Interference Contrast (DIC)

PRIMARY COMPONENTS OF A MICROSCOPE

- Eyepiece(s)
- Camera Port
- Objective
  - Lenses
  - Turret
- Condenser
  - Diaphragm
- Light source
- Stage
  - Focus
  - XY Adjustment

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EDMUND OPTICS PRODUCT OFFERING

- Infinity Corrected Objectives
- Finite Conjugate Objectives
- Reflective Objectives
- Stereo Microscopes
- Miscellaneous
  - Accessories, eyepieces, relay lenses, couplers, reticles, micrometers, pocket and direct microscopes, simple magnifiers

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MICROSCOPE ILLUMINATION

- Afocal/Nonfocused Illumination
  - Systems that do not form an image of the light source at a specific point in the optical pathway. Early microscopes utilized natural sunlight and cloud cover for crude diffusion.
- Critical/Nelsonian Illumination
  - Relies on substage condenser to produce a focused image from a homogeneous light source along the specimen plane to achieve even illumination over the entire field of view
- Current Techniques
  - Incandescent Lamps
  - Arc Lamps
  - LEDs
  - Lasers

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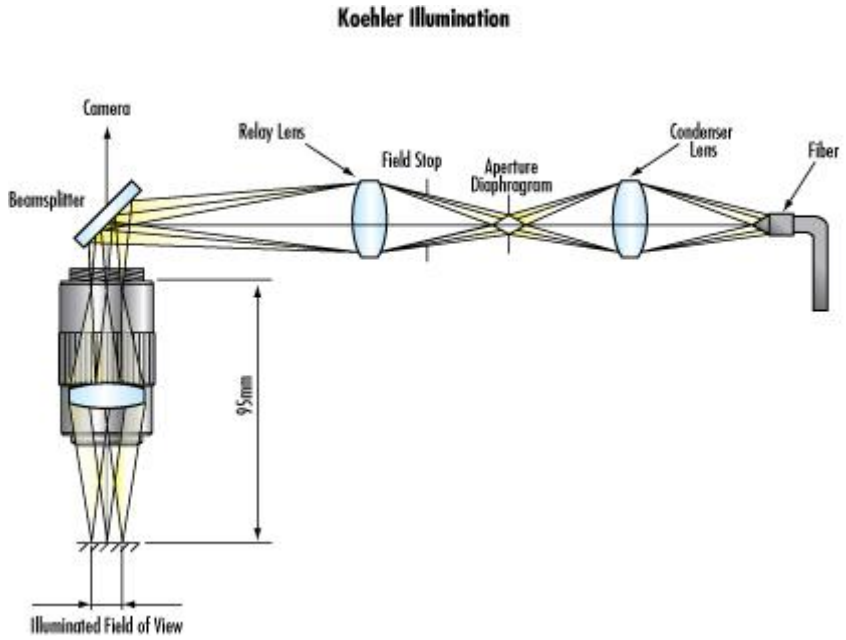
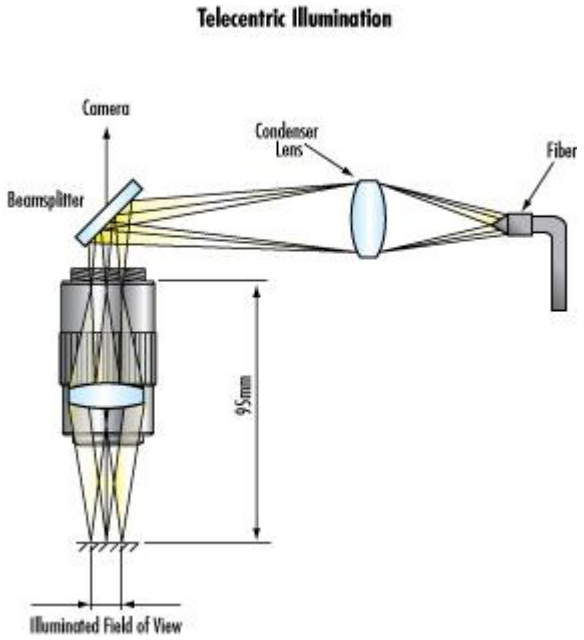
KOEHLER ILLUMINATION

Koehler illumination overcomes the disadvantages of other illumination setups by causing parallel rays to light the specimen so that, because they will not be in focus, the image of the specimen will not include an image of the light source.



