



**University of Al-Maarif**



**Medical Laboratory Techniques Department**  
**Laboratory instruments**  
**First stage**  
**Lab(2)**

**Microscope**



**Dark field Microscope**

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## 2- Dark field Microscope: -

Is a simple method to make transparent, unstained samples clearly visible.

These objects often have refractive indices very close in value to those in their surroundings and are difficult to image in conventional bright field microscopy. For example, many small aquatic organisms have a refractive index of 1.2 to 1.4, which results in a small optical difference from the surrounding aqueous medium. This technique is very suitable for these types of samples.



**Dark field Microscope**

### Parts:

They are the same parts of a light microscope, but it differs from a light microscope in that it has a modified piece, which is:

### Opaque disk:

Located under the condenser.

It prevents light from entering the sample with high brightness.

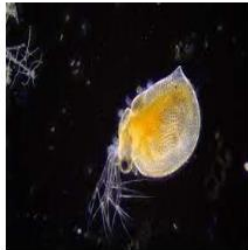
### Principle of the Dark field Microscope:

A hollow cone of light is focused on the specimen in such a way that unreflected rays do not enter the objective. The only light that has been reflected by the specimen forms an image.

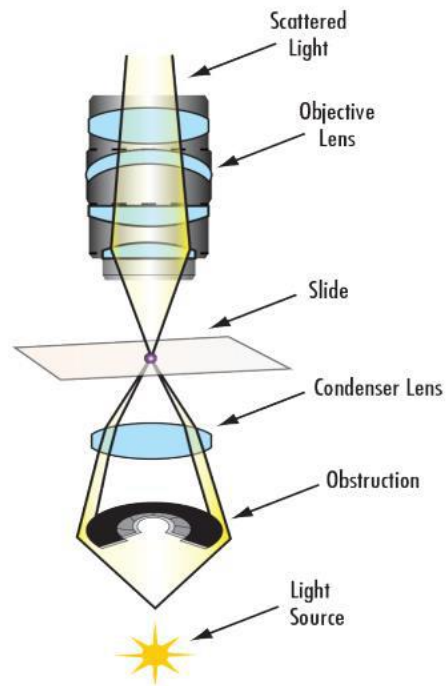
The field surrounding a specimen appears black or dark, while the object itself is brightly illuminated.



