Types of microscopes :-

Microscopes are classified according to illumination used into:

1. Optical (light) microscope: uses light to observe the magnified image of a specimen or object. Optical microscopes function on the basis of optical of lenses by which it can magnifies the image obtained by the movement of a light wave through the sample. The waves used in optical microscopes are electromagnetic and that in electron microscopes are electron beams.

Light microscopes can be classified into:

A. Bright field microscope.
B. Phase contrast microscope.
C. Dark field microscope.
D. Fluorescence microscope.
E. Polarizing microscope.

2. Electron microscope: uses an electron beam to illuminate the specimen to produce a highly magnified image. There is two major types of electron microscope:

A. Scanning Electron Microscope(SEM),
B. Transmission electron microscope (TEM).

Dark field and phase contrast microscopes

Introduction

- Live cells in generally lack sufficient contrast to be studied, since the internal structures of the cell are colorless and transparent.
- The most common way to increase contrast is to stain the different structures with selective dyes, but this often involves killing and fixing the sample.
- The human eye is not sensitive to the difference in light phase when passing into two different media with different refractive indices, but clever optical solutions have been thought out to change this difference in phase into a difference in light intensity.

Illumination techniques

Many techniques are available which modify the light path to generate an improved contrast image from a sample.

Dark field microscopy: is a technique for improving the contrast of unstained, transparent specimens by using a carefully aligned light source to minimize the quantity of directly transmitted (unscattered) light passing the sample surroundings and collecting only the light scattered by the sample.

Principal:

The steps are illustrated in the figure where an upright microscope is used:

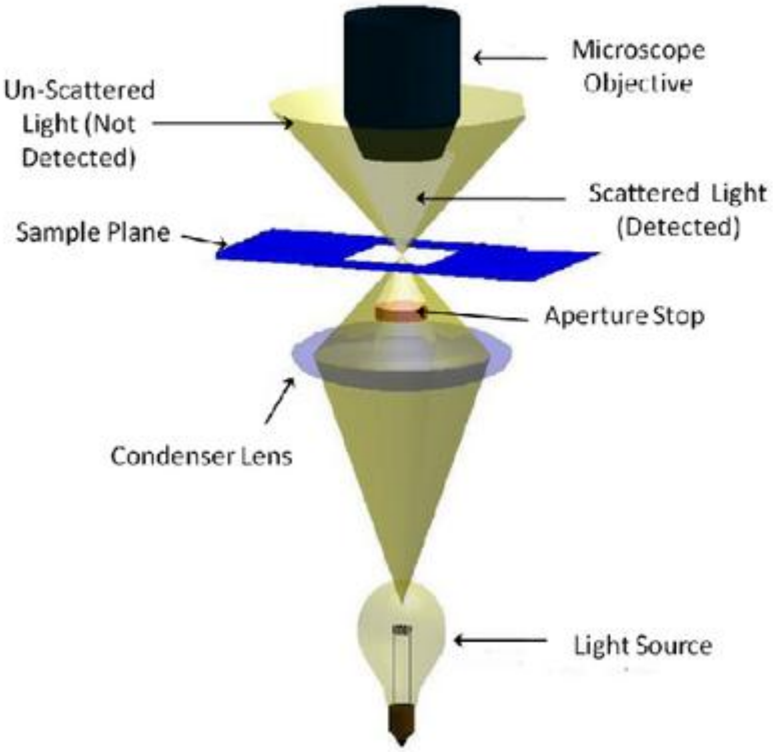


Diagram illustrating the light path through a dark field microscope.

1. Light enters the microscope for illumination of the sample.
2. A specially sized disc, the *patch stop* (see figure) blocks some light from the light source, leaving an outer ring of illumination.
3. The condenser lens focuses the light towards the sample.

4. The light enters the sample. Most is directly transmitted, while some is scattered from the sample.

5. The **scattered light (diffracted light)** enters the objective lens, while the **directly transmitted light** simply misses the lens and is not collected.
6. Only the scattered light goes on to produce the image, while the directly transmitted light is omitted.

Advantage: Dark field can dramatically improve image contrast – especially of transparent objects – while requiring little equipment setup or sample preparation.

