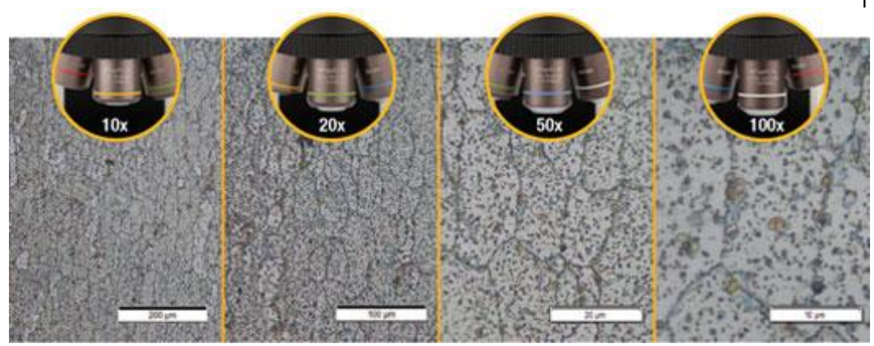
CCAM's Selection of

Zeiss Microscope Objectives

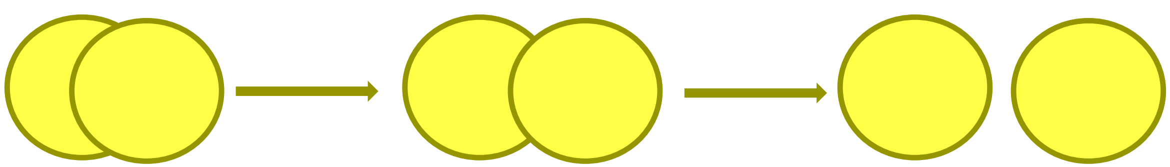
Things to consider when selecting an objective

1. Magnification

   Image scale



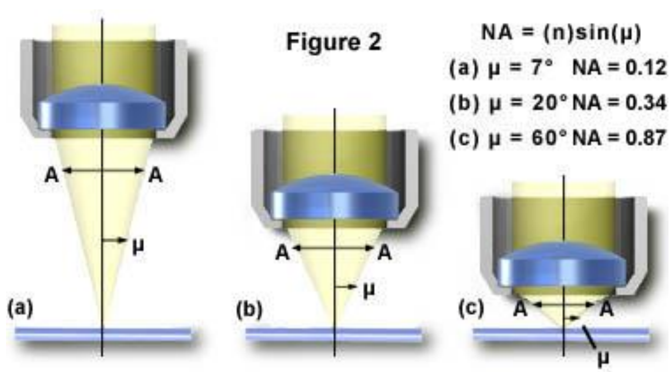
2. Resolution
   - The minimum separation distance between two points that are clearly resolved.
   - The resolution of an objective is limited due to diffraction and the nature of light
   - Defined by Abbe's formula
   - $d = \lambda / 2NA$ (λ = wavelength of light used, NA = the numerical aperture of the objective)



Things to consider when selecting an objective

3. Numerical Aperture (NA)

   Objective's ability to collect light and resolve specimen detail at a fixed distance.



(https://micro.magnet.fsu.edu/primer/anatomy/numaperture.html)

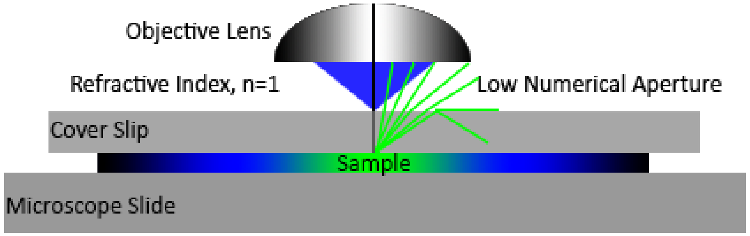
n = refractive index of medium between front lens element and cover slip.

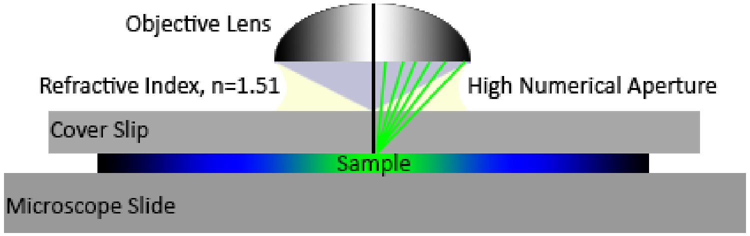
μ = ½ the angular aperture (A)

Things to consider when selecting an objective

3. Numerical Aperture/Refractive index (cont.)

- The refractive index is the limiting factor in achieving numerical apertures greater than 1.0.
- To obtain a higher numerical aperture, a medium with a higher refractive index must be used.
- Highly corrected lenses are designed with higher numerical apertures.

