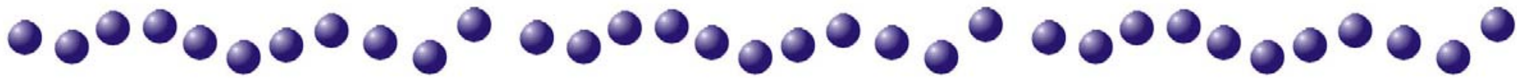

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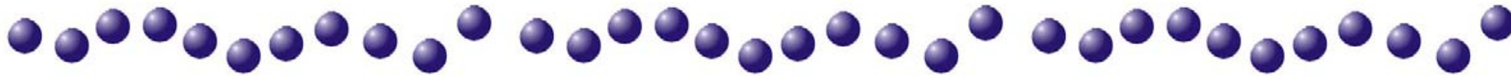

1. Microscopy system

- History of Microscope
- Simple Optical microscope
- Microscope components
- Transmitted and Reflected light microscope
- Optical magnification and optical resolution
- Numerical aperture

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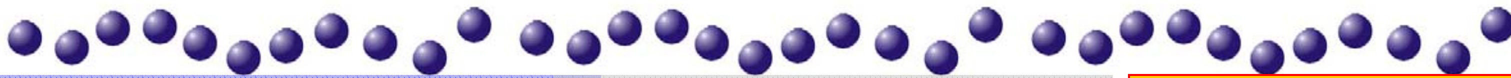

Timeline of microscope technology

1500s – 1700s

1590 - Dutch spectacle-makers,Hans Janssen and his son Zacharias Janssen,
claimed by later writers (Pierre Borel
1620 - 1671 or 1628 - 1689 and Willem Boreel 1591 - 1668) to have invented a compound microscope.
1609 - Galileo Galilei develops an *occhiolino* or compound microscope with a convex and a concave lens.
1612 - Galileo presents *occhiolino* to Polish king Sigismund III.
1619 - Cornelius Drebbel (1572 - 1633) presents, in London, a compound microscope with two convex lenses.
1622 - Drebbel presents his invention in Rome.
1624 - Galileo presents his *occhiolino* to Prince Federico Cesi, founder of the *Accademia dei Lincei* (in English
, *The Linceans*).
1625 - Giovanni Faber of Bamberg (1574 - 1629) of the Linceans coins the word *microscope* by analogy with
telescope.
1665 - Robert Hooke publishes *Micrographia*, a collection of biological micrographs. He coins the word *cell* for the
structures he discovers in cork bark.
1674 - Anton van Leeuwenhoek improves on a simple microscope for viewing biological specimens.

1500s – 1700s

1877- Ernst Abbe together with Carl Zeiss defining resolving distance of an objective (Abbe's Law)
1931 - Ernst Ruska builds the first electron microscope.
1953 - Frits Zernike, professor of theoretical physics, Nobel Prize in Physics for his invention of the
phase contrast optical microscope.
1981 - Gerd Binnig and Heinrich Rohrer develop the scanning tunneling microscope.

4 http://en.wikipedia.org/wiki/

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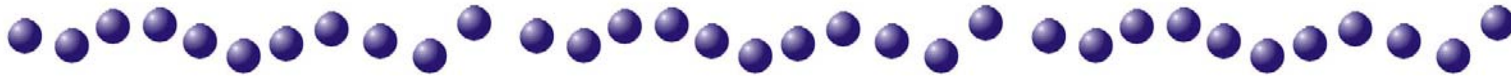

A **simple microscope**, as opposed to a standard compound microscope (see below) with multiple lenses, is a microscope that uses only one lens for magnification. Van Leeuwenhoek's microscopes consisted of a single, small, convex lens mounted on a plate with a mechanism to hold the material to be examined (the sample or specimen). This use of a single, convex lens to magnify objects for viewing is still found in the magnifying glass, the hand-lens, and the loupe.

