Optical Microscopy Overview

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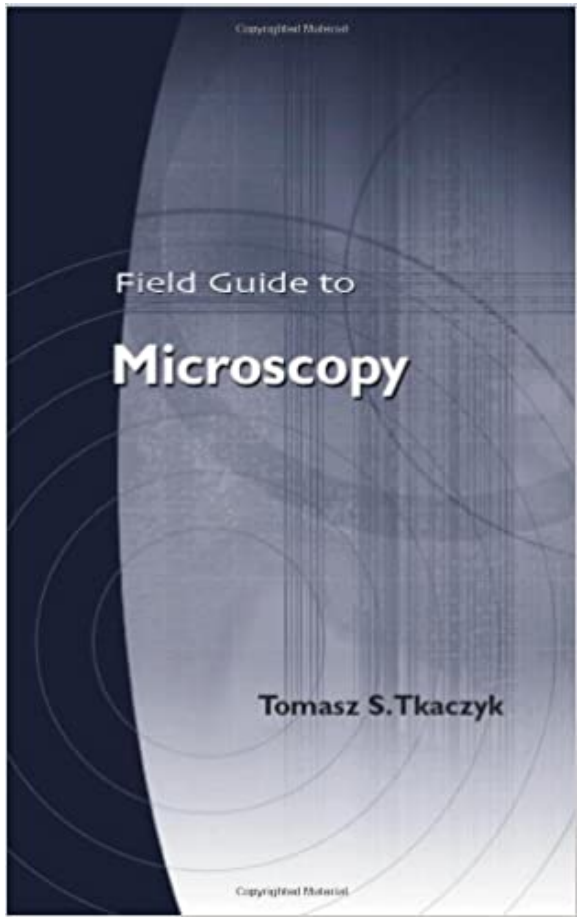
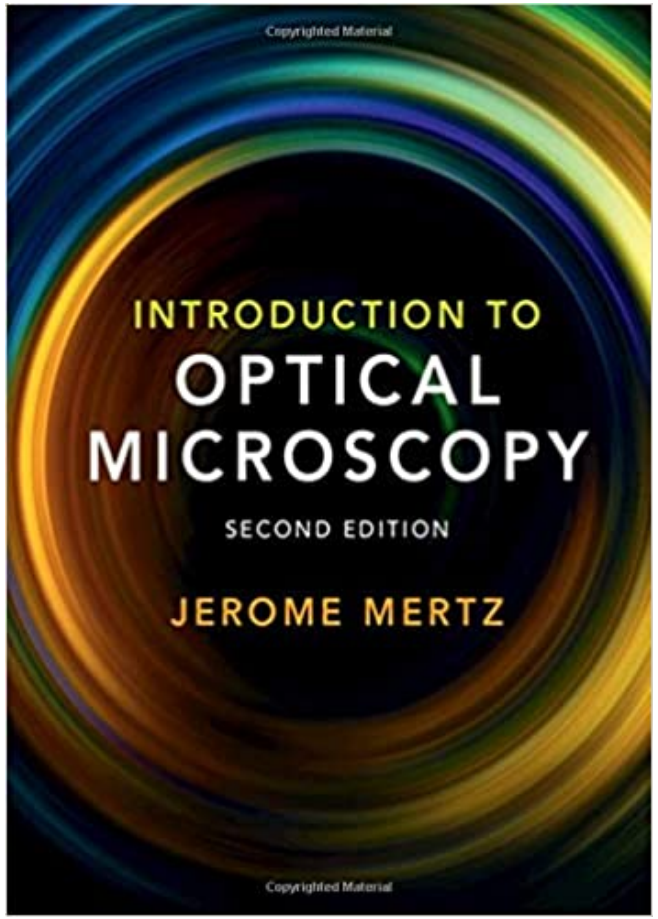
UR Boyd Group Meeting

