ECE 5984-Introduction to Modern Optical Microscopy

Homework-III

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In the first section of this report, objective lenses in Zeiss online micro-shop are studied and compared. The comparison is based on the price and related specifications of each lens to understand what makes an objective lens more expensive.

In the second section of the report, a brief summary of Nobel prizes related to optics and photonics and their impact on microscopy is detailed.

Objective lenses

In microscopy, the objective lenses are the optical elements closest to the specimen. It relays a real image of the object to the eyepiece and produces the base magnification. Objective lenses are the most complex part of the microscope due to their multi-element design. In addition to the magnification, indexes to determine the performance of an objective lens includes the numerical aperture, working distance, lens to image distance, and cover slip thickness correction.

In general, objective lenses are responsible for:

- Primary image formation
- Determine the quality of the image produced
- The total magnification
- The overall resolution

Objectives lenses greatly vary in design and quality. As such, they can be roughly classified based on:

- Intended purpose
- Microscopy method
- Performance
- Magnification
- Aberration correction

Classification based on Microscopy Method

- Reflected dark field objectives have a special construction that consists of a 360 degree hollow chamber that surrounds the centrally located lens element
- Differential interference contrast (DIC objectives) use stain-free optical elements and relies on the action of Nomarski prisms (or Wollaston prism) which influence optical path differences between sheared light beams at the rear focal plane.
- Fluorescence objectives designed with quartz and special glass with high transmission from ultraviolet to the infrared regions.
- Phase contrast objectives are divided in to several categories depending on construction and neutral density of internal phase ring. These include; dark low objectives (DL) Dark low low objectives (DLL) Apodized dark low objectives (ADL) Dark medium objectives (DM) Bright medium objectives (BM).

Classification based on Magnification

- Scanning Objective Lens (4x,5x and 10x) :low magnification power and gives the observer a good overview of the entire slide and sample.
- Intermediate magnification objectives (20x to 50x).
- High magnification objectives (100x). Immersion oil is used with high magnification lenses to obtain high resolving power.

Classification based on Aberration Correction

Light through a lens generates color aberration (color bleeding), which has a different refractive index according to the wavelength. There are two main levels of correction related to chromatic abberation correction. These are the achromatic and apochromatic correction.

Achromatic objectives

- simplest, least expensive and most commonly used objectives
- These objectives are designed to provide corrections for chromatic aberration in both the red and blue wavelengths. They are also corrected for spherical aberration in the green wavelength
- the chromatic aberration correction is limited and so is the flat field of view.
- low performance power
- better suited for monochromatic applications

Semi-apochromatic lens(fluorite lens)

- Designed to make the refractive indexes of three wavelengths (colors) of light the same.
- Used for fluorescence observation because the transmission ratio is ensured for ultra-violet light with a wavelength of about 340 nm.

Apochromatic objectives

- have a higher precision and more expensive.
- chromatically corrected for red, blue and yellow
- corrected for spherical aberration for two and three wavelengths in addition to a higher numerical aperture and long working distance.
- high performance power
- Because of their better design, apochromatic objectives are ideal for white light applications.

Both achromatic and apochromatic objective designs, however, suffer significantly from distortion and field curvature and this worsens as objective magnification increases. Therefore, it is always important to focus on the complete system performance, rather than just objective performance alone.

Classification based on field curvature abberation

- Field curvature aberration is when the off-axis image cannot be brought to focus in a flat image plane, resulting in a blurred image as it deviates from the optical axis.
- Field curvature often results in blurred images and correction for this helps produce good quality images.
- The lens is corrected for field curvature aberration so that not only the lens center but also the periphery are focused.
- If the lenses listed above have their field curvature aberrations corrected, they are respectively called plan achromatic lens, plan fluorite lens, and plan apochromatic lens.

Figure 1 is an example of field flatness measured radially from the center in achromatic, semi-plan, and plan objective designs. Achromatic objectives have a flat field in the center 65% of the image. Plan objectives correct best overall and display better than 90% of the field flat and in focus. Semi-plan objectives are intermediate to the other two types with 80% of the field appearing flat.