4 http://en.wikipedia.org/wiki/

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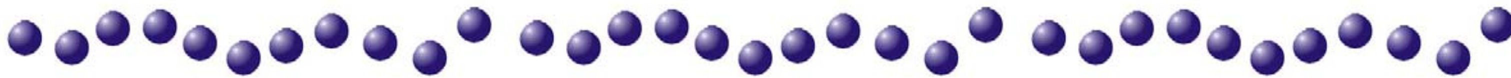

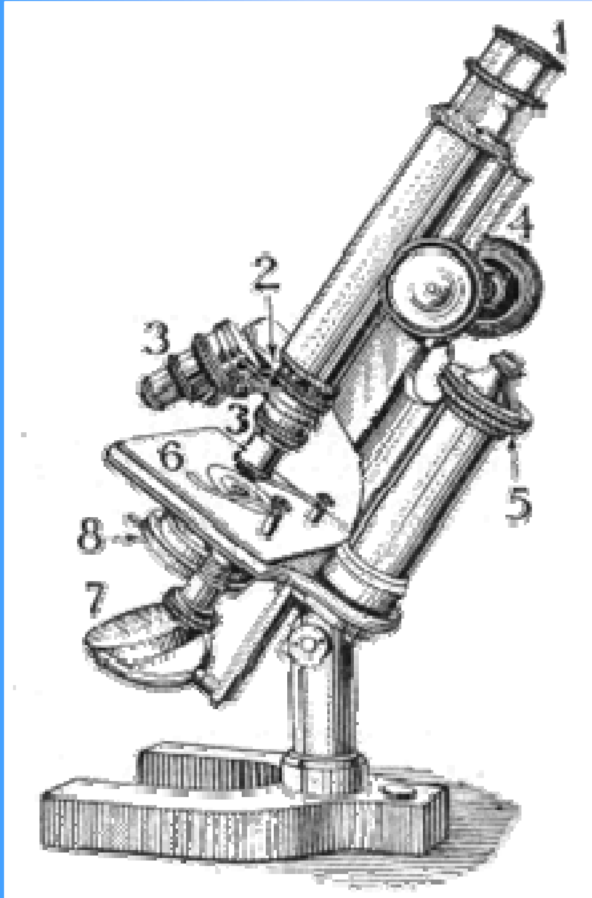

Basic Components

- ocular lens or eye-piece
- objective turret, or nosepiece
- objective lenses
- coarse adjustment knob
- fine adjustment knob
- object holder or stage
- mirror
- diaphragm and condenser

4 http://en.wikipedia.org/wiki/

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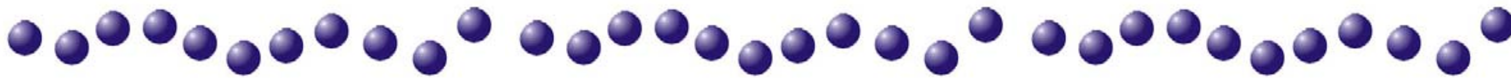
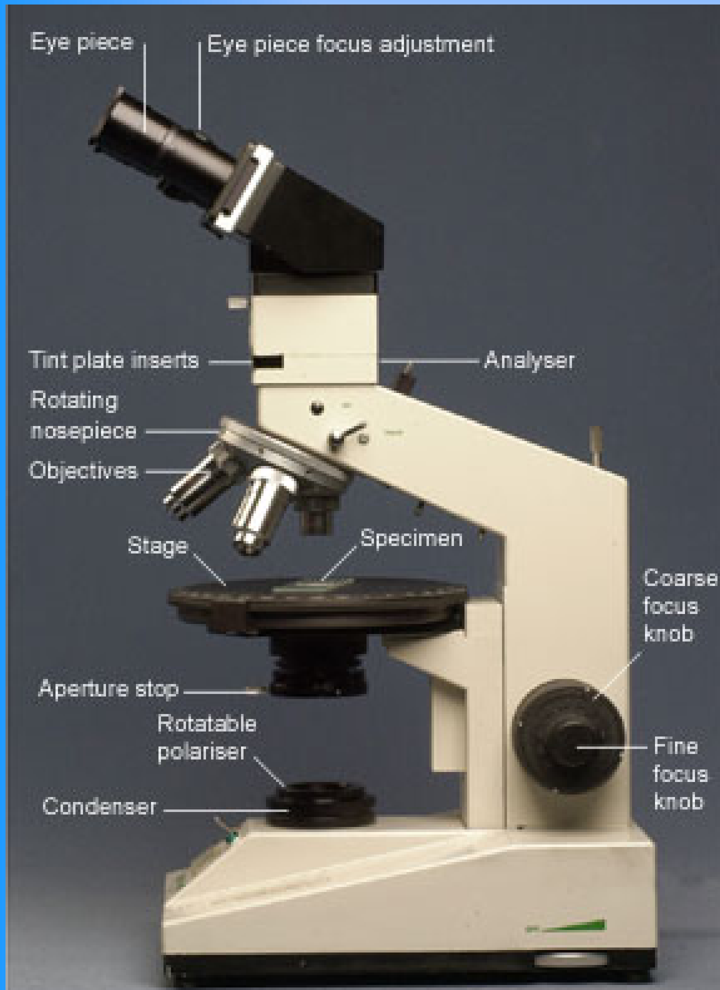