**Opaque disk**



Pictures under dark field microscope



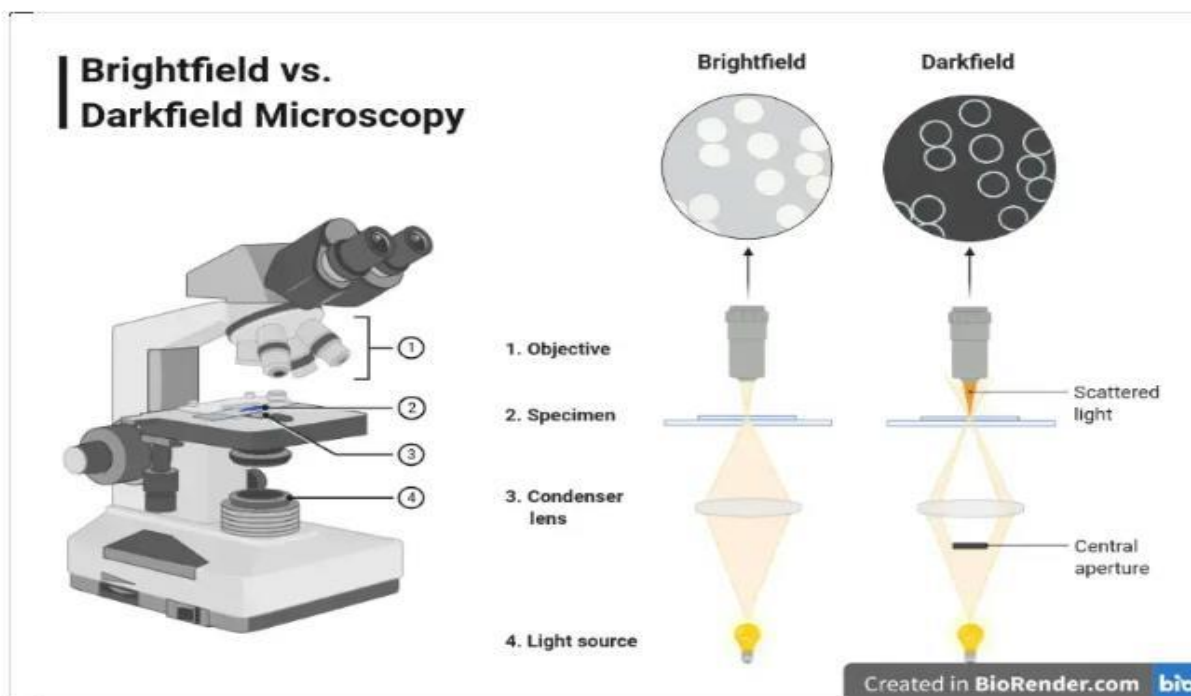
Principle of the Dark field Microscope

### How does Dark field Microscope work?

- 1) Light enters the microscope to illuminate the specimen.
- 2) A special disc, called patch stop blocks some light from the light source, leaving an outer ring of illumination.
- 3) Now the condenser lens focuses the light on the specimen.
- 4) The light enters the specimen.
- 5) Most of the light rays are directly transmitted, while some of them are scattered from the sample.
- 6) Only Scattered light started to enter the objective lens and creates an image of the specimen.
- 7) While the directly transmitted light simply misses the lens and is not collected due to a direct-illumination block and they omitted.

## Uses:

- We Can observe the living and unstained cells.
- Used to identify bacteria like thin and shape, identify algae and observe the blood cells.
- Study of some gelatinous organisms such as some invertebrates and hydrogels.
- Used to study the diamonds and other precious stones.
- Monitoring motility of unicellular organisms suspended in water or taken from its cultures.
- Examination of lightly stained prepared slides.



Components of Bright field microscope and difference in illumination with darkfield microscope.  
(Image source: Created with BioRender.com)

## Limitations of Darkfield Microscope

- The main limitation of dark-field microscopy is the low light levels seen in the final image.
- The sample must be very strongly illuminated, which can cause damage to the sample.

### 3- Phase Contrast Microscope:

It is an optical microscopy technique in which phase differences are converted into changes in light intensity, giving a clear image of the sample.

#### Parts:

They are the same parts of the optical microscope, but this microscope differs from the optical microscope in that it has two additional pieces that help it to perform its work properly which are:

#### 1- The annular diaphragm.

Located below the condenser.

Made up of a circular disc having a circular annular groove.

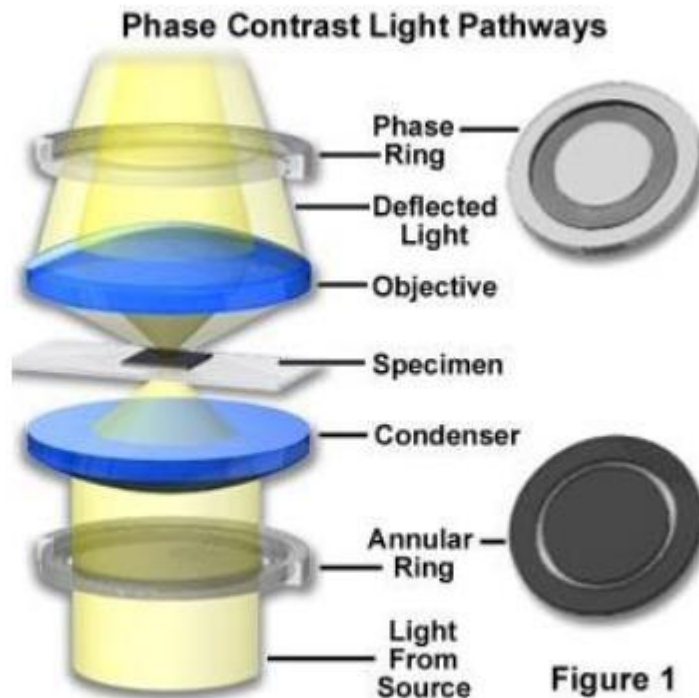
Allows light rays to pass through the groove.