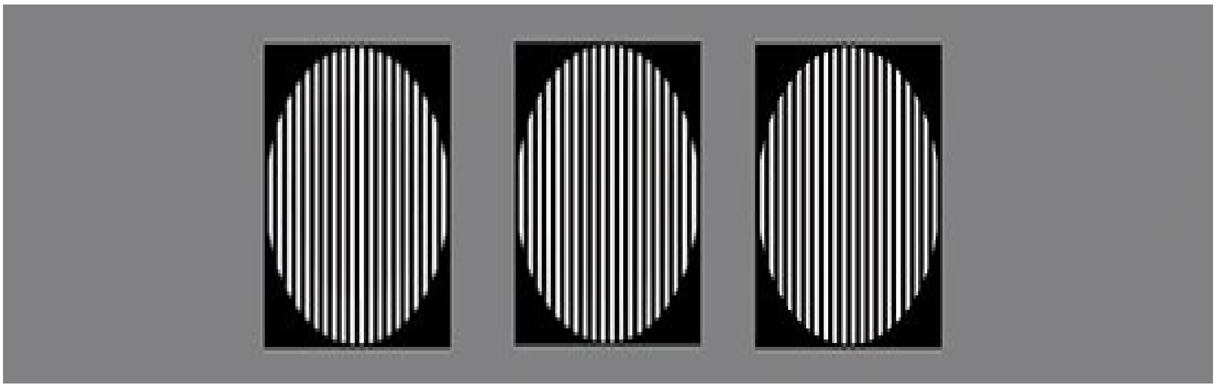


Figure 1: Flat Field Correction: Achromatic 65% (Left) vs. Semiplan 80% (Center) vs. Plan 90% (Right)

Refractive and Reflective Objectives Lenses

Refractive lenses

- Most common objectives.
- Light is bent (refracted) by the optical elements, which are designed in a manner that reduces back reflections thereby improving the overall light passing through.
- Often used in applications that require resolution of highly fine details.
- For refractive objectives, designs may range from two elements in the basic achromatic objectives to fifteen elements in plan-apochromatic objectives.

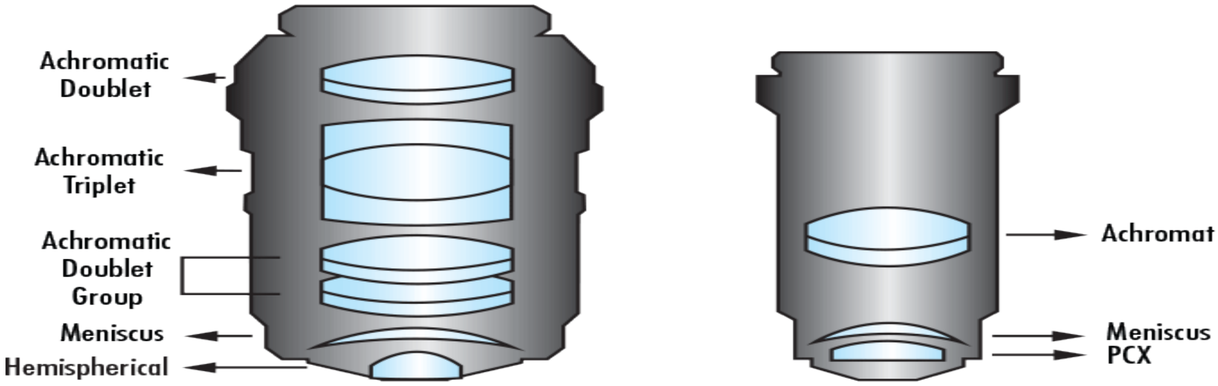


Figure 2: Apochromatic (Left) vs. Achromatic (Right) Objective Design

Reflective objectives

- typically use reflective/mirror based design.
- not commonly used but overcome a number of problems found in the design of refractive objectives.
- no additional designs are necessary to overcome aberrations
- they produce higher light efficiency and better resolving power, which is excellent for fine detail imaging.
- they allow for working deeper into either the ultra-violet or infrared spectral regions given that they use mirrors.

Classification based on finite conjugate/infinity conjugate

Finite conjugate

- light from a source (not at infinity) is focused down to a spot
- the image of the object under inspection is magnified and projected onto the eyepiece
- Used in applications where cost and ease of design are major concerns.