http://www.doitpoms.ac.uk/tlplib/optical-microscopy/rmicroscope.php

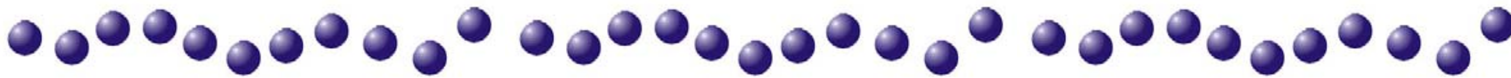
Compound Microscope

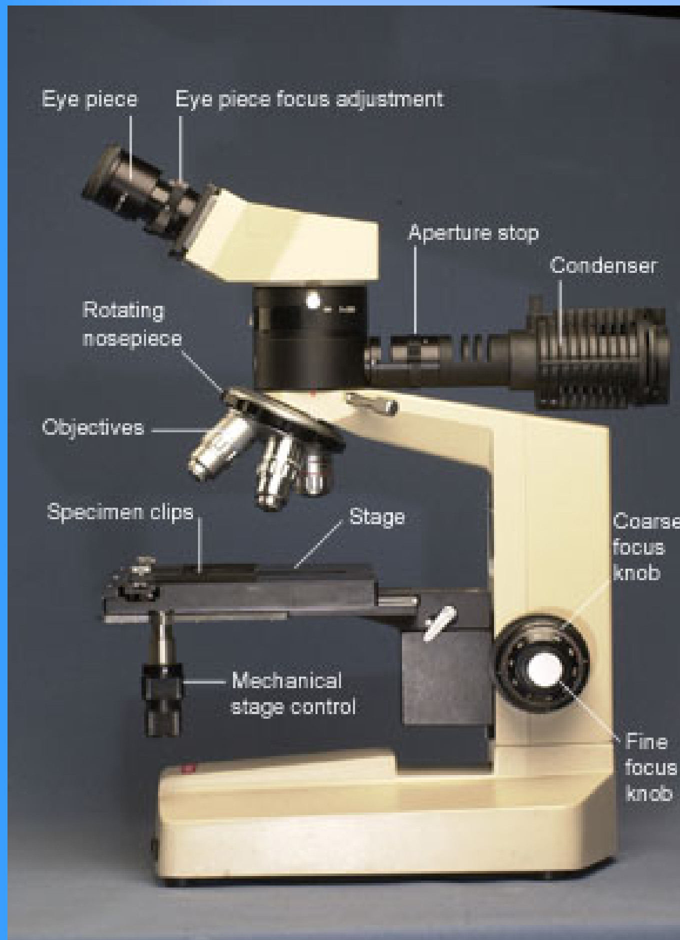
Compound optical microscopes can magnify an image up to 1000× light passing through the sample from below and special techniques are usually necessary to increase the contrast in the image to useful levels (see *contrast methods*).

Typically, on a standard compound optical microscope, there are three objective lenses : a scanning lens (4×), low power lens (10×), and high power lens (40×). Advanced microscopes often have a fourth objective lens, called an oil/water immersion lens. Immersion lens usually has a power of 100×.

The actual power or magnification is the product of the powers of the ocular (eyepiece), usually about 10×, and the objective lens being used.

