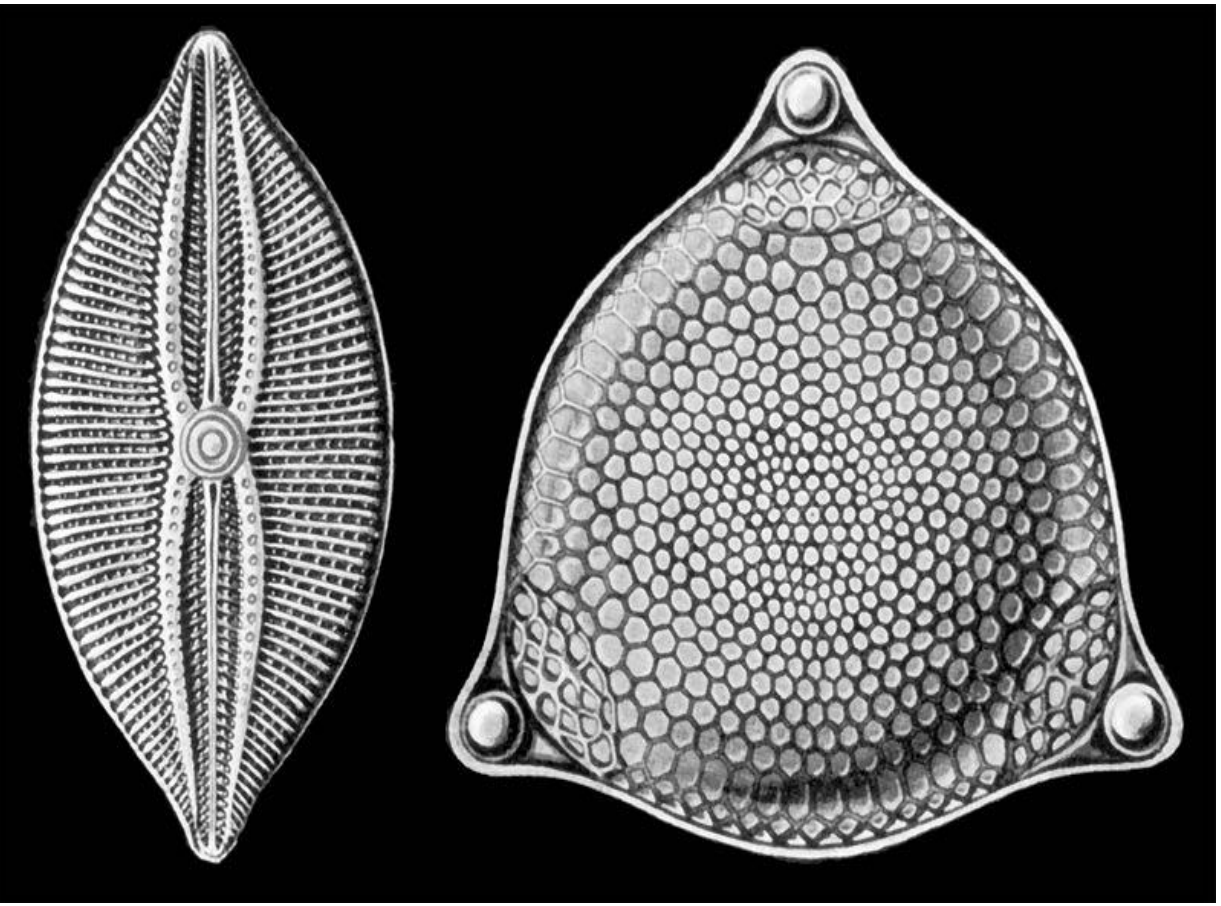