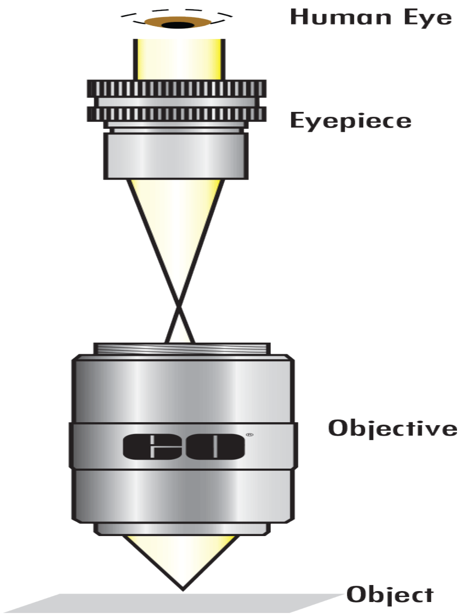


Figure 3: Finite conjugate

Infinite Conjugate (Infinity Corrected)

- light from a source placed at infinity is focused down to a small spot
- the spot is the object under inspection and infinity points toward the eyepiece
- utilizes an additional tube lens between the object and eyepiece in order to produce an image
- allows for the introduction of optical components such as filters, polarizers, and beamsplitters into the optical path
- provides flexibility to vary magnification according to specific application needs by varying the tube lens focal length

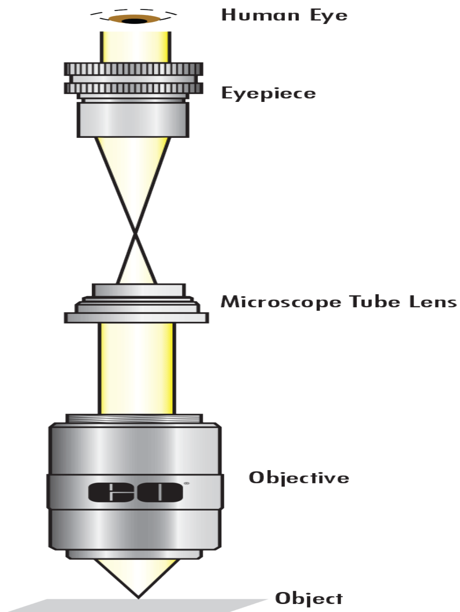
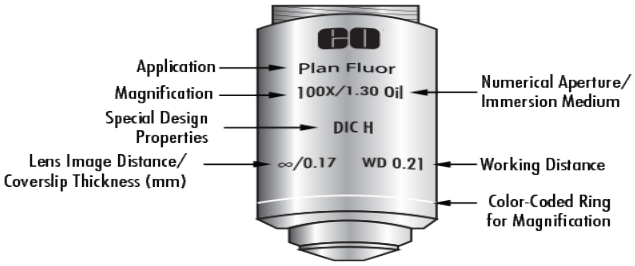


Figure 4: Infinite conjugate

Specifications

Specifications of the objective are usually listed on the body of the objective



Magnification	1X	2X	3X	4X	10X	20X	40X	60X	100X
Color Code	Black	Gray	Red	Yellow	Green	Light Blue	Light Blue	Dark Blue	White

Figure 5: Transmissive Microscope Objective

Objective standard

- Objective standards as DIN or JIS will be listed on the body of the objective.
- This shows the required specification present in the system.The DIN has 160mm distance from objective frange to the frange of the eyepiece while JIS has 170mm distance

Magnification

- Usually denoted by an X next to a numeric value (100X, 10X etc)
- Objectives also have a colored band around the circumference of the objective that indicates the magnification of the objective, as shown in the above figure

Numerical aperture (NA)

- Refers to the function of focal length and entrance pupil diameter and labeled next to the magnification of the objective
- A large numerical aperture (more than 1) means that that immersion oil may have to be used given that the highest NA that can be achieved. Without immersion oils (in air), the highest possible NA is 1.

Cover slip thickness

- Denoted by a number (such as 0.17mm) ,the cover slip thickness is labeled on the objective to note the type of cover slip that should be used
- A cover slip changes the way light is refracted from the specimen. Therefore, it is important to ensure that the right cover slip is used in order to produce good quality image.