4 http://en.wikipedia.org/wiki/



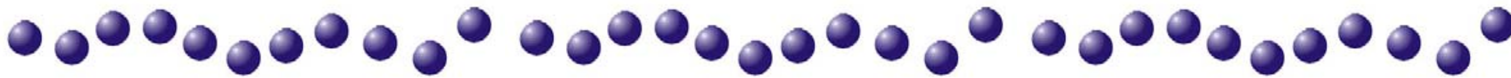


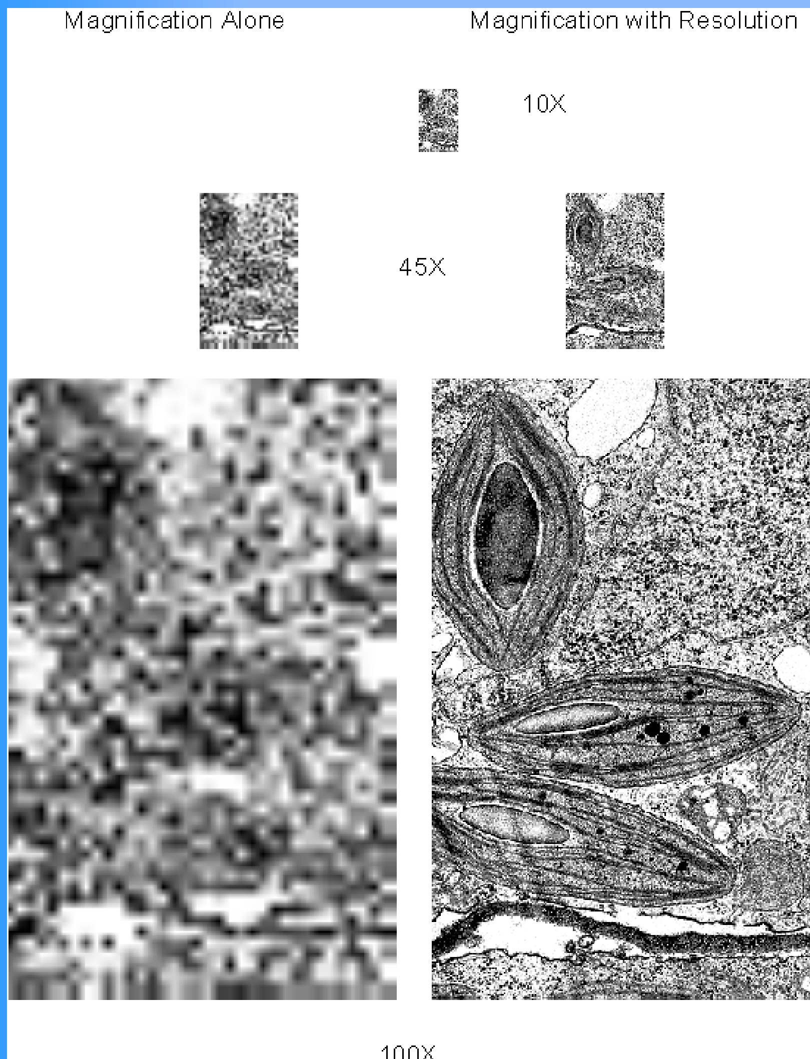
http://www.doitpoms.ac.uk/tlplib/optical-microscopy/rmicroscope.php

Reflected Microscope

To study the thin structure of metals (see metallography) and minerals, another type of microscope is used, where the light is *reflected* from the examined surface. The light is fed through the same objective using a semi-transparent mirror.

4 http://en.wikipedia.org/wiki/





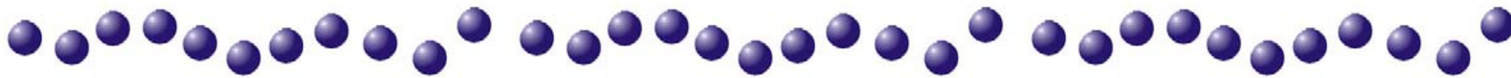
http://homepages.gac.edu/~cellab/chpts/chpt1/figure1-1.html