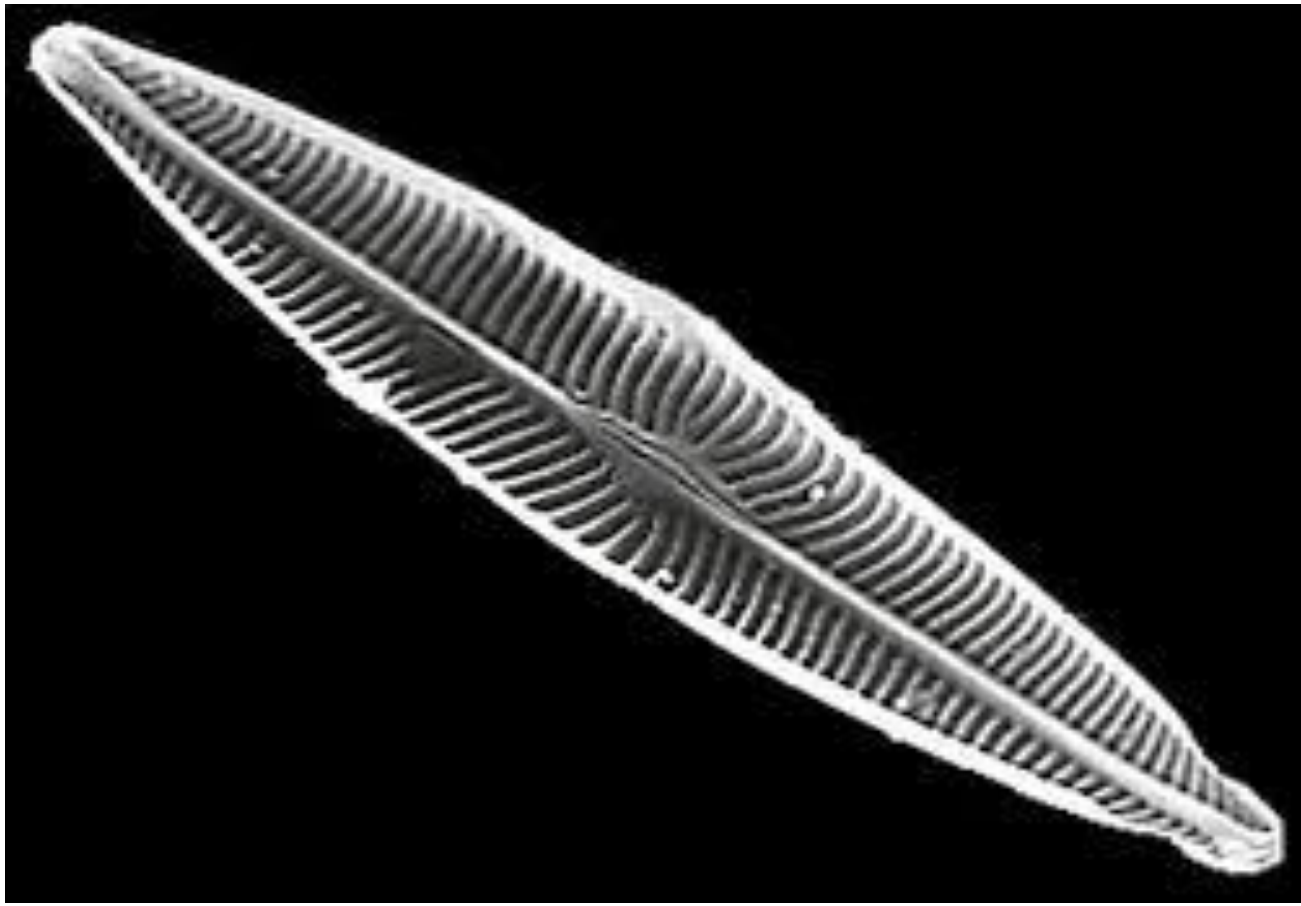
DARK FIELD MICROSCOPY