Quality correction

- Quality correction such as achromatic, apochromatic, plan and semi-plan are often denoted on the objective

Field of View

- Field of View is the area of the object that is imaged by a microscope system.
- The size of the field of view is determined by the objective magnification.
- When using an eyepiece-objective system, the field of view from the objective is magnified by the eyepiece for viewing.

Cost analysis in Zeiss online mircro shop

Based on the parameters studied in detail previously, I expect an expensive objective lenses to have the following properties

- Apochormatic - chromatically corrected for red, yellow and blue
- Plan lens - corrected for field curvature to provide flatness
- Infinity corrected
- Medium to high magnification. It should be noted that distortion and field curvature will worsen with magnification.
- Corrected for cover slip thickness
- Provide high numerical aperture (greater than or equal to 1) and field of view
- Excellent correction for aberrations.
- Provide high resolving power, color purity and rich contrast
- Suitable for reflective light work
- Minimal stray light
- High and uniform transmission in the desired wavelength range
- Long working distance

The cost of the EC Plan-Neofluar 40x/0.75 objective is $2142.00.

Based on the search using Zeiss objective assistant, the most expensive objective lenses, costing $19,617 each, are,

- Objective LD SC Plan-Apochromat 20x/1.0 Corr M32 85mm
- Objective Clr Plan-Apochromat 20x/1.0 Corr nd=1.38 M32 85mm
- Objective Clr Plan-Neofluar 20x/1.0 Corr nd=1.45 M32 85mm

As expected the specification of the lenses match the expected criteria.

- They are all plan-apochormatic lenses corrected for chromatic aberrations and field curvature aberrations whereas the cheaper lens is plan- semiapochromatic.
- The expensive lenses have a magnification of 20x.
- They are all infinity corrected

- They provide excellent transmission in the desired wavelength range, close to 90% . In the cheaper lenses, we have non-uniform transmission in the wavelength range. An example transmission curve of expensive and cheap lenses is provided in figure 6 and 7
- They have high free working distance(5.6mm) compared to cheaper lens(0.17mm)
- High numerical aperture (equal to 1) compared to cheaper lens (0.75) and thus better resolving power than the cheaper lens.
- Expensive lenses in general have high magnification (40X to 100X) whereas the cheaper ones have low magnification (5X to 10X)
- VIS-NIR Correction collar for compensation of spherical aberrations is provided for the expensive lens
- High parfocal length of 85mm compared to the 45.06mm provided by cheaper lens
- Provides excellent flatness and color correction compared to cheap lens
- They have correction ring and long distance which are not included in the cheaper lens
- Multi-channel,UV transmission and infra-red transmission is better in the expensive variety
- The cheaper ones require additional accessories like sliders and cover slips to improve performance whereas the expensive ones are in general standalone
- The objectives have immersion liquid to provide high resolving power and numerical aperture
- Expensive lenses find application in confocal microscopy, fluorescence, bright field and have better performance than the cheaper one.