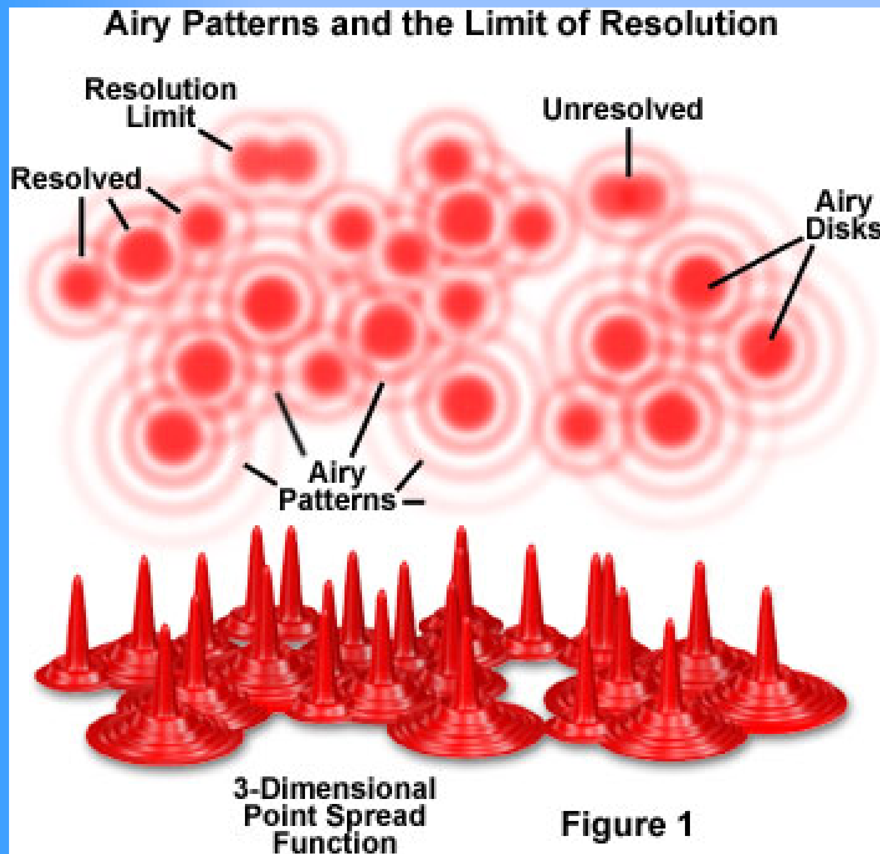
Magnification is simply the number of times an image's size is enlarged where size is measured in the degrees of an angle formed by lines running from either end of the image to the vertex at the observer's eye - link to illustration.

In practice this simply means that if we view a 1cm line at 2x it appears to us as a 2cm line.

Resolution is a measurement of how well the smallest details of an image can be discerned. Magnification beyond the resolving power of an optical system is empty magnification. This is often encountered with extremely cheap microscopes like those bought at a toy store. On the other hand a certain level of magnification is necessary to see the details resolvable in a given image

4 http://en.wikipedia.org/wiki/



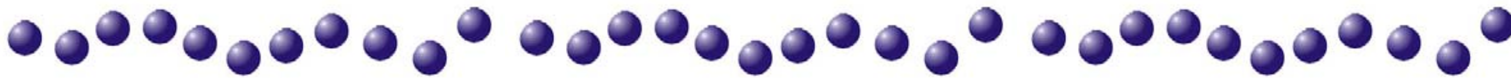


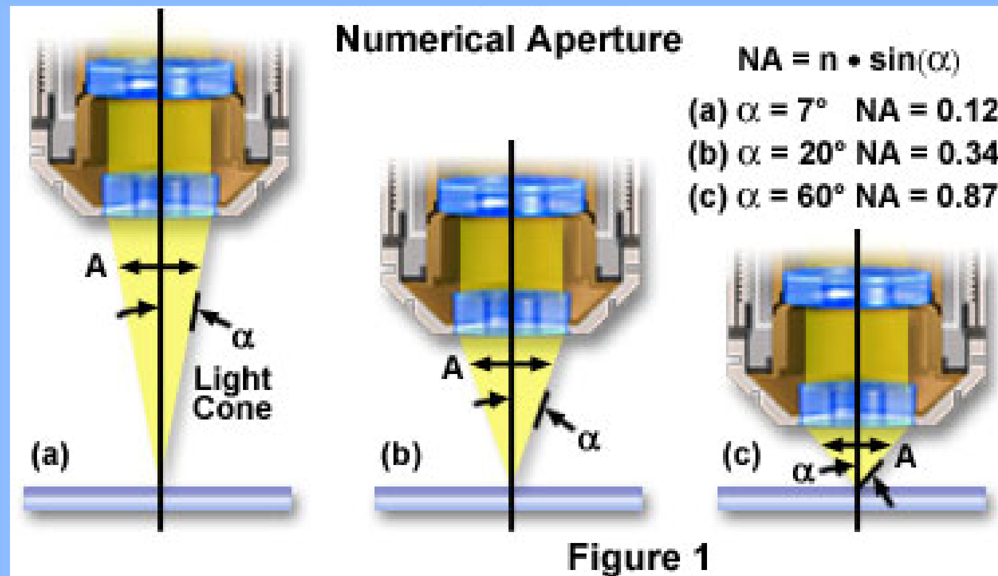
The resolution of an optical microscope is defined as the shortest distance between two points on a specimen that can still be distinguished by the observer or camera system as separate entities.

