Microscopy

Group 2
Cabatit, Mendoza, Ramos, Rodriguez, Tan

Compound Light Microscope

- A device that transmits light through several lenses to produce an enlarged image of a microscopic specimen.
- Modern compound light microscopes, under optimal conditions, can magnify an object from 1000X to 2000X (times) the specimens original diameter.

Bright Field Microscopy

Bright Field Microscopy

- Simplest optical microscopy illumination technique
- Uses visible light as source of illumination
- *> the shorter the wavelength, the greater the resolution (blue is the best)*
- Contrast comes from absorbance of light in the sample, or from staining.
- When the diaphragm is wide open the image is brighter and contrast is low.

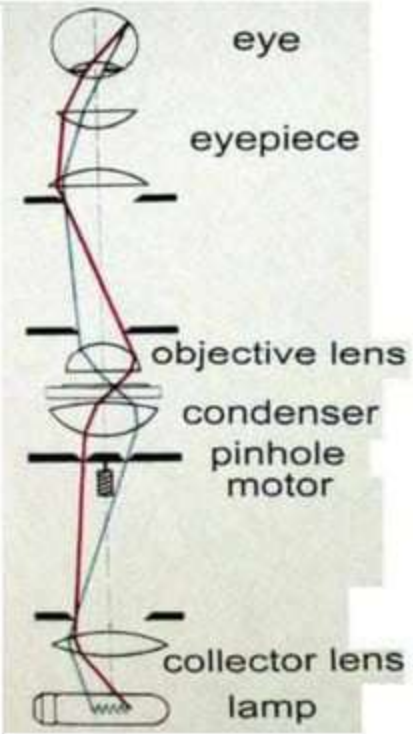
Bright Field Microscopy

- Sources of illumination: *Lamp on the base*
- Types of image produced: *Relatively large internal structures and outline can be seen*

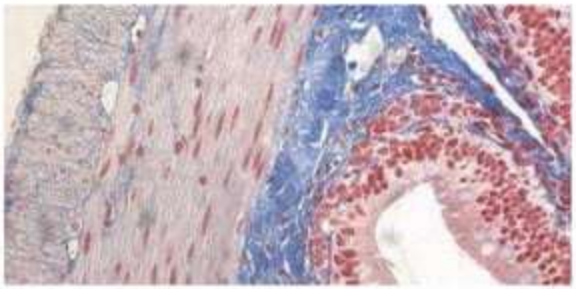
Bright-field

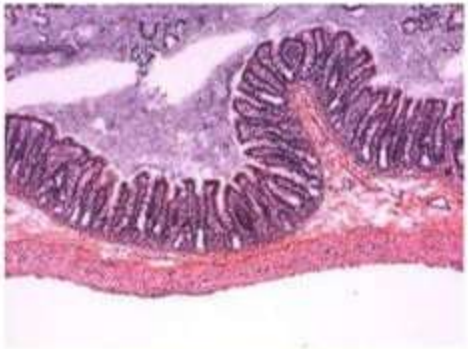
Colored or clear specimen against bright background

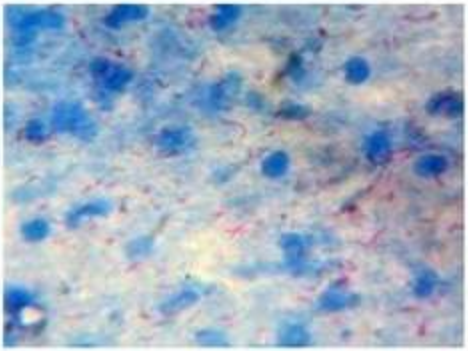
- Total Magnification: *(if 10x ocular magnification is used)* *Range: 10x-1000x*
- Resolution: *Up to 200nm (white light)*