Observing Living Organisms Through a Microscope

Historical Background

- In the 1840's, the ultimate test objects for light microscopes were diatoms, in particular the species then known as Navicula spencerii
- This diatom was named after Charles Spencer, the New York lens maker whose lenses could resolve this diatom's striae (linear marks, a number of similar parallel features)
- It was found that the use of oblique light was required to resolve the striae
- Oblique illumination was the first step toward dark field microscopy
- In dark field, light seems to radiate from the specimen while all the rest of the field is black
- Resolution is as good as that in bright field while contrast is enhanced.
- Syphilis spirochete is made dark field popular and promoted the use of dark field by the medical profession and subsequently by biologists in general





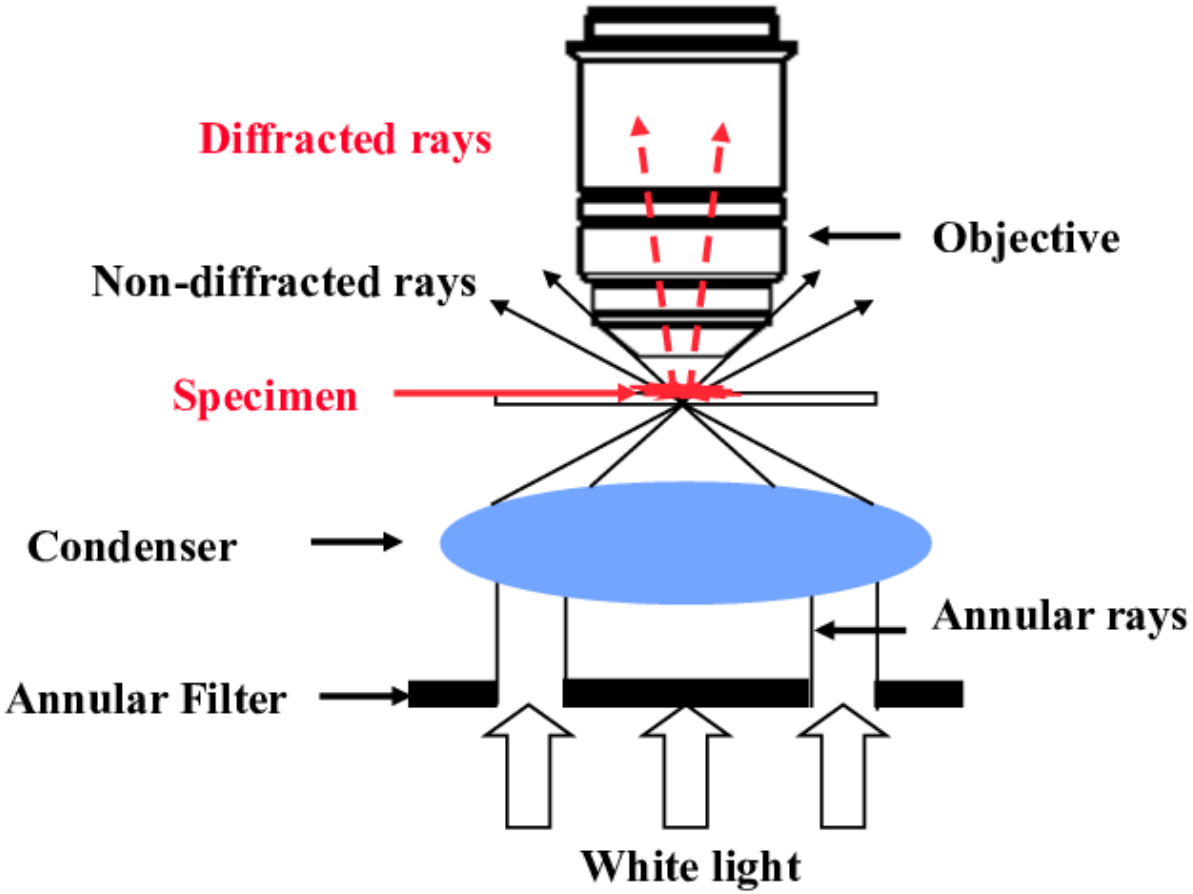
Dark Field Microscope

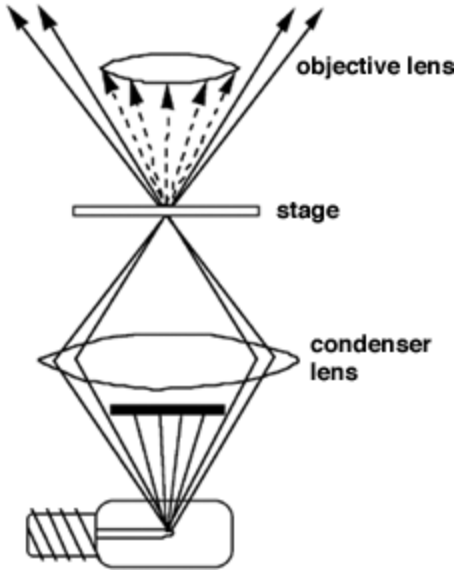
- This is similar to the ordinary light microscope; however, the condenser system is modified so that the specimen is not illuminated directly
- The condenser directs the light obliquely so that the light is deflected or scattered from the specimen, which then appears bright against a dark background
- Living specimens may be observed more readily with darkfield than with brightfield microscopy