Disadvantage:

1. The technique suffers from low light intensity in final image of many biological samples.

2. Low resolution.

Phase contrast microscope

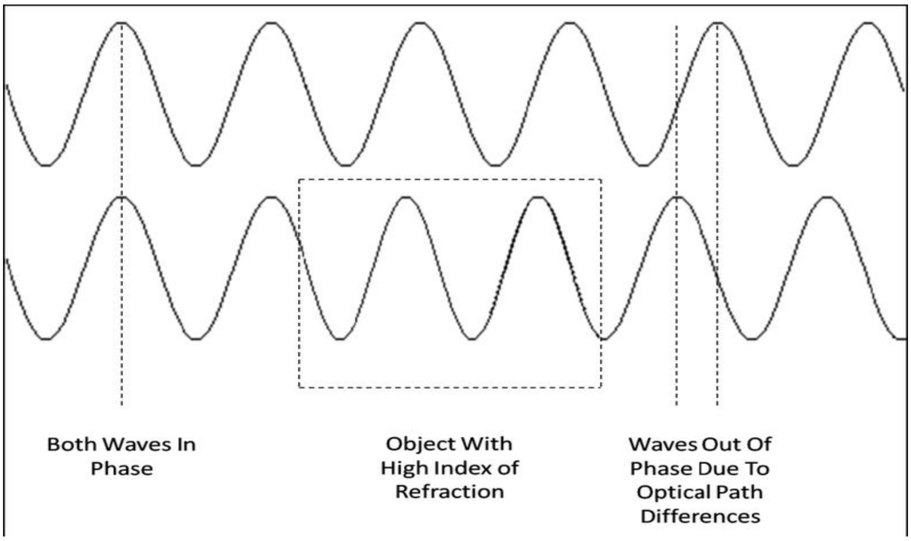
The phase contrast microscope is widely used for examining such specimens as biological tissues. It is a type of light microscopy that enhances contrasts of transparent and colorless objects by influencing the optical path of light. The phase contrast microscope is able to show components in a cell or bacteria, which would be very difficult to see in an ordinary light microscope.

Altering the Light Waves

The phase contrast microscope uses the fact that the light passing through a transparent part of the specimen **travels slower** and, due to this is shifted compared to the uninfluenced light. This difference in phase is not visible to the human eye. However, the change in phase can be increased to half a wavelength by a transparent phase-plate in the microscope and thereby causing a difference in brightness. This makes the transparent object shine out in contrast to its surroundings

The Invisible Can Be Seen

The phase contrast microscope is a vital instrument in biological and medical research. When dealing with transparent and colorless components in a cell, dyeing is an alternative but at the same time stops all processes in it. The phase contrast microscope has made it possible to study living cells, and cell division is an example of a process that has been examined in detail with it. The phase contrast microscope was awarded with the Nobel Prize in Physics, 1953.



- Internal details and organelles of live unstained organisms can be seen clearly with this microscope (**e.g. mitochondria, lysosomes, and the Golgi body**).Light passing through cellular structures, such as chromosomes or mitochondria is retarded because they have a higher refractive index than the surrounding medium.

Interaction of Light Waves with Phase Specimens

An incident wavefront present in an illuminating beam of light becomes divided into two components upon passing through a phase specimen. The primary component is an undeviated (or undiffracted; **zeroth-order**) planar wavefront, commonly referred to as the **surround** (**S**) wave, which passes through and around the specimen, but does not interact with it. In addition, a deviated or **diffracted** spherical wavefront (**D**-wave) is also produced, which becomes scattered over a wide arc (in many directions) that passes through the full aperture of the objective.