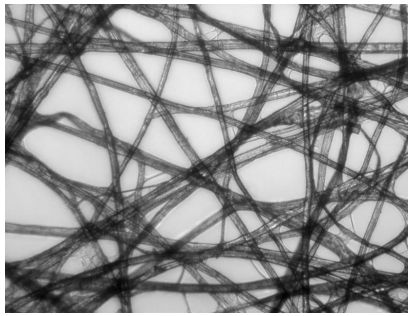
TELECENTRIC ILLUMINATION

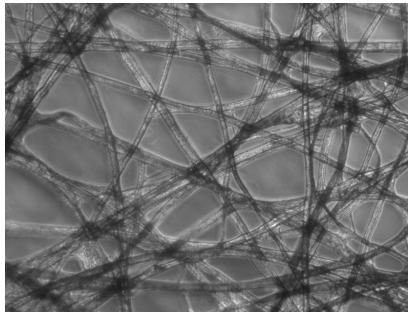
Telecentric illumination is designed so that principal light passes through the focal point. This system has the advantage of retaining the size of the image center even if it is out of focus (although the circumference of the image is defocused). This illumination system provides an even illumination intensity over the entire field of view, and is typically used for lower magnifications: 2x-5x.

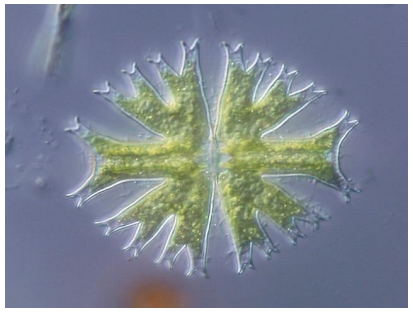


MICROSCOPY TECHNIQUES

- Brightfield
- Darkfield
- Phase Contrast
- Polarization Contrast
- Differential Interference Contrast (DIC)
- Fluorescence
- Interference Reflection (TIRF)







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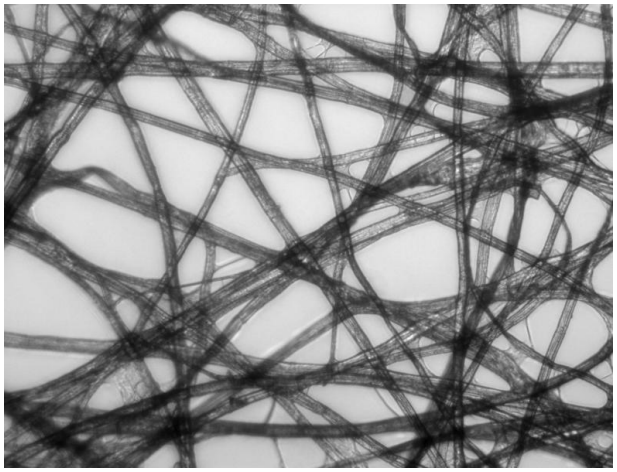
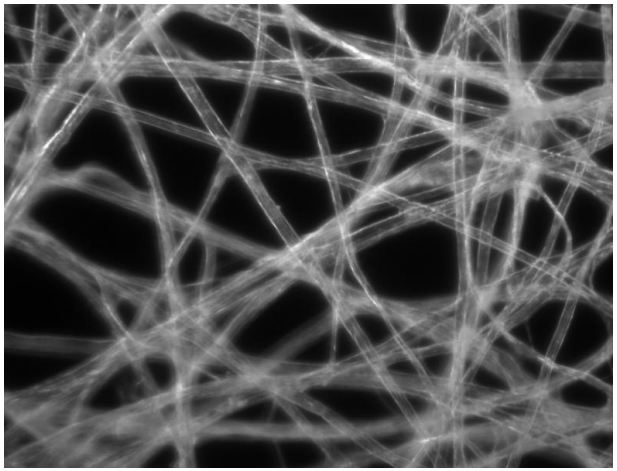
BRIGHTFIELD - DARKFIELD

- Brightfield Illumination
  - Sample contrast comes from *absorbance* of light in the sample
  - One of the simplest techniques in light microscopy
  - Typical appearance is a *dark sample* on a *bright background*
- Darkfield Illumination
  - Sample contrast comes from light *scattered* by the sample
  - Technique used to enhance contrast in unstained samples
  - Typical appearance is a *dark/black background* with *bright objects up front*