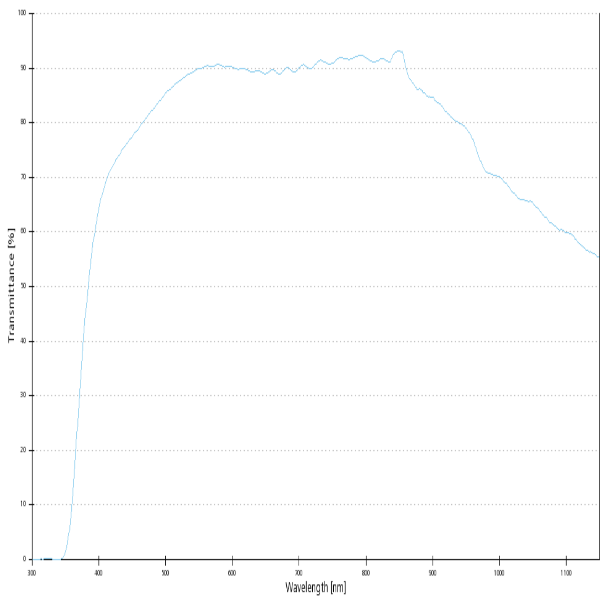


Figure 6: Uniform transmission curve of an expensive lens

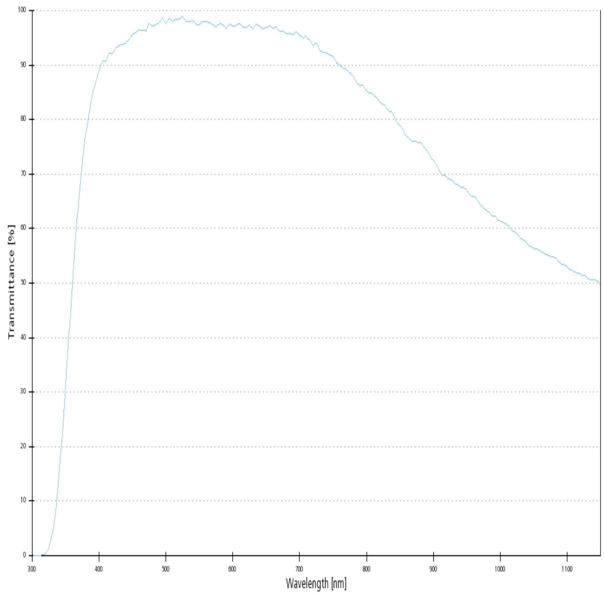


Figure 7: Non-uniform transmission of cheap lens

Cost of Zeiss Observer D1

The axial observer d1 is a coded semi-motorized inverted microscope manufactured by Carl zeiss. Axio Observer microscopes are inverted light microscopes for universal use. They are used primarily for examining cell and tissue cultures and sediments in culture flasks, Petri dishes and microtiter plates under transmitted and reflected light. The overall cost is estimated by summing up the cost of the individual components

Basic Stand	7 771.00 $
Z-drive operation,flat; right	54.00$
6 piece objective nosepiece	2522.00$
1-position tube lens mount, fixed	222.00$
Aqua Stop II	664.00$
Side port 60N L 3 positions	1 454.00$
Path deflection to the tube	239.00$
Condenser module DIC II/0.55 with polarizer	1 642.00$
Mechanical stage 130x85 R/L	2 750.00$
Carrier, transmitted-light illumination, LCD display, Shutter	2 522.00$
LD condenser 0.55 H ,6 positions	4 170.00$
Reflected light illumination,slider and shutter	1 686.00$
Universal mounting frame K	666.00$
Conversion filter	31.00 $
Eyepieces	770.00 $
Auxiliary microscopes	490.00 $
Eyepiece eyecup	36.00 $
Objective LD A-Plan 10x/0.25 Ph1 M27	806.00 $
Objective LD Plan-Neofluar 20x/0.4 Corr Ph2 M27	3 276.00 $
Objective LD Plan-Neofluar 40x/0.6 Corr Ph2 M27	3 701.00 $
laser safety port	461.00$
6-position reflector turret for PC modules	948.00$

Excitation filter wheel	2 976.00$
FL attenuator, discrete	727.00$
Analyzer module DIC ACR PC for transmitted light, discrete	476.00$
FluoArc control	229.00$
DIC slider LD PN 40x/0.60 II	1 302.00$
Dust cover for Axio Observer / Axiovert 200/200M	27.00$
Shutter FL, internal for stands Axio Observer 3 / 5 / 7 / D1 / Z1	158.00$
Antiglare screen for Axio Observer/Axiovert 200	205.00 $
Iris stop slider for incident-light equipment	585.00 $
Objective LD A-Plan 5x/0.15 M27	520.00 $
HAL 100 illuminator with quartz collector	920.00 $

Table 1: Cost of each component

An estimate of the total cost of the microscope including optional accessories is $45,006. Many accessories are optional and the cost can decrease if not all accessories are included in the purchase.

Nobel Prizes in optics and photonics

In this section, the Nobel prizes related to optics and photonics are listed. In addition, the breakthrough discoveries and inventions that expanded the boundaries of microscopy are discussed. The criteria with the prizes are chosen to be listed is their relevance to the broad field of optics including optical imaging, electro-optics, optical fibers, optical metrology and spectroscopy, and microscopy.