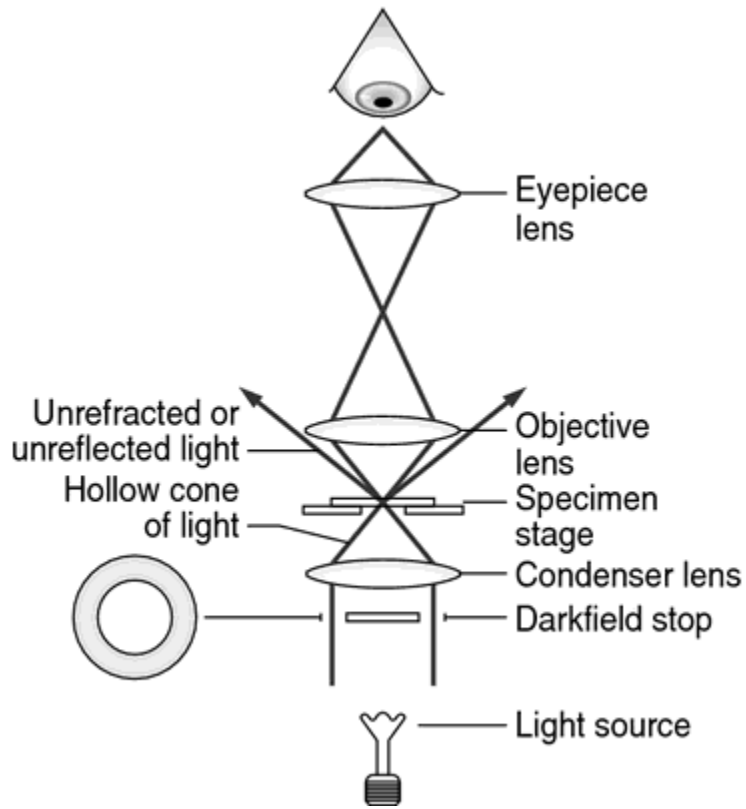
Principle

- A dark field microscope is arranged so that the light source is blocked off, causing light to scatter as it hits the specimen
- When light hits an object, rays are scattered in all directions
- The design of the dark field microscope is such that it removes the dispersed light, so that only the scattered beams hit the sample
- The introduction of a condenser and/or stop below the stage ensures that these light rays will hit the specimen at different angles, rather than as a direct light source above/below the object
- The result is a “cone of light” where rays are diffracted, reflected and/or refracted off the object, ultimately, allowing the individual to view a specimen in dark field

- **The dark-field condenser with a central circular stop, which illuminates the object with a cone of light, is the most essential part of the dark-ground microscope which prevents light from falling directly on the objective lens**
- **This microscope uses reflected light instead of transmitted light used in the ordinary light microscope**
- **Light rays falling on the object are reflected or scattered onto the objective lens with the result that the microorganisms appear brightly stained against a dark background**


Diffracted rays
Objective
Non-diffracted rays
Specimen
Condenser
Annular rays
Annular Filter
White light


objective lens
stage
condenser lens


Eyepiece lens
Unrefracted or unreflected light
Objective lens
Hollow cone of light
Specimen stage
Condenser lens
Darkfield stop
Light source

Types of Specimens for Dark Field:

- The best specimens for dark field are those that have refractive objects scattered about with empty space between them
- No dark field occurs if objects are too crowded or if a thick solid specimen turns light into the microscope
- Very thin histological sections can be used if unstained or if only certain components are stained, as in silver stains
- Biological fluids from animals and plants, cell cultures, microbes, foods, fibers, crystals, colloids, and sub-microscopic particles are all suitable for dark field microscopy
- Preparations of autoradiography and gold labeling are also suitable

Low Magnification Dark Field Condensers:

- This is an opaque disk of the proper diameter placed in its front focal plane
- The diameter of the opaque disk must be just large enough to prevent any direct light from entering the objective