#### 2- The phase plate.

Located above the objective lens.

It is a transparent disc with dark ring.

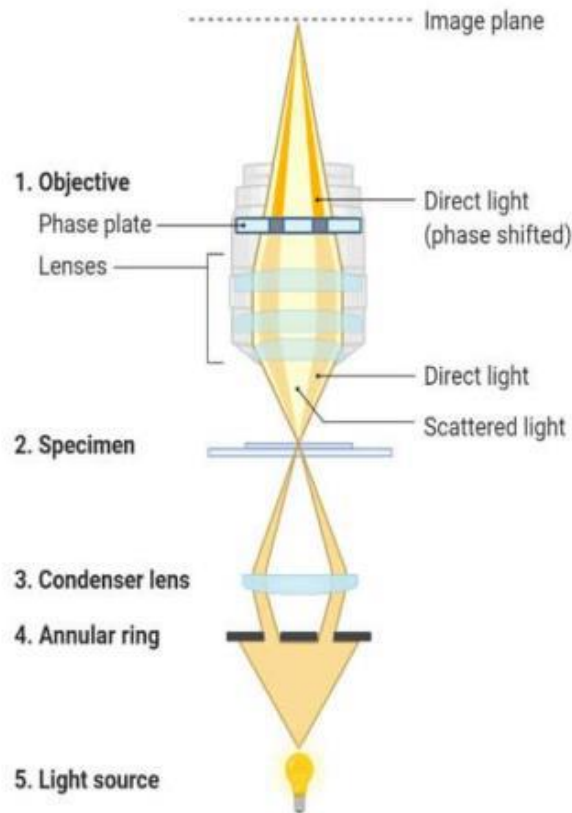
Separates direct rays from scattered rays.



The annular diaphragm & The phase plate.

## Principle of the Phase Contrast Microscope:

This microscope can increase the contrast between the transparent particles inside the living cell according to the difference in the density of these particles.



**Principle of the Phase Contrast Microscope**

### How does Phase Contrast Microscope work?

- 1) Light rays enter the annular diaphragm at its source.
- 2) Then it passes through the condenser lens, which focuses the rays on the sample.
- 3) Light travels through the sample and then enters the objective lens where an image of the sample is created.
- 4) As the light travels through the sample, it produces both direct and indirect light rays.
- 5) The rays pass out if they fall on the transparent part of the ring.
- 6) While the rays hit the dark part and come out in different phases.
- 7) As a result, direct and indirect rays give different images of the sample.

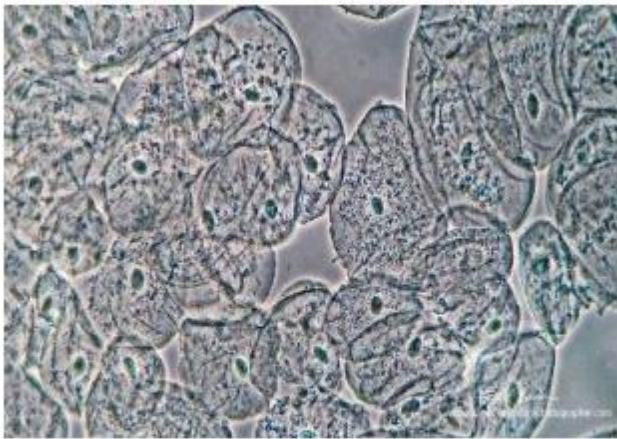


## Uses:

- 1- To view the living cells used up in cultures.
- 2- It gives a clearer picture of the transparent and unstained microorganism structures.
- 3- Study the reproduction and development of living organisms during their life stages.



**Phase Contrast Microscope**



**Pictures under phase contrast microscope**

## Limitations of Phase Contrast Microscope

- Not ideal for thick specimen
- Halo effect and shade-off are common. Condenser annulus limit the aperture, hence decrease resolution