Introduction

Polarization microscopy is an optical technique used to make **birefringent structures** like starch grains or cellulose visible without staining.

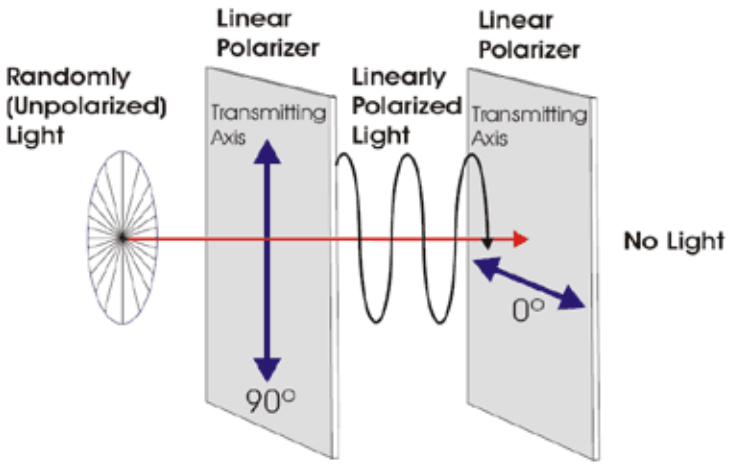
Birefringence is the optical property of a material having a refractive index that depends on the polarization and direction of light.

When illuminated with polarized light, birefringent structures can produce specific color effects which can be easily visualized by using a polarizing microscope.

Component of polarized microscope:

Besides optical component like the condenser aperture diaphragm and objective, some additional components have to be inserted into the light path to achieve polarized microscopy:

1. Polarizer: is an optical filter located under the condenser that passes light oscillating within single plane" linearly polarized light".
2. The analyzer: another polarizer filter located after the objective lens, and rotated 90° relative to the polarizer.



Principle:

1. The first polarizer produces polarized light which is focused on the specimen by the condenser
2. When the polarized light passes through **birefringent material** in the specimen, the polarization plane of a portion of the light is turned 90° ,light that did not pass through birefringent material can have any polarization plane.
3. The image is then magnified by the objective lens
4. The light then will pass through the analyzer which is rotated 90° compared to the first polarizer, light that did not pass through birefringent material will be filtered out since it does not have a polarization plane of 90° .while light passing by birefringent material will be able to pass through the polarizer since it is rotated by 90° and continuous to produce the image.

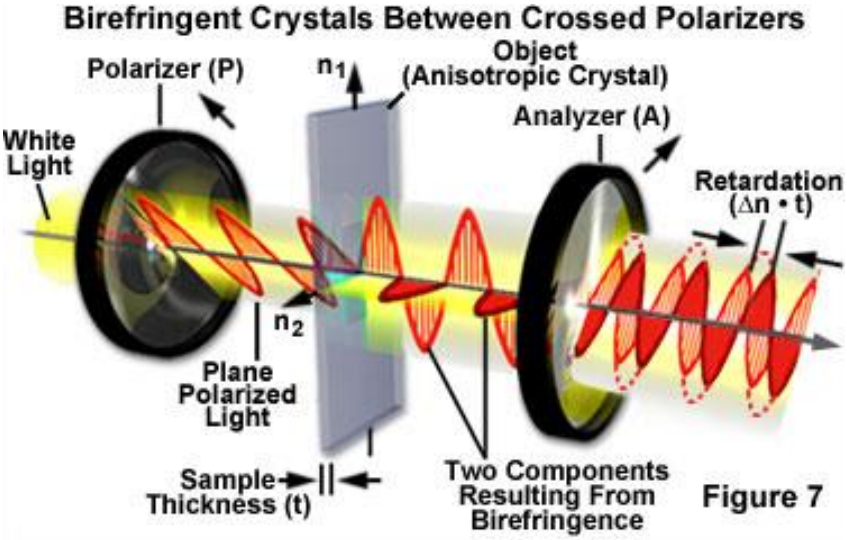


Figure 7

Fluorescent Microscopy

Fluorescence Microscopy is a special form of light microscopy. It uses fluorescence to highlight structures in fixed and living biological specimens instead of using absorption.