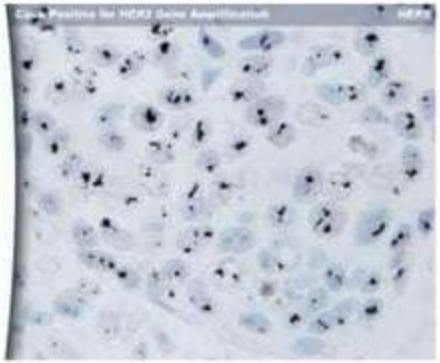


Source:
http://www.austincc.edu/biocr/1406/labm/ex3/prelab_3_8.htm









Dark Field Microscopy

Dark Field Microscopy

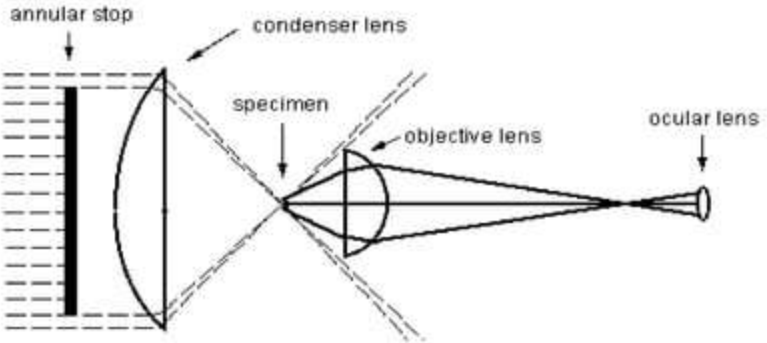
- Type of microscopy which is the exact opposite of a bright field microscope
- Dark background/field with the specimen being the only one illuminated.
- Used in observing unstained specimens
- Most microscopes have the potential to do dark field microscopy such as compound or stereomicroscopes.

Dark Field Microscopy

- Light source: Light bulb from a microscope
- Condenser type: Specialized to block most light from the source; contains an annular/patch stop which disperses the light in various directions, resulting to a “cone of light”
- Image formed: Dark background with illuminated specimen; may be inverted or not depending on microscope used

Dark Field Microscopy

- Total Magnification: Can range from those of compound microscopes (10x to 1000x)



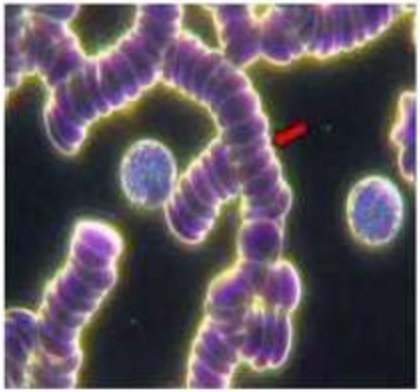
Pros and Cons of Dark Field Microscopy

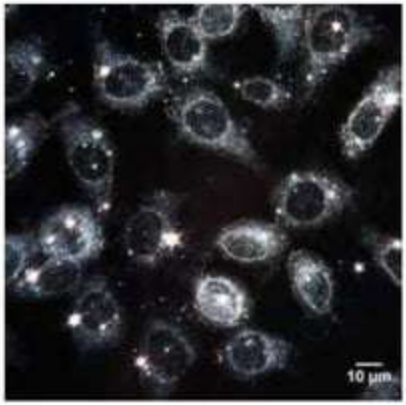
Advantages

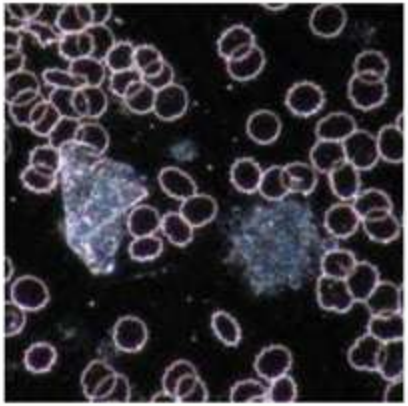
- Used to view unstained specimens more clearly.
- Can be used to study various live bacteria, protists, algae, fungi, and many other cultures.
- Can examine the external of the specimen with detail

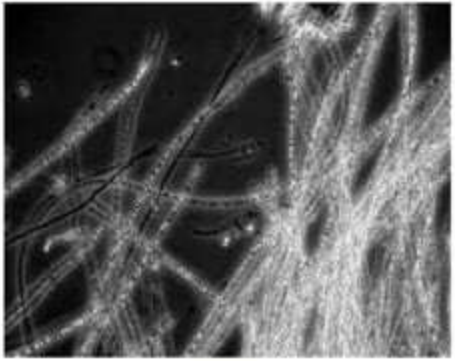
Disadvantages

- Can be inaccurate compared to other methods.
- Special care if more needed for this type of microscopy to prevent aberrations.
- Needs intense amount of light which can hurt the eyes and cause glare.
- Air bubbles in the slide can cause problems.