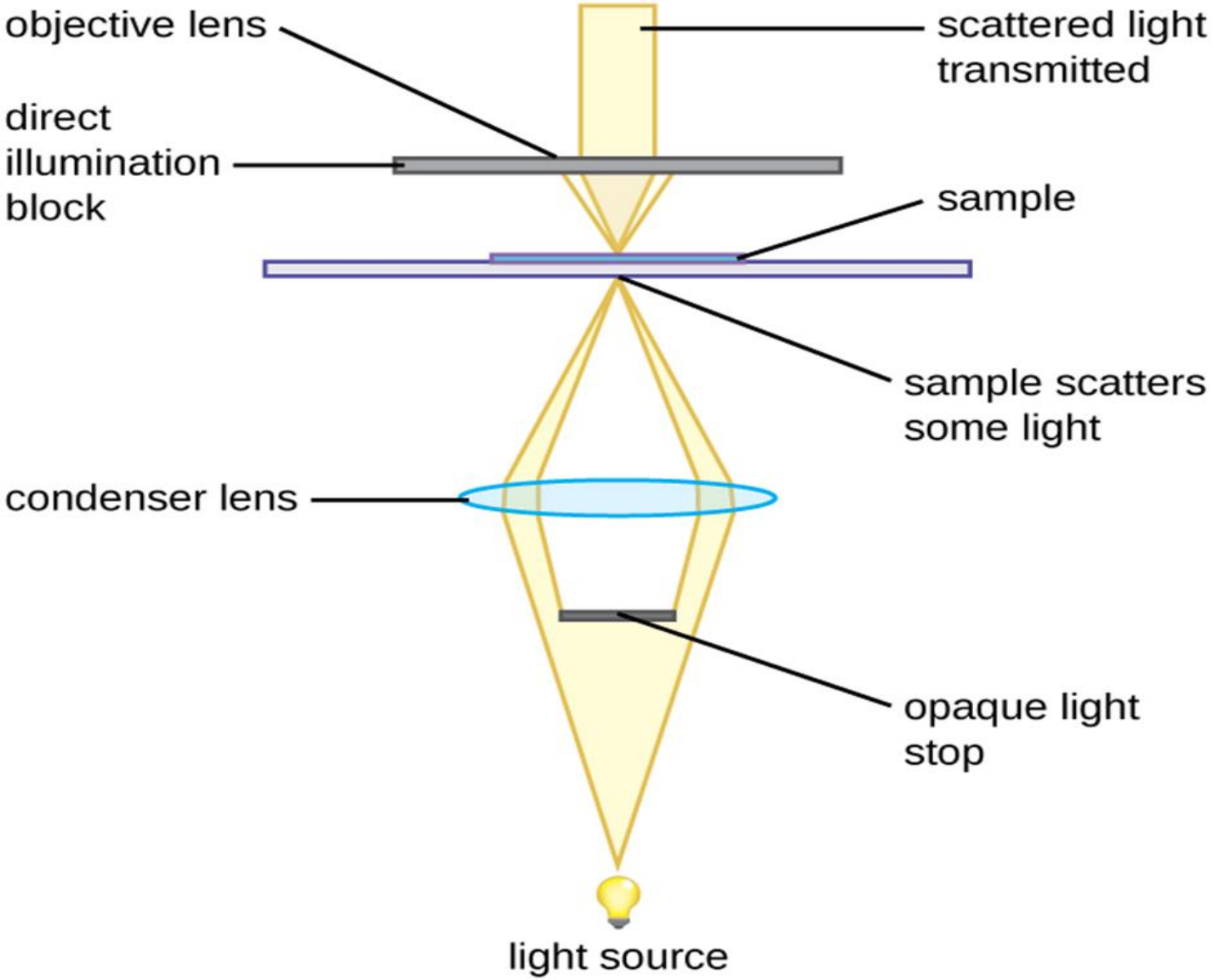
High Numerical Aperture Dark Field Condensers:

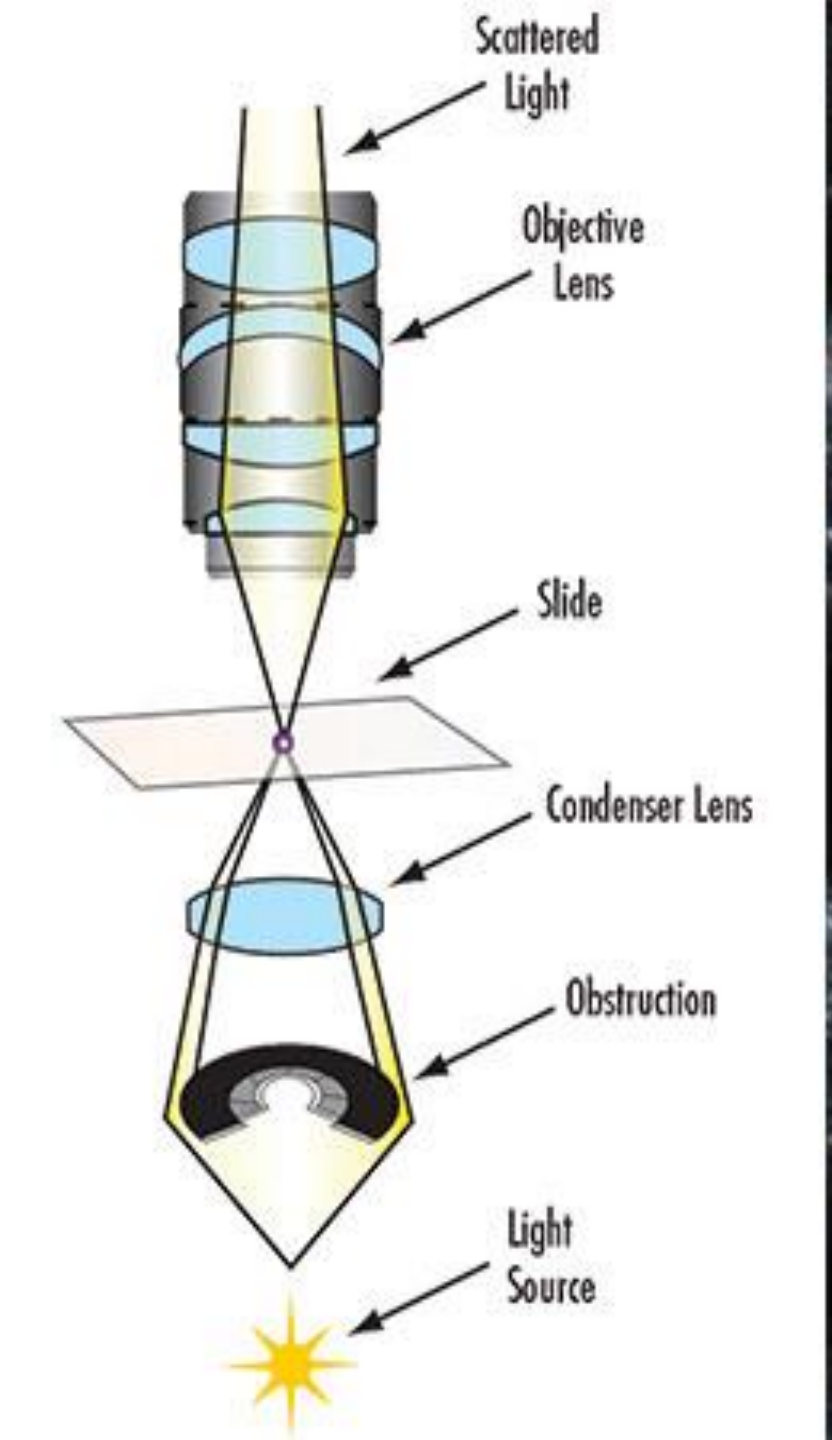
- This condenser used a parabolic glass reflector to create a hollow cone of light
- The result is a more finely focused spot of light

Dark field Microscopy

- ❖Useful to examine live or unstained specimens
- ❖Light sensitive organisms
- ❖Specimens that lack contrast with their background.
- ❖Darkfield condenser with opaque disc blocks light that would enter objective lens directly
- ❖Light reflects off specimen at an angle.
- ❖Only light reflected by specimen enters objective lens.
- ❖No direct background light.
- ❖Image : Light specimen against dark background