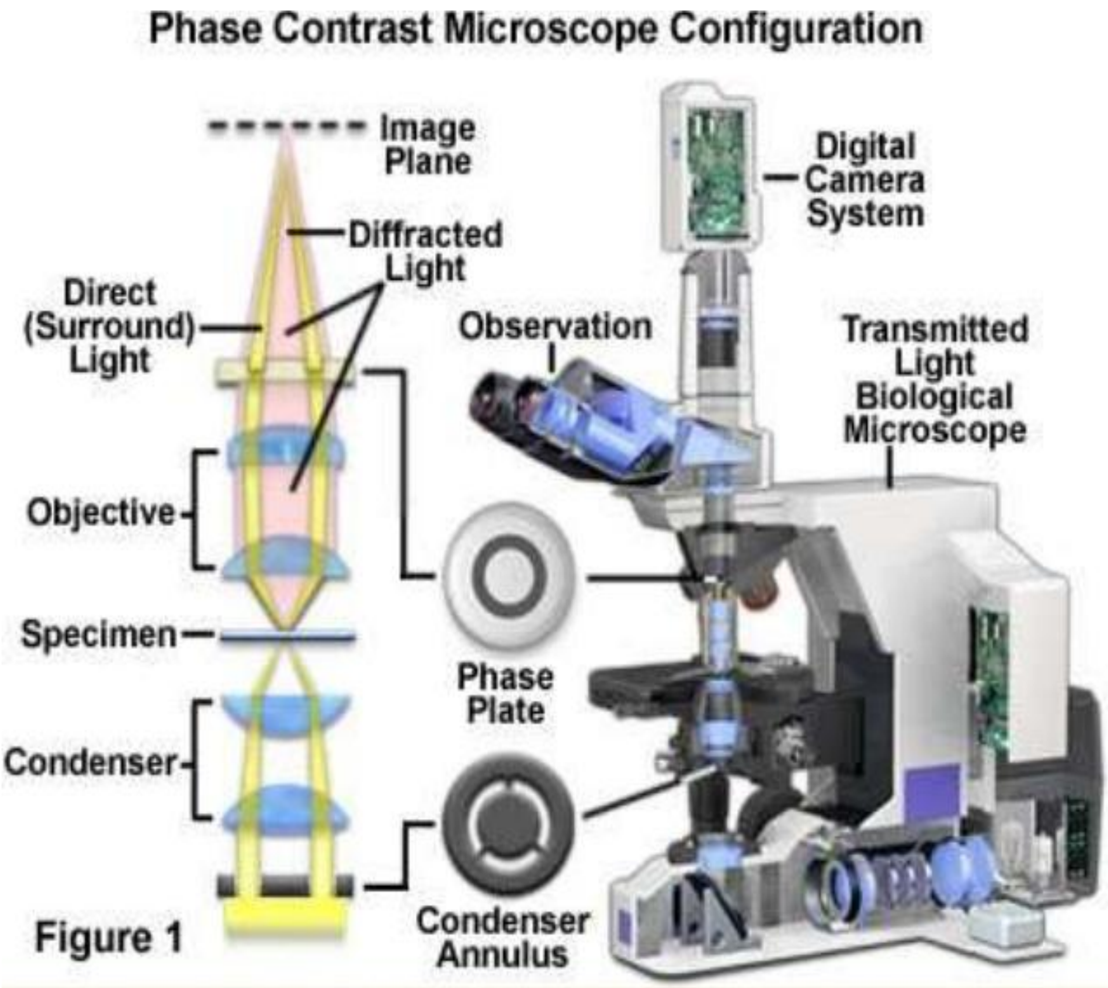
After leaving the specimen plane, **surround and diffracted light waves** enter the objective front lens element and are subsequently focused at the intermediate image plane where they combine through interference to produce a resultant **particle** wave (often referred to as a **P**-wave). The mathematical relationship between the various light waves generated in phase contrast microscopy can be described simply as:

$$\mathbf{P = S + D}$$

Detection of the specimen image depends on the relative intensity differencesof the particle and surround (**P** and **S**) waves. If the the particle and surround waves are significantly different in the intermediate image plane, then the specimen acquires a considerable amount of contrast and is easily visualized in the microscope eyepieces.

Advantage:

- Phase-contrast microscopy allows the visualization of living cells in their natural state with high contrast and high resolution.


Phase Contrast Microscope Configuration
Image Plane
Diffracted Light
Direct (Surround) Light
Observation
Digital Camera System
Transmitted Light Biological Microscope
Objective
Specimen
Phase Plate
Condenser
Condenser Annulus
Figure 1