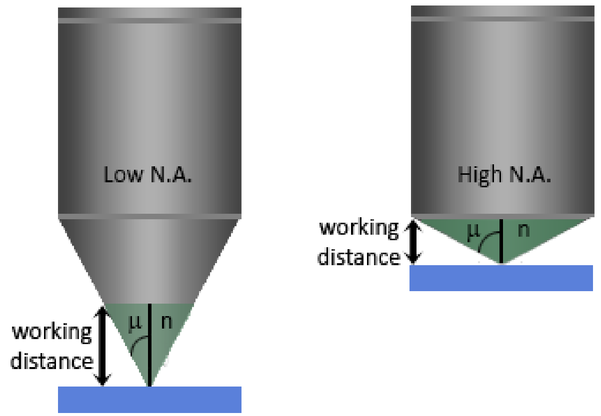




Things to consider when selecting an objective

4. Working Distance

Distance between the front lens of the objective and the cover glass of the specimen.



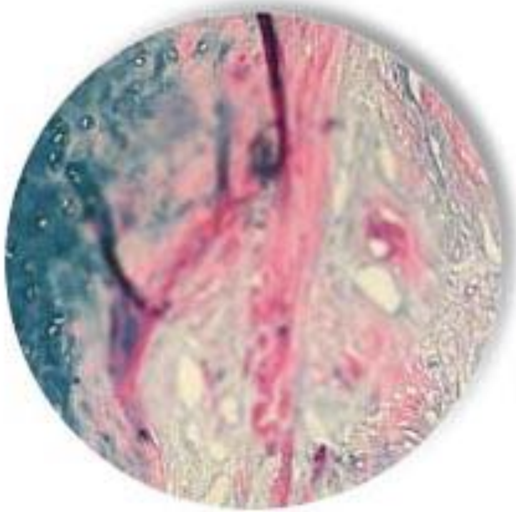
- Note the working distance is reduced with the increase in numerical aperture and magnification.

Things to consider when selecting an objective

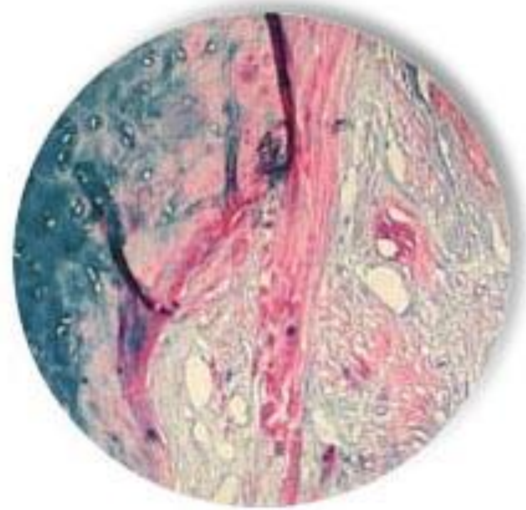
4. Flatness of Field

   Correction of field curvature

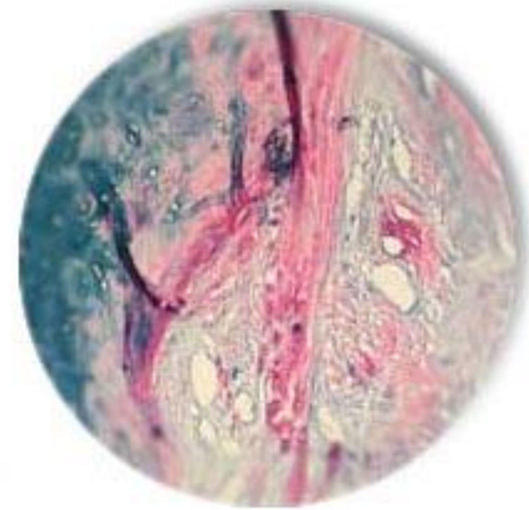
   - Objectives provide a common focus through the field of view.
   - Such objectives are traditionally named as "plan"



Edges in focus



Entire field in focus



Center in focus

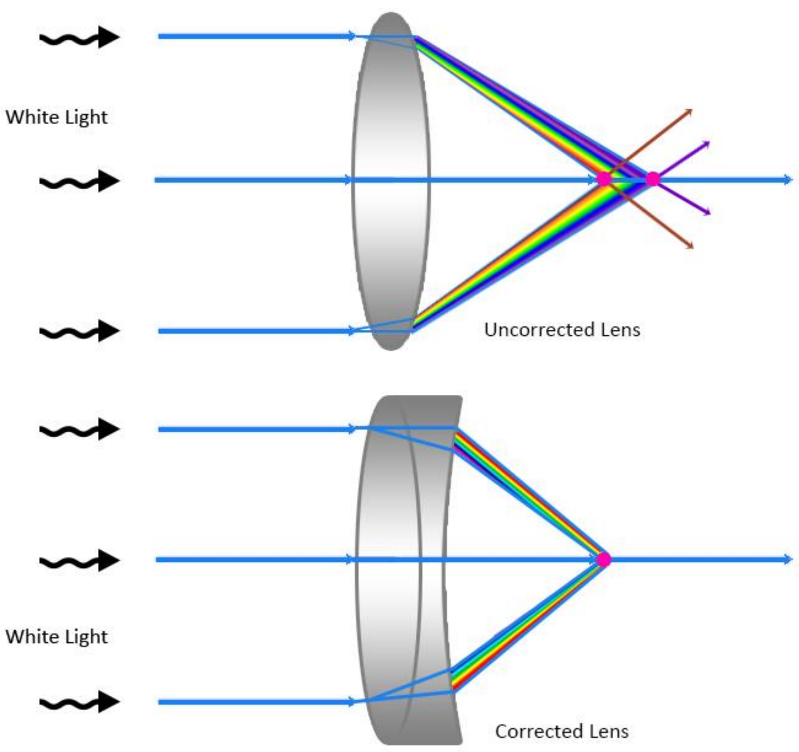
https://micro.magnet.fsu.edu/primer/anatomy/fieldcurvature.html