The limit of resolution of a microscope objective refers to its ability to distinguish between two closely spaced Airy disks in the diffraction pattern (noted in the figure).

Three-dimensional representations of the diffraction pattern near the intermediate image plane are known as the **point spread function**, and are illustrated in the lower portion of Figure 1. The specimen image is represented by a series of closely spaced point light sources that form Airy patterns and is illustrated in both two and three dimensions.

http://www.microscopyu.com/tutorials/java/imageformation/airyna/index.html





The numerical aperture of a microscope objective is a measure of its ability to gather light and resolve fine specimen detail at a fixed object distance. Image-forming light waves pass through the specimen and enter the objective in an inverted cone as illustrated in Figure.1

http://www.microscopyu.com/tutorials/java/objectives/nuaperture/index.html

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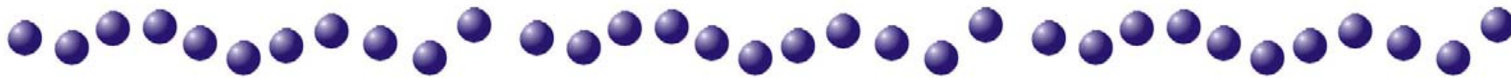
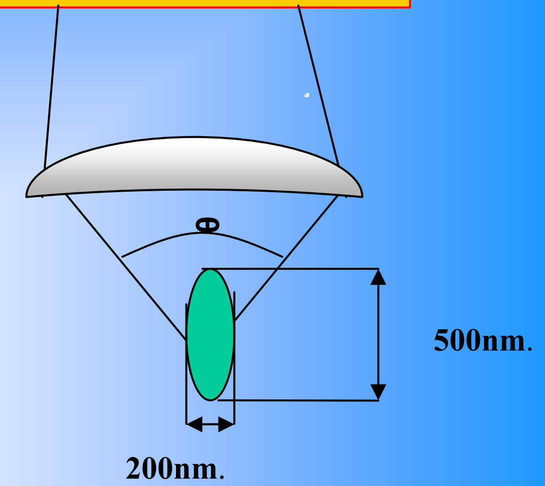


Resolution and Numerical Aperture by Objective Type

	Objective Type					
	Plan Achromat		Plan Fluorite		Plan Apochromat	
Magnification	N.A.	Resolution (µm)	N.A.	Resolution (µm)	N.A.	Resolution (µm)
4x	0.10	2.75	0.13	2.12	0.20	1.375
10x	0.25	1.10	0.30	0.92	0.45	0.61
20x	0.40	0.69	0.50	0.55	0.75	0.37
40x	0.65	0.42	0.75	0.37	0.95	0.29
60x	0.75	0.37	0.85	0.32	0.95	0.29
100x	1.25	0.22	1.30	0.21	1.40	0.20

N.A. = Numerical Aperture



- $D_{lateral} = 0.61\lambda/NA$
- $D_{axial} = 2\lambda/NA^2$
- $NA = n.\sin(\theta)$

which is clearly influenced by the objective numerical aperture. Note that lower values of **D** indicate higher resolution.