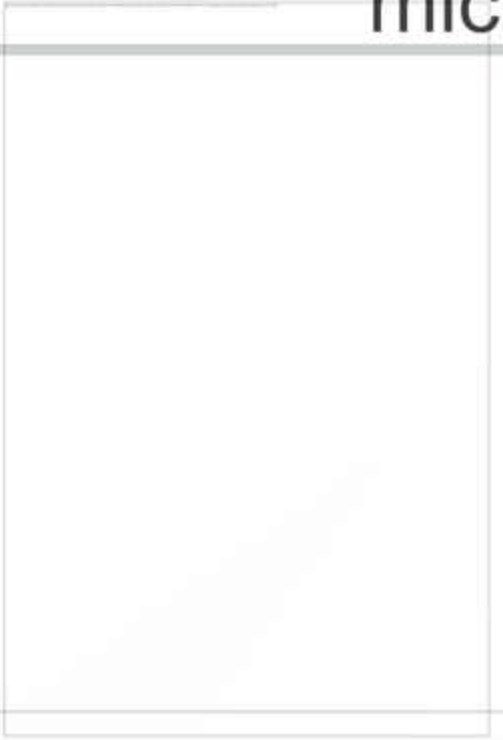

10 µm

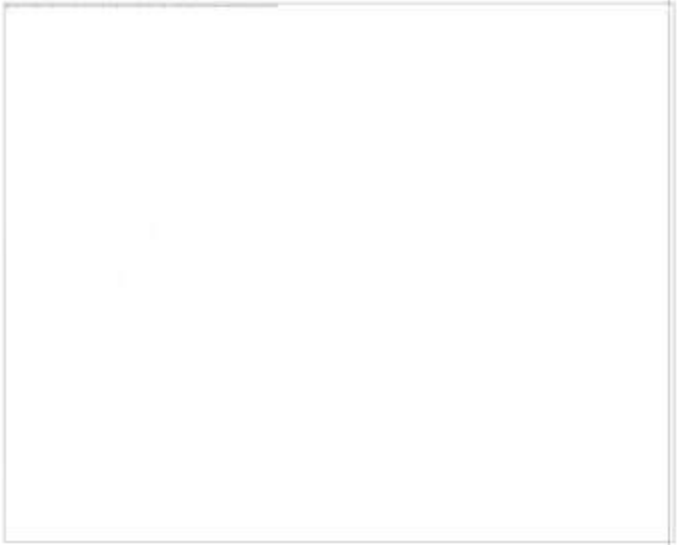




Phase Contrast Microscopy

Phase-contrast microscope





Phase-contrast microscope

- Type of light microscope
- Enhances contrast in micrographs by converting phase shifts of light waves into brightness
  - Offers more contrast than brightfield microscopy
- Does not require the use of staining procedures which usually kill cells
  - Especially useful for examining living, unpigmented cells

Source of illumination

- Visible light from an illuminator

TOTAL MAGNIFICATION

- Phase contrast objective lenses (Nikon) come in 4x, 10x, 20x, and 40x powers, so the total magnification for phase contrast microscopes range from **40x to 400x**

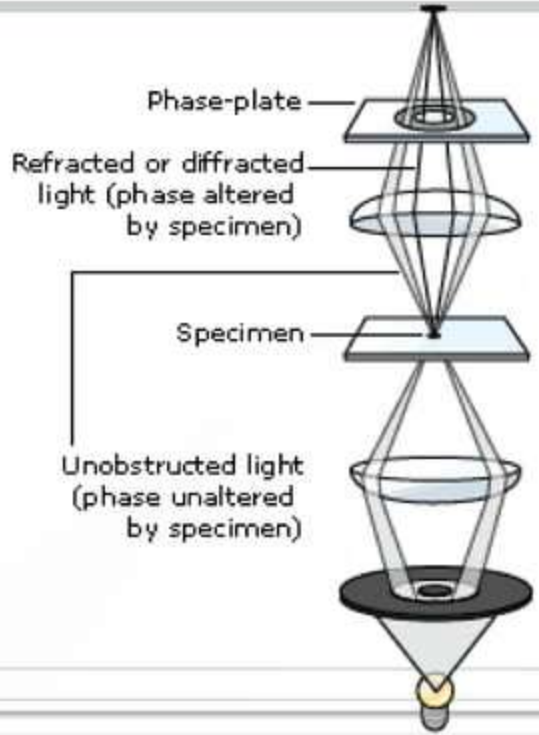
Principle

- Differences in **density** (Campbell et. al., 1999) or **refractive index** (Tortora et. al., 2007) within the specimen or cell causes light waves to be diffracted at different degrees
- **Diffraction** of light waves implies a **change in the phase** of their wavelength