Things to consider when selecting an objective

6. Chromatic aberration

   Color correction, focuses different wavelengths of light to the same point

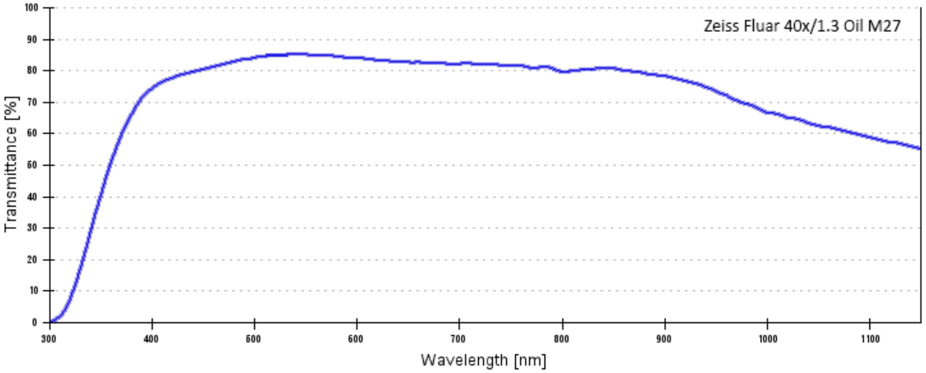
- Achromatic – focuses two wavelengths of light, e.g. red and blue
- Apochromatic – focuses three wavelength of light, e.g. red, green and blue

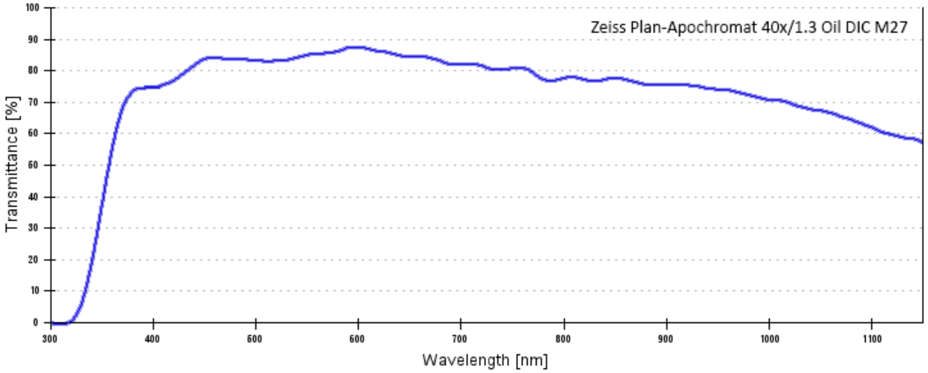


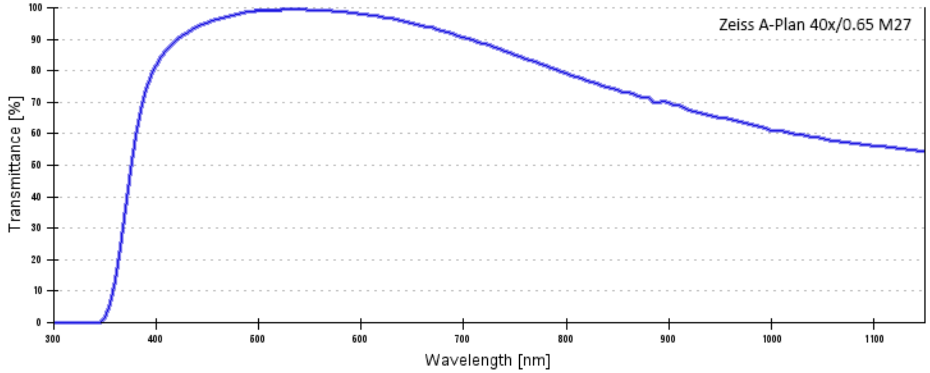
Things to consider when selecting an objective

7. Light Transmission

   The various classes of objectives transmit wavelengths of light, with different efficiencies.