An important fact to note is that magnification does not appear as a factor in any of these equations, because only numerical aperture and wavelength of the illuminating light determine specimen resolution

http://www.microscopyu.com/tutorials/java/objectives/nuaperture/index.html

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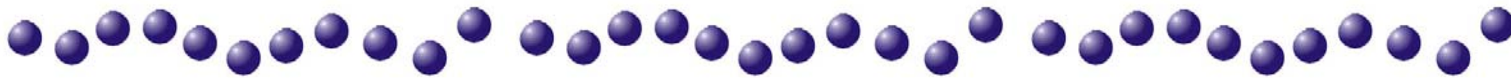

Resolution versus Wavelength

Wavelength (nanometers)	Resolution (micrometers)
360	.19
400	.21
450	.24
500	.26
550	.29
600	.32
650	.34
700	.37

The resolving power of a microscope is the most important feature of the optical system and influences the ability to distinguish between fine details of a particular specimen.

As discussed above, the primary factor in determining resolution is the

1) objective numerical aperture, but resolution is also dependent upon the type of specimen,
2) coherence of illumination,
3) degree of aberration correction,
4) and other factors such as contrast enhancing methodology either in the optical system of the microscope or in the specimen itself.

http://www.microscopyu.com/tutorials/java/objectives/nuaperture/index.html

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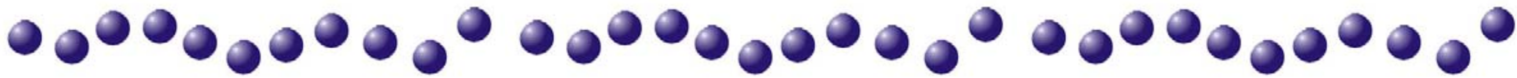

Types of Transmitted Light Microscopy

1. Light Microscopy: the contrast issue
   - Bright field
   - Dark field
   - Phase contrast
   - Differential interference contrast (DIC)
2. Fluorescence Microscopy
3. Confocal scanning
4. Electron microscopy

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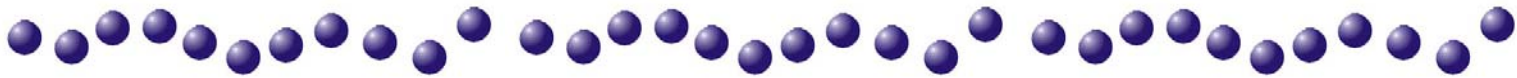

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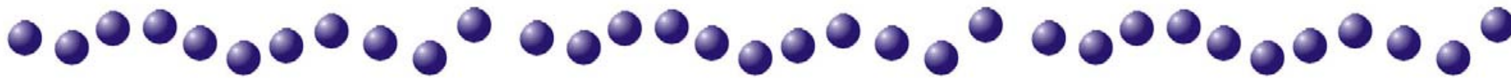

Terminology for different types of microscope objective

Plan stands for flat field.
Lenses which are uncorrected for flatness of field will have the center of the field in focus and the outer edges out of focus (or vice versa depending on how you focus the lens). So Plan means the lens is corrected to allow the whole field to be in focus.

Achroplans are best for transmitted light while
Epiplans are designed for reflected light use. Some microscope manufacturers will list their flat field achromatic lenses as simply **"Plan"**.

Achromat lenses have good color correction for two wavelenths of light. They are budget priced lenses.

Planachromats are achromats with correction for flatness of field as well as the aforementioned color correction.

Plan-Neofluar or **Plan-Fluotar** lenses are semiapochromatic lenses. They have good color correction for at least three wavelengths and also have the all around flatness of field. They are excellent for polarization microscopic techniques such as differential interference. As they also transmit UV very well, they are excellent lenses for all types of fluorescence microscopy.

Any lens with the term **fluar** in it has fluorite elements in it and all of these are very good for fluorescence work. Zeiss recently introduced a new line of semiapochromatic lenses named

Fluar lenses. These are objectives without a flat field made especially to increase the brightness of fluorescence. The image from a fluar lens is approximately 10% brighter than the equivalent Plan-Neofluar. In the UV range , the light transmission increases by 25-50%. This line of objective lenses was introduced about two years ago.

Apochromatic lenses (**Planapochromat**)are the most highly color corrected objectives: they are corrected for four wavelengths and are top of the line in objective lenses. These most often have the highest numerical apertures (see below). Be careful in using these lenses for fluorescence, however. They do not transmit UV light. They work very well for visible light excitation in the blue and green ranges.

http://www.microscopyu.com/tutorials/java/objectives/nuaperture/index.html