The fluorescence is achieved either by inorganic dyes, proteins, synthetic beads or by auto fluorescent structures within a sample. The most prominent difference between fluorescence microscopy and bright field-based contrasting methods is that fluorescence microscope employs incident light instead of transmitted light.

Principle

The basic task of the fluorescence microscope is to let high energy excitation light illuminate the specimen and then filter out the much weaker emitted light from the image, the light beam can be illustrates by the following steps

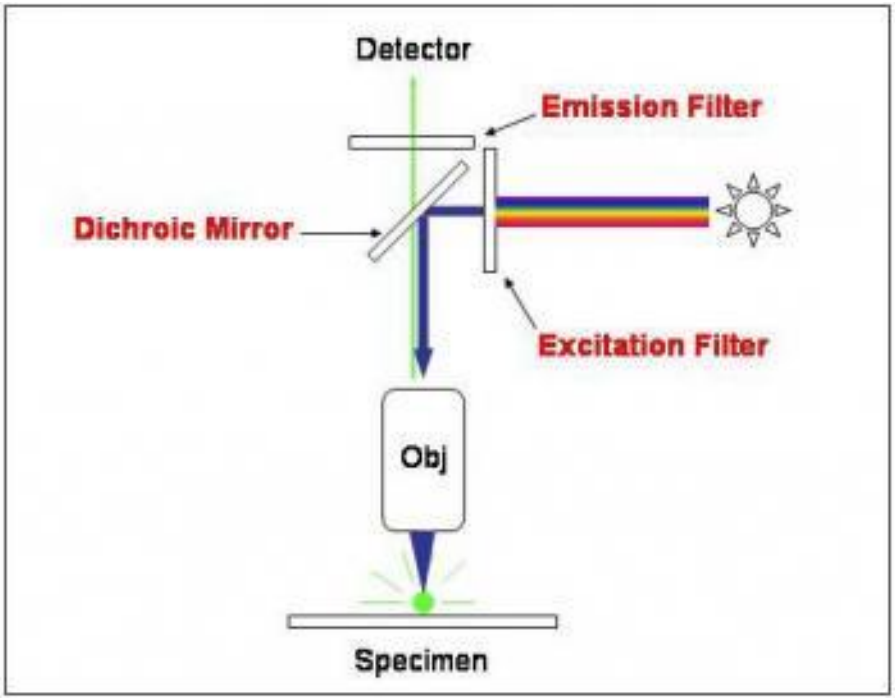
1. The microscope has a filter that only lets light with the specific wavelength that matches your fluorescing material.
2. The light interferes with the atoms in specimen and electrons are excited to a higher energy level. When they relax to a lower level, they emit light.
3. To become visible to the human eye the fluorescence emitted from the sample is separated from the excitation light in a second filter. This works because the emitted light is of lower energy and has a longer wavelength than the light that is used for illumination.

4. The majority of fluorescence microscopes, especially those used in the life sciences are of the epifluorescence design which mean that excitation Light is focused on the specimen through the objective lens.
5. The fluorescence emitted by the specimen is focused to the detector by the same objective that is used for the excitation.
6. Since most of the excitation light is transmitted through the specimen, only reflected light reaches the objective together with the emitted light.
7. An additional barrier filter between the objective and the detector can filter out the remaining excitation light from fluorescent light.

Applications:

These microscopes are often used for -

- Imaging structural components of small specimens, such as cells
- Conducting viability studies on cell populations (are they alive or dead?)
- Imaging the genetic material within a cell (DNA and RNA)
- Viewing specific cells within a larger population with techniques such as FISH.


Detector
Emission Filter
Dichroic Mirror
Excitation Filter
Obj
Specimen