2018	Physics	Awarded for groundbreaking inventions in the field of laser physics, in particular for the method of generating high-intensity, ultra-short optical pulses
2018	Physics	Awarded for groundbreaking inventions in the field of laser physics", in particular for the optical tweezers and their application to biological systems
2014	Physics	Awarded for the invention of efficient blue light-emitting diodes which has enabled bright and energy-saving white light sources

2009	Physics	Awarded for groundbreaking achievements concerning the transmission of light in fibers for optical communication
2005	Physics	Awarded for the contributions to the development of laser-based precision spectroscopy, including the optical frequency comb technique
2005	Physics	Awarded for the contribution to quantum theory of optical coherence
1997	Physics	Awarded for development of methods to cool and trap atoms with laser light.
1986	Physics	Awarded for the design of the scanning tunneling microscope
1986	Physics	Awarded for fundamental work in electron optics, and for the design of the first electron microscope
1981	Physics	Awarded for development of methods to cool and trap atoms with laser light.
1997	Physics	Awarded for the contribution to the development of laser spectroscopy
1971	Physics	Awarded for the invention and development of the holographic method
1953	Physics	Awarded for the demonstration of the phase contrast method, especially for the invention of the phase contrast microscope
1907	Physics	Awarded for the optical precision instruments and the spectroscopic and metrological investigations carried out with their aid
2017	Chemistry	Awarded for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution
2014	Chemistry	Awarded for the development of super-resolved fluorescence microscopy
1991	Chemistry	Awarded for contributions to the development of the methodology of high resolution nuclear magnetic resonance (NMR) spectroscopy
1982	Chemistry	Awarded for the development of crystallographic electron microscopy and structural elucidation of biologically important nucleic acid-protein complexes
1979	physiology	Awarded for the development of computer assisted tomography

Table 2: Nobel prizes related to optics and photonics

Notable inventions related to Microscopy

Out of the listed Nobel prizes awarded inventions, few inventions pushed the boundaries of microscopy thereby having a tremendous impact in the field of microscopy

- 2017 Nobel Prize in chemistry for inventing cryo-electro microscopy to see individual atoms within biological molecules. This invention made it possible for life's molecular building blocks to be captured mid-movement and allowed scientists to visualise processes that had never before been seen thereby moveing biochemistry into a new era. It was used in important application such as to determine the structure of the Zika virus and image Salmonella's "injection needle" for attacking cells

- 2014 Nobel Prize in chemistry for the development of super-resolved fluorescence microscopy. The inventors used fluorescent molecules to circumvent Abbe's physical limit of 0.2 micrometers, thereby enabling to see things at much higher levels of resolution previously thought not possible. The techniques of super resolved microscopy include Stimulated emission depletion (STED) microscopy and single-molecule microscopy. Electron microscopy had resolution of 10nm but it involved killing the cells. But the newly invented techniques made it possible to see live cells with super resolution.

- 1953 Nobel prize in physics for invention of phase contrast microscopy. This was a breakthrough invention that opened doors previously thought were closed. The principle is to convert phase shifts in light passing through a transparent specimen to brightness changes in the image. It revealed many cellular structures that are not visible with a simpler bright-field microscope. Traditionally, these structures were made visible by staining which resulted in killing the cells. The phase-contrast microscope made it possible to see living cells and to understand proliferation through cell division. Also, it is one of the few methods that does not use fluorescence. This technique paved the way for many techniques such as differential interference contrast (DIC) microscopy and Hoffman modulation contrast microscopy
- 1986 Nobel prize in physics for inventing Scanning Tunneling Microscope. The principle based on quantum tunneling enabled imaging surfaces at the atomic level with a resolution of 0.01nm depth resolution. The advantage of STM over other measurements of the density of states is its ability to make extremely local measurements.
- 1986 Nobel prize in physics for invention of electron microscope. It works on the principle that a short coil carrying an electric current can deflect electrons in the same way that a lens deflects light. Before this invention, light microscope was widely used but observing anything in greater detail is limited by the wavelength of light. It was impossible for visible light to produce an image of objects such as proteins and atoms that are smaller than its wavelength. The development of the electron microscope opened up this previously hidden world. As electrons have a much smaller wavelength than light, this microscope made it possible to see details many times smaller than with a light microscope.