Dark Field Microscope




Scattered Light
Objective Lens
Slide
Condenser Lens
Obstruction
Light Source

Applications

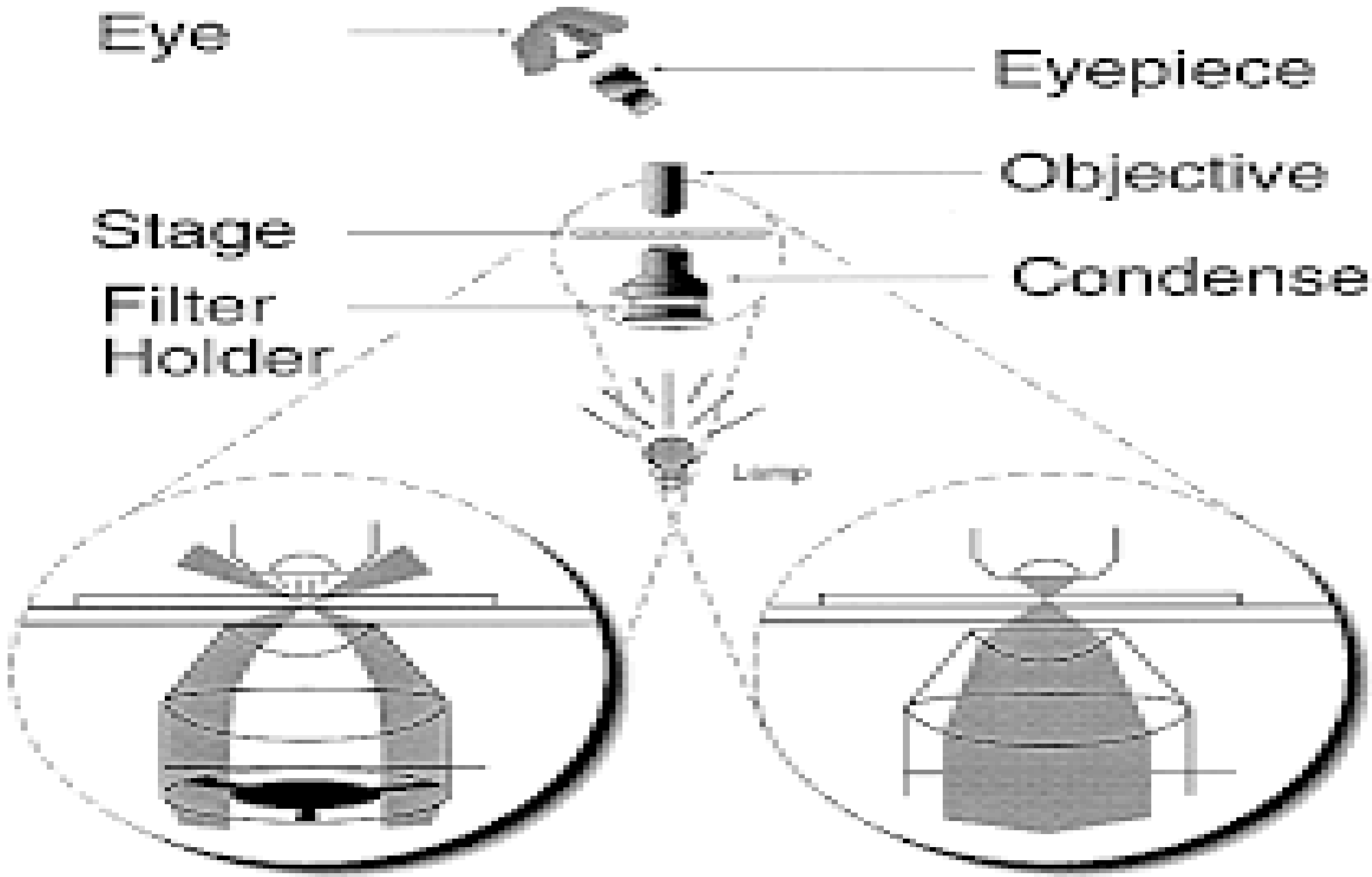
- It is useful for the demonstration of very thin bacteria not visible under ordinary illumination since the reflection of the light makes them appear larger
- This is a frequently used method for rapid demonstration of *Treponema pallidum* in clinical specimens resulting siphilis
- It is also useful for the demonstration of the motility of flagellated bacteria and protozoa
- Darkfield is used to study marine organisms such as algae, plankton, diatoms, insects, fibers, hairs, yeast and protozoa as well as some minerals and crystals, thin polymers and some ceramics.
- Darkfield is used to study mounted cells and tissues
- It is more useful in examining external details, such as outlines, edges, grain boundaries and surface defects than internal structure

Advantages:

- It is well suited for uses involving live and unstained biological samples, such as a smear from a tissue culture or individual, water-borne, single-celled organisms
- Considering the simplicity of the setup, the quality of images obtained from this technique is impressive

Disadvantages/ Limitations:

- 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
- If the specimen's density differs across the slide or is not thin enough, it can create artifacts throughout the image.
- The slide, stage, nose, and light source must free from dust
- It cannot measure the specimen accurately
- Liquid bubbles can be formed during uses of oil or water on the condenser and/or slide, which is almost impossible to avoid


Eye
Eyepiece
Objective
Stage
Condense
Filter
Holder
Lamp
Darkfield
Brightfield