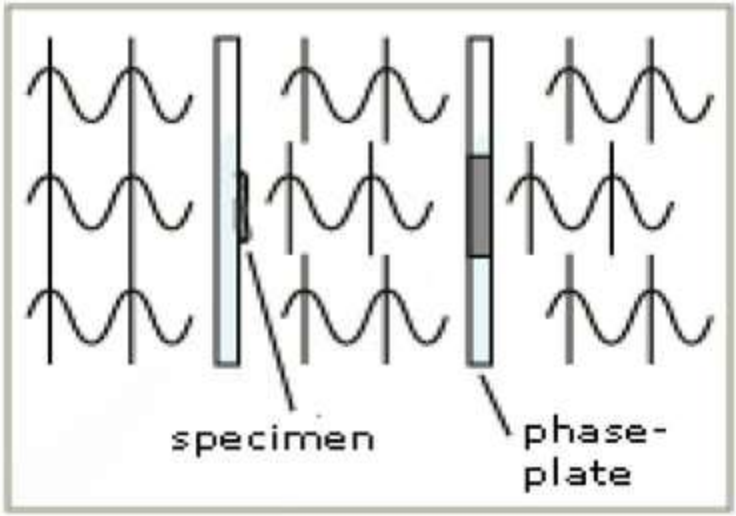
Principle

- A unique part of the phase-contrast microscope, called the **phase-plate**, amplifies this change in phase to one-half wavelength
- When both the direct (undiffracted) and reflected (diffracted) types of light waves converge at the ocular lens, **constructive and destructive interference** occurs

Principle


Phase-plate
Refracted or diffracted light (phase altered by specimen)
Specimen
Unobstructed light (phase unaltered by specimen)

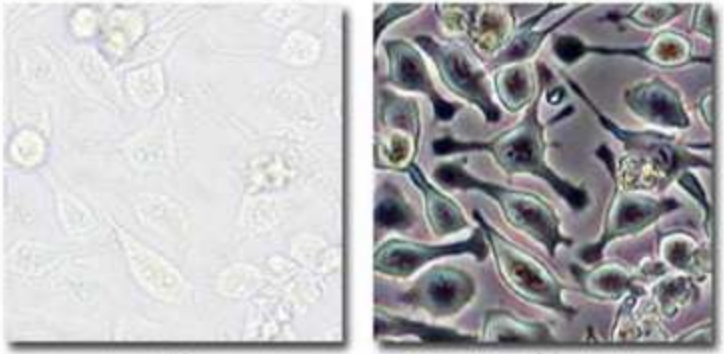
Principle



Principle

- Constructive interference corresponds to bright spots in the field of view
- Destructive interference corresponds to dark spots

Living Cells in Brightfield and Phase Contrast



(a) (b)

Figure 2

Type of image produced

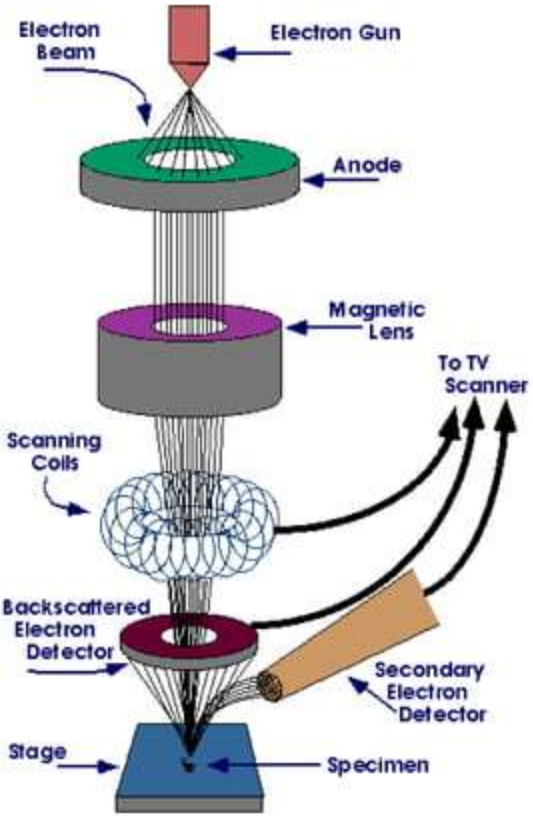
- The end result is a magnified and highly contrasted view of a living, unstained, normally transparent specimen