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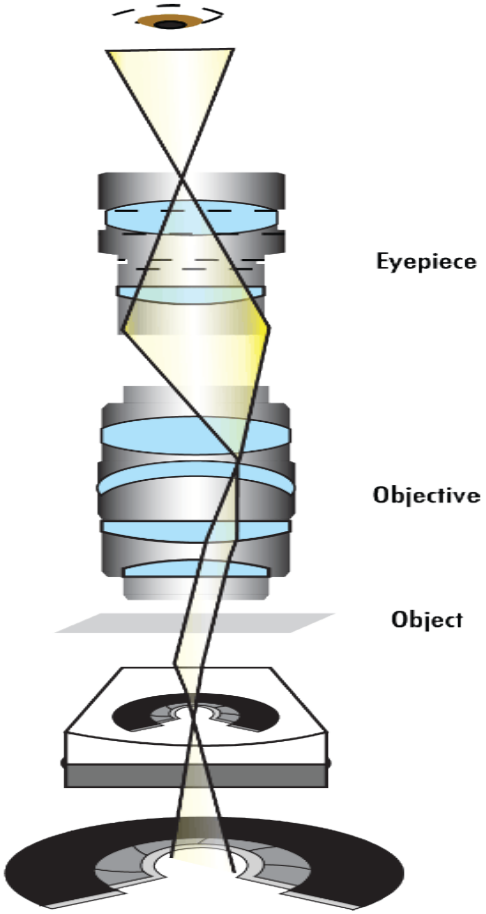
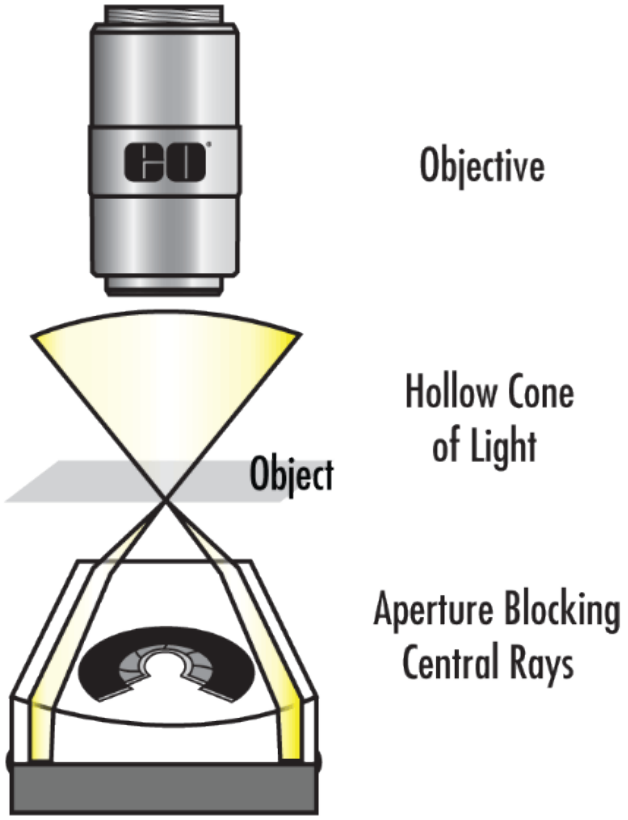
BRIGHTFIELD AND DARKFIELD SETUP



Brightfield



Darkfield

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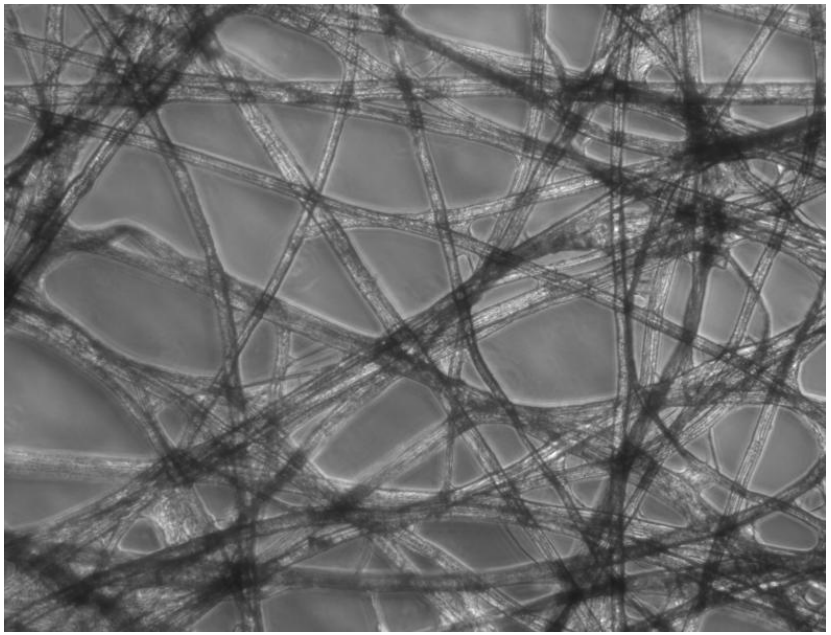
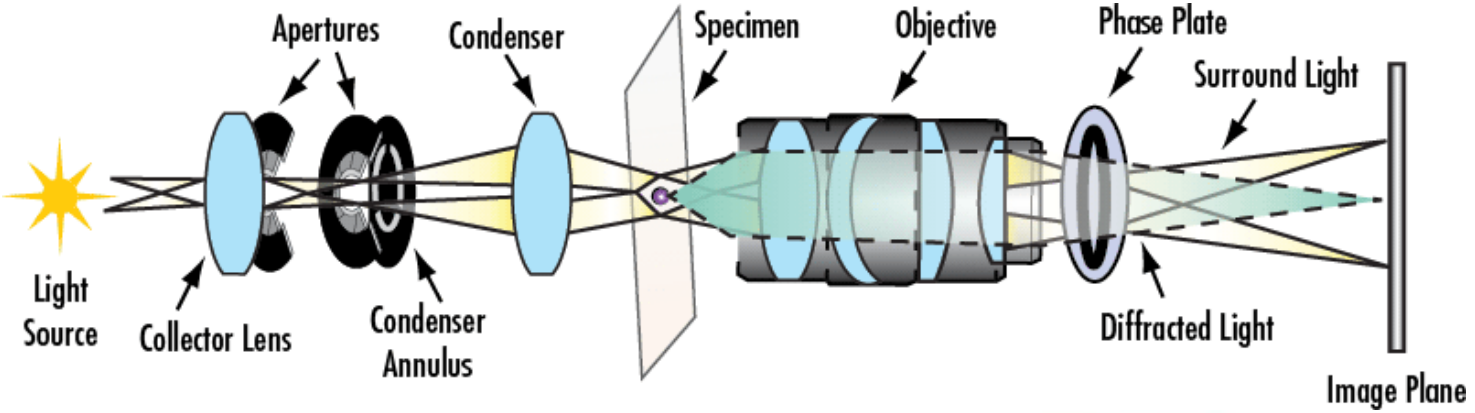
PHASE CONTRAST

- Displays proportional differences in optical density
- Shows differences in refractive index as contrast difference
  - Nucleus appears darker than surrounding ECM
- Typically a grey background with light and dark features - these features represent change in optical density





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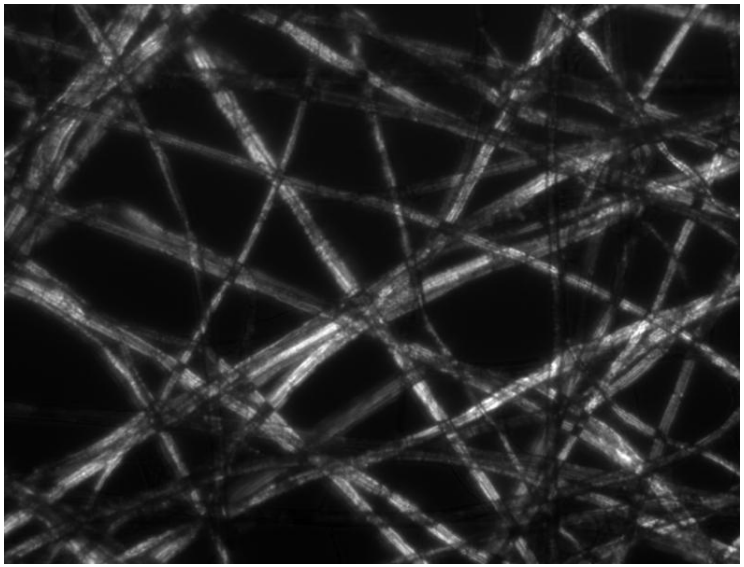
POLARIZATION CONTRAST

- Any optical microscope involving polarized light
- Complimentary to DIC and TIRF
- Identifies varying refractive indices much like brightfield
- Can distinguish between isotropic and anisotropic substances
- Must be equipped with a *polarizer* in the light path and an *analyzer* in the image path
- Image contrast arises from interaction with a *birefringent* specimen



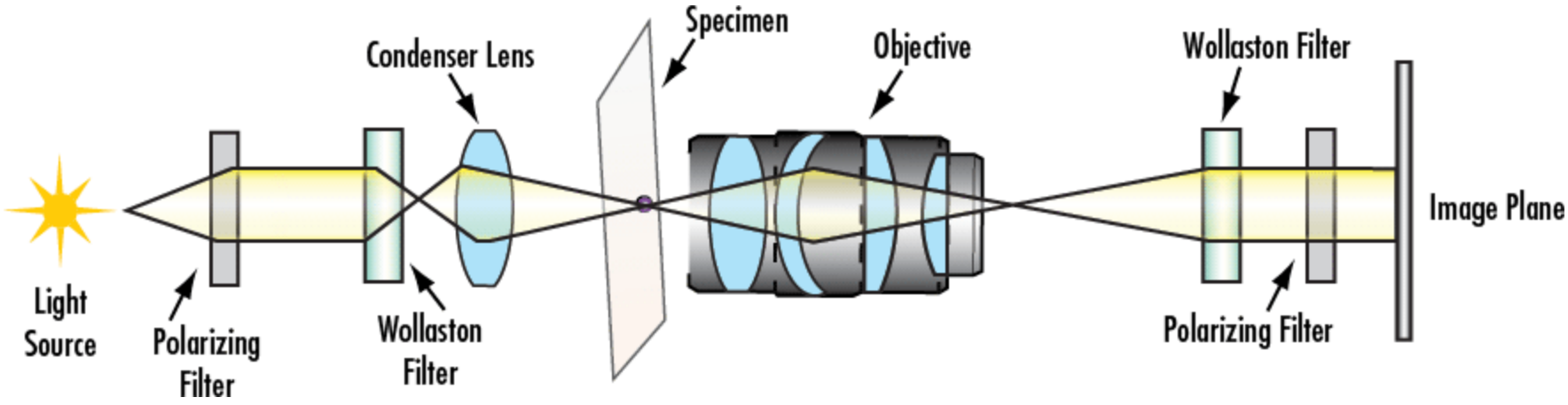
DIFFERENTIAL INTERFERENCE CONTRAST (DIC)

Enhances the contrast in unstained, transparent samples. This technique works on the principle of interferometry to access the optical path length of the sample and enhances what were once invisible features. Similar to phase contrast without the illuminated diffraction halo.

- Fully utilizes NA of the system
- No confusing halos as seen in Phase images
- Images appear vibrant in color due to optical staining
  - 3D shadowed like appearance
- Low cost plan achromats or achromats can be utilized
- Very high resolution system
- Can focus on a thin section of thicker specimen while avoiding noise around the image plane

Micrasterias furcata - Transmitted DIC Microscopy

DIC OPTICAL PATHWAY



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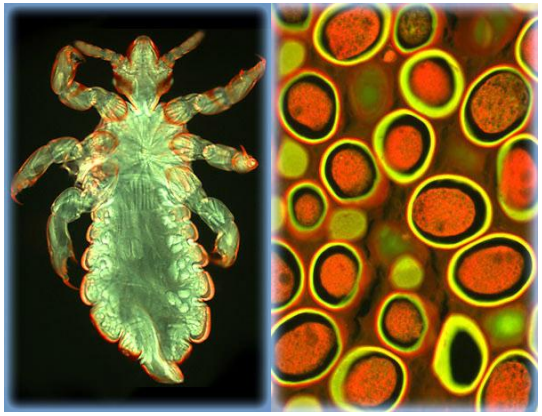
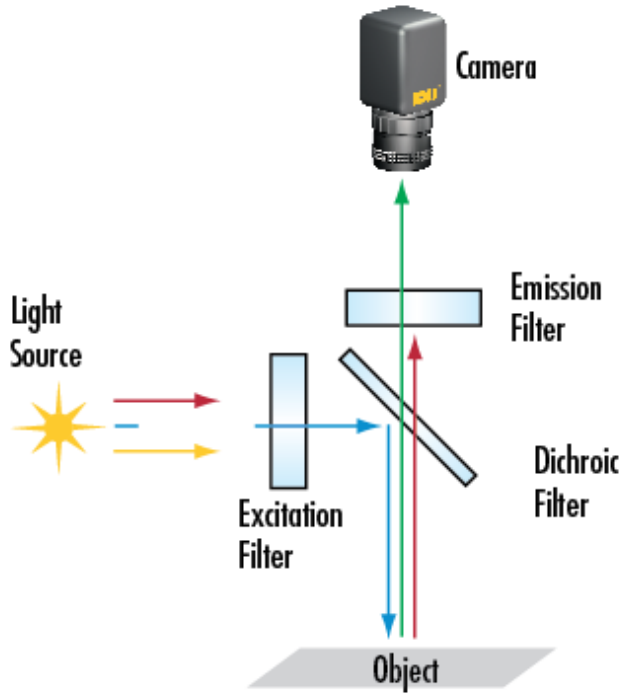
FLUORESCENCE

- **Photoluminescence** – when specimens absorb and subsequently re-radiate light
- **Phosphorescence** – when the emission of light persists for up to a few seconds after excitation has ceased
- **Fluorescence** – describes light emission that continues only during the absorption of excitation light



Head lice Round Worm



Specimen emits a longer wavelength than the light used to excite for fluorescence.

Utilizes three filters for best image:

1. Excitation
2. Emission
3. Dichroic

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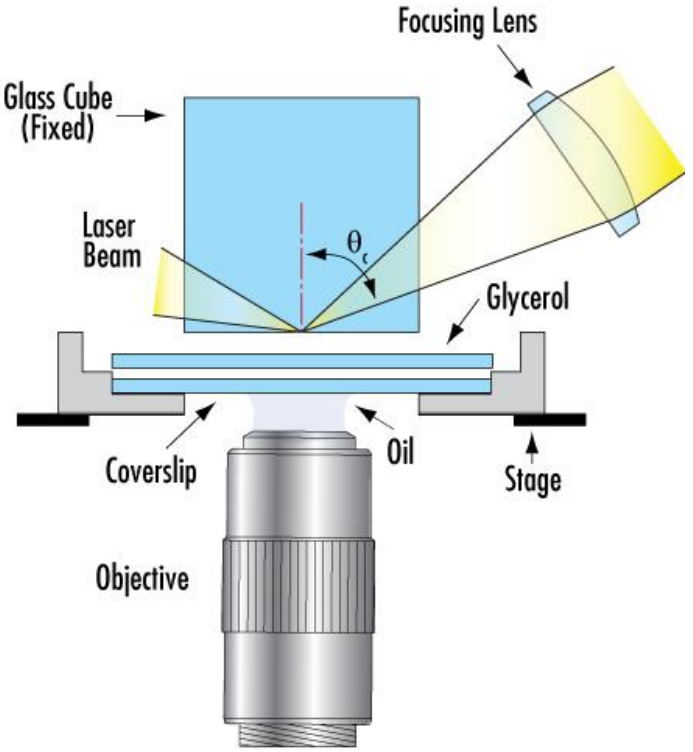
TOTAL INTERFERENCE REFLECTION FLUORESCENCE (TIRF)

TIRF is an extremely elegant optical microscopy technique primarily used to observe single molecule fluorescence at adhesion points on a surface. This interaction between molecules and a surface is an area of importance for multiple disciplines such as cellular and molecular biology.

- Employs the properties of an induced evanescent wave to illuminate/excite fluorophores
  - Molecules/fluorophores are immediately adjacent to the glass-water interface in most cases
- Simple concept requiring only an excitation light beam at high incident angle to sample
- Refractive index difference between glass and water regulate light refraction/reflection
- The evanescent wave/field intensity exponentially decays as distance from the surface increases



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TIRF OPTICAL PATHWAY