Some good references

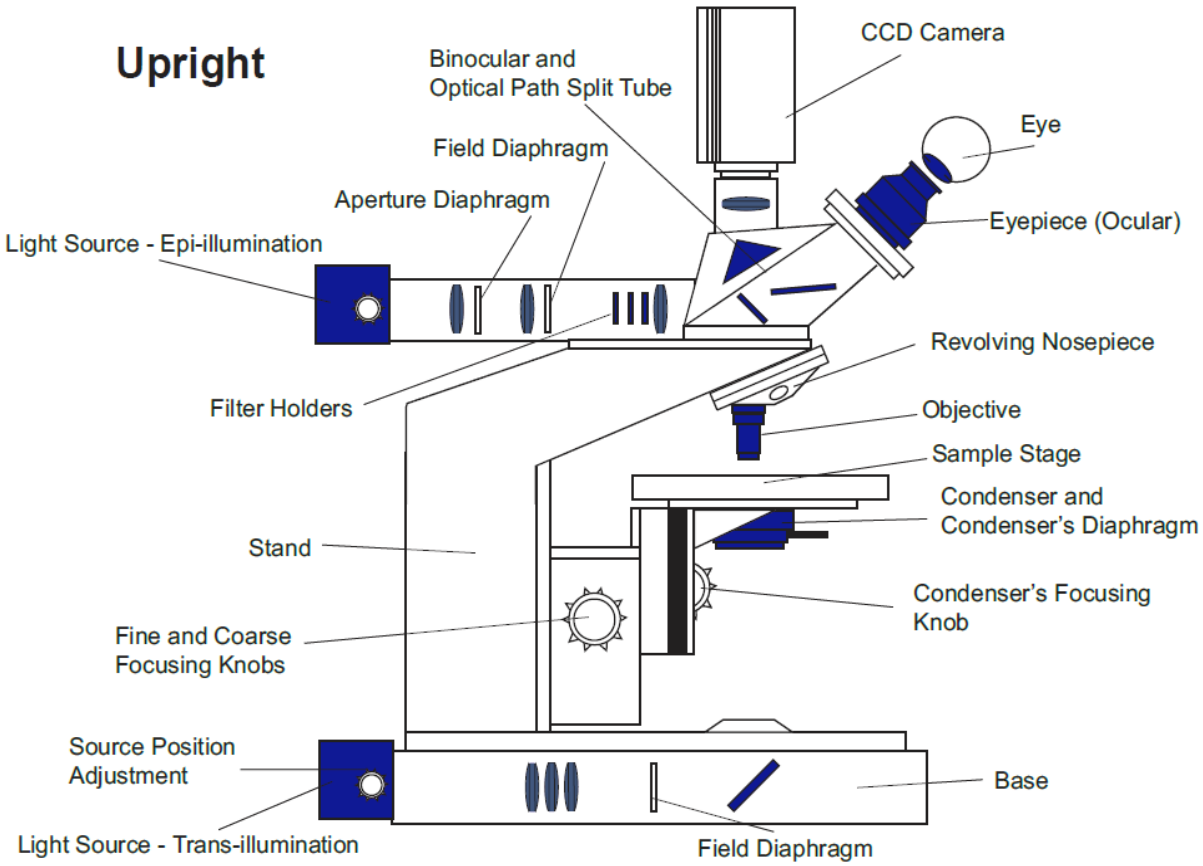


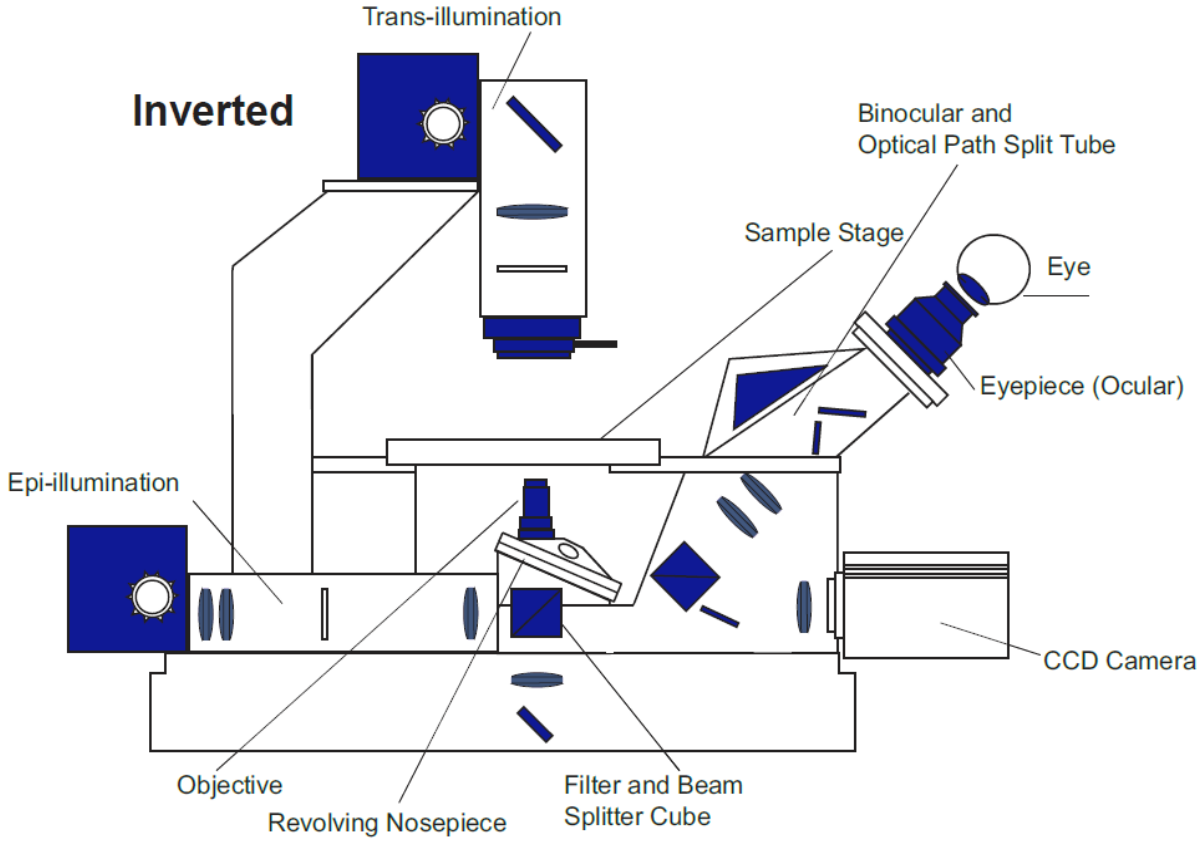
Brief Overview



Thorough Treatment

Upright vs Inverted Microscopes





Reflection vs Transmission Microscopy

Reflection

- Illumination and viewing on same side of sample
- Objective also used for illumination
- Also known as “epi”
- Configuration of choice for fluorescence microscopy (“epifluorescence”) when paired with a dichroic beamsplitter
- Also useful for surface profiling

Transmission

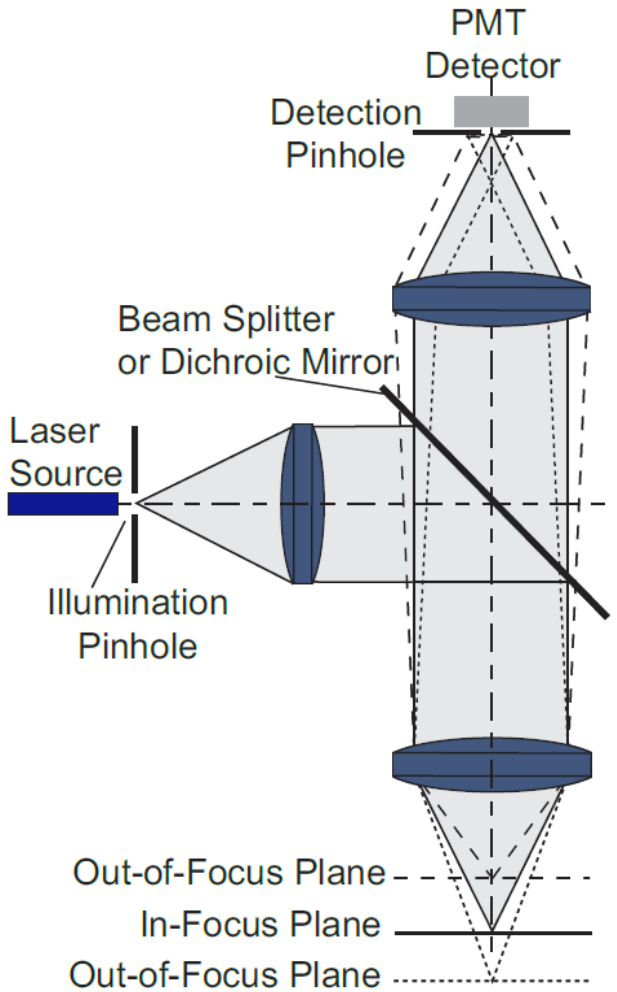
- Illumination and viewing on opposite sides of sample
- Objective for imaging, condenser for illumination
- Configuration of choice for semi-transparent samples (phase objects especially)
- Typical for imaging unlabeled biological specimens

Widefield vs Confocal Microscopy

In widefield microscopy, the entire region of interest (ROI) is illuminated at once

In confocal microscopy, a pinhole is used to reject light from out-of-focus planes, allowing optical sectioning. A translation stage or galvo-mirror system is used for 3D imaging

Confocal microscopy yields a slight resolution improvement for an appropriately sized pinhole. Deconvolution algorithms can be used to provide sectioning in widefield systems



Overview of Microscopy Techniques

Technique	Type of sample
Bright-field	Amplitude specimens, reflecting specimens, diffuse objects
Dark-field	Light-scattering objects
Phase contrast	Phase objects, light-scattering objects, light-refracting objects, reflective specimens
Differential interference contrast (DIC)	Phase objects, light-scattering objects, light-refracting objects, reflective specimens
Polarization microscopy	Birefringent specimens
Fluorescence microscopy	Fluorescent specimens
Laser scanning, Confocal microscopy, and Multi-photon microscopy	3D samples requiring optical sectioning, fluorescent and scattering samples
Super-resolution microscopy (**RESOLFT**, **4Pi**, **I^{5}M**, **SI**, **STORM**, **PALM**, and others)	Imaging at the molecular level; imaging primarily focuses on fluorescent samples where the sample is a part of an imaging system
Raman microscopy, **CARS**	Contrast-free chemical imaging
Array microscopy	Imaging of large FOVs
SPIM	Imaging of large 3D samples
Interference Microscopy	Topography, refractive index measurements, 3D coherence imaging

Overview of Sample Types

Sample type	Sample Example
Amplitude specimens	Naturally colored specimens, stained tissue
Specular specimens	Mirrors, thin films, metallurgical samples, integrated circuits
Diffuse objects	Diatoms, fibers, hairs, micro-organisms, minerals, insects
Phase objects	Bacteria, cells, fibers, mites, protozoa
Light-refracting samples	Colloidal suspensions, minerals, powders
Birefringent specimens	Mineral sections, crystallized chemicals, liquid crystals, fibers, single crystals
Fluorescent specimens	Cells in tissue culture, fluorochrome-stained sections, smears and spreads

Performance Criteria

Numerical aperture $= \mathrm{NA} = n \sin u$

Abbe resolution $= \Delta x = \dfrac{\lambda}{NA_{objective} + NA_{condenser}} = \dfrac{\lambda}{NA}$

Depth of field $= 2\Delta z = \dfrac{n\lambda}{NA^2}$, depth of focus is in the image plane

Field of view is the diameter of the total transverse sample area being imaged in the object plane, usually in millimeters

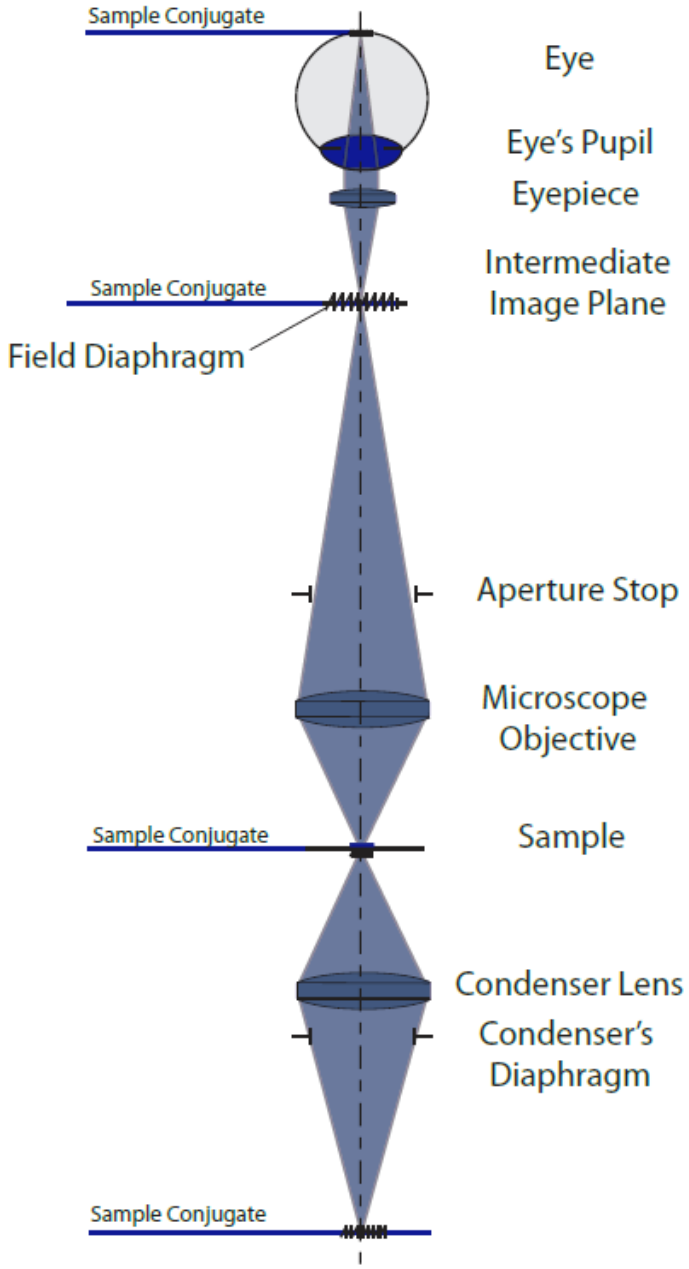
Magnification also an important objective parameter

Critical Illumination

In critical illumination, the source is imaged onto the object plane

Generally disadvantageous as any non-uniformities in the source (filament structure, Gaussian beam profile, etc.) show up in the image

Virtually all modern microscopes do not use this technique

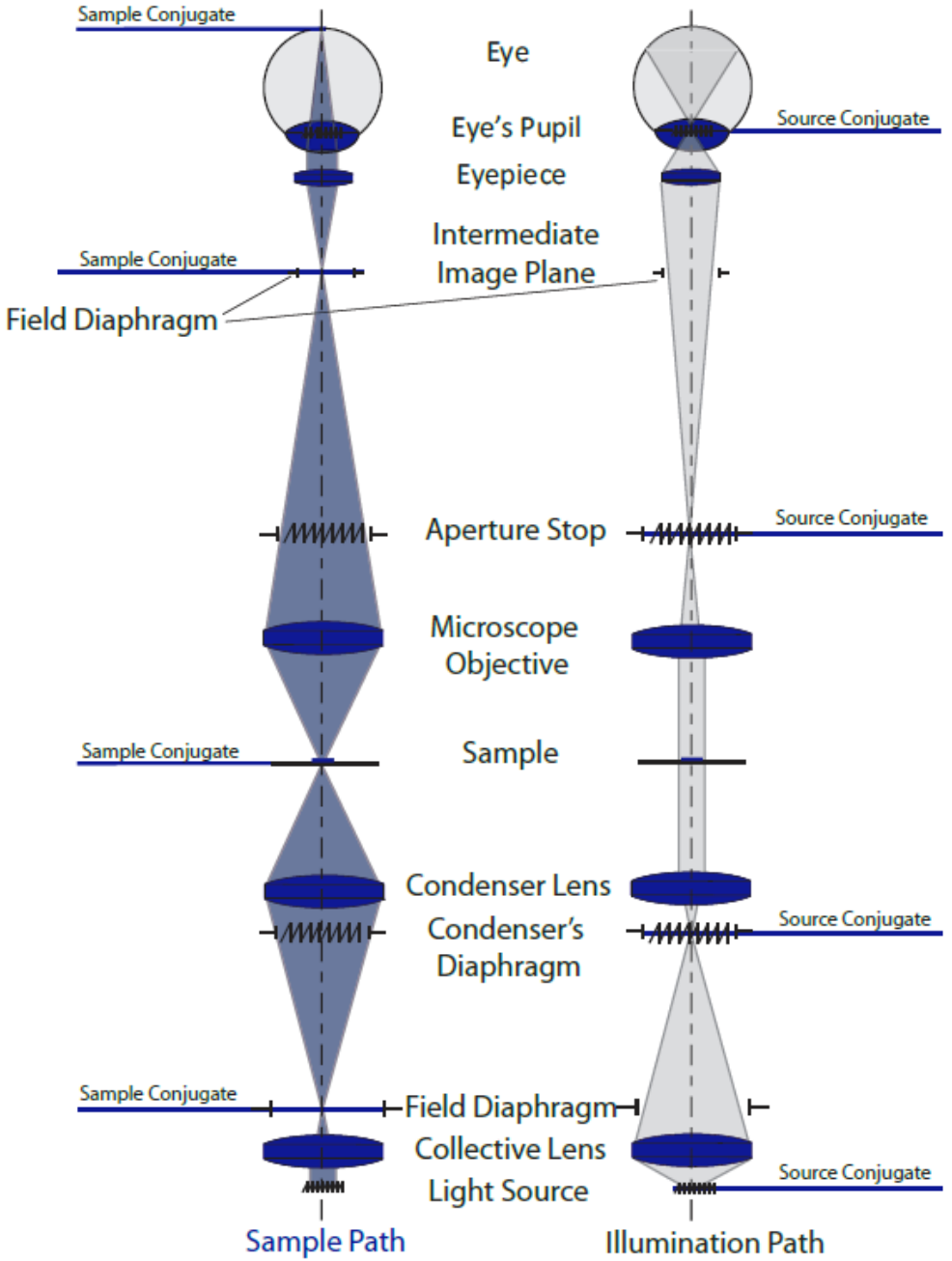


Kohler Illumination

Modern microscope systems use Kohler illumination

Kohler illumination uses collective and condenser lenses to put the light source at infinity with respect to the sample

This ensures bright, even illumination



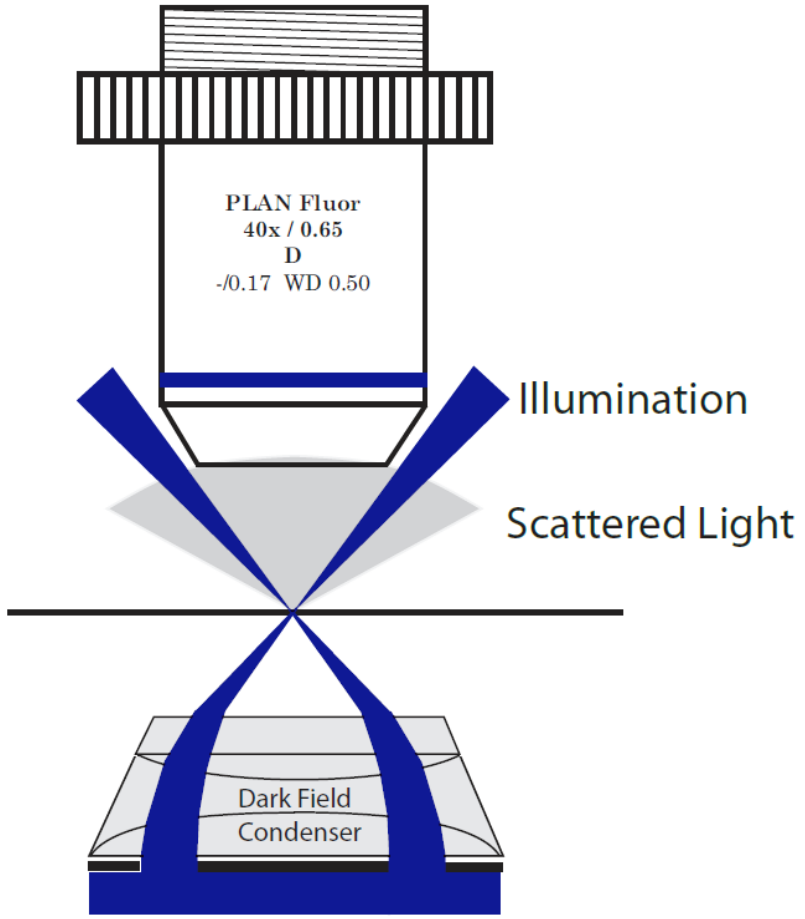
Brightfield vs Darkfield Microscopy

Darkfield microscopy uses oblique illumination, such that only scattered light is collected by the objective

This makes it well-suited for imaging highly scattering objects

Requires an annular mask in the condenser and an objective with a lower NA than the illumination cone

In contrast, the more typical brightfield approach uses on-axis illumination and gathers un-scattered light through the objective

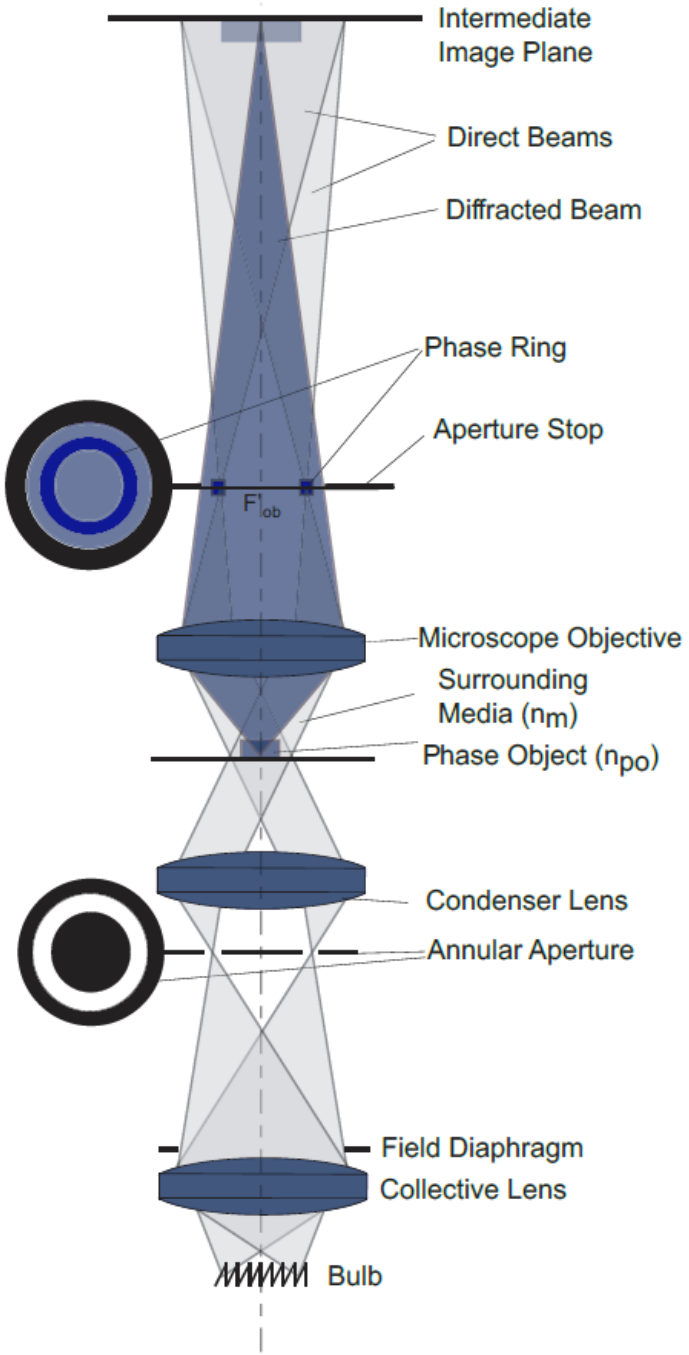


Phase Contrast Microscopy

Phase shift in the aperture stop of the objective provides phase contrast through interference

Using an SLM to step the phase and collect 4 separate images allows one to quantitatively reconstruct the phase (quantitative phase microscopy or phase-shifting holography)

Use of annular phase masks and illumination in contrast to just an on-axis phase mask optimizes light throughput in the instrument



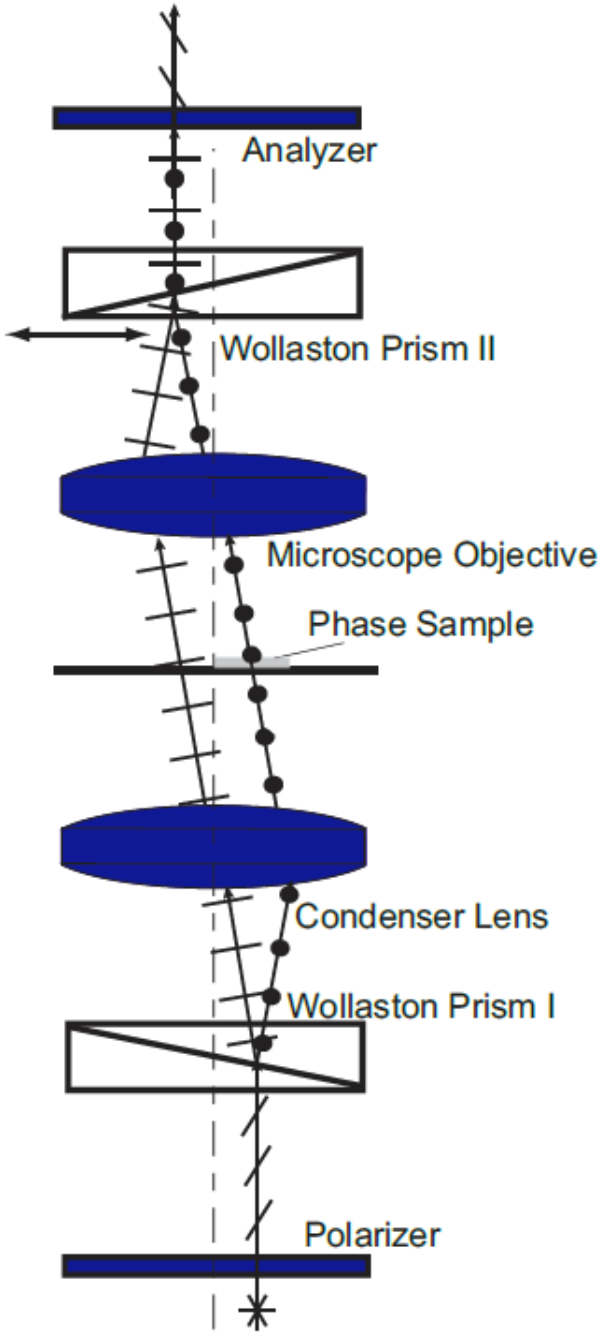
Differential Interference Contrast

DIC microscopes can image thicker phase objects and better resolve boundaries than phase contrast microscopes, although are unable to handle birefringent samples

Basically shearing interferometry with orthogonally polarized beams

Image contrast depends on the phase gradient, rather than the phase itself

Offers (slightly) superior resolution to phase contrast microscopy as well as optical sectioning capability



What else?

Super-resolution: Limited to a factor of 2 for unlabeled specimens, theoretically unlimited in fluorescence microscopy but practical limits set in around ~25 nm

Coherent vs incoherent illumination: Generally get an extra factor of 2 in resolution for incoherent illumination, but lose phase information (also other performance metrics, like contrast, might be worse)

Chemically selective imaging: Raman and CARS/SRS microscopy very useful for biomedical imaging with molecular specificity