Electron Microscopy

Scanning Electron Microscope

- Illumination: electrons
- Magnification: ~100,000x
- How it works: Detect electrons back-scattered by the sample.
- Image: Monotone (but may be color enhanced), 3-D surface of specimen

- Magnetic lens—focuses electron beam
- Scanning coils—for systematic scanning (left to right, then down)
- Backscattered Electron Detector– detects electrons that bounced off the film
- Secondary Electron Detector– detects electrons emitted by



Source: http://www.purdue.edu/rem/rs/graphics/sem2.gif

Pros

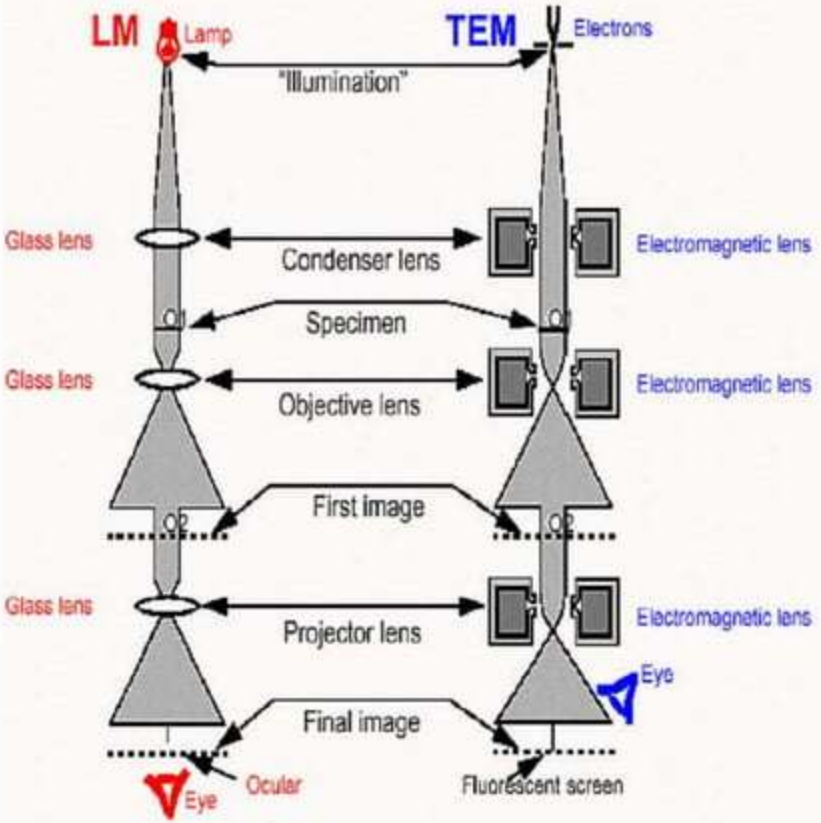
- High magnification
- High resolution
- Shows the surface of specimen

Cons

- Needs specimen to be in vacuum
- Needs living cells and tissues and whole, soft-bodied organisms to be treated, usu. coated w/ gold film
- No color
- Cannot examine live specimen
- Really. Big. And Expensive. Equipment.

Transmission Electron Microscope

- Illumination: electrons
- Magnification: ~100,000x
- How it works: Detect electrons scattered as they move through the sample.
- Image: Monotone (but may be color enhanced), 2-D structure of specimen



Source: http://www.lab.anhb.uwa.edu.au/hb313/main_pages/timetable/lectures/Image6.gif

Pros

- High magnification
- High resolution
- Shows small structures that cannot be seen under light microscopes

Cons

- Needs specimen to be in vacuum
- Needs specimen to be covered in gold film
- Specimen <100nm thick (obviously cannot observe live specimen)
- No color
- Really. Big. And. Expensive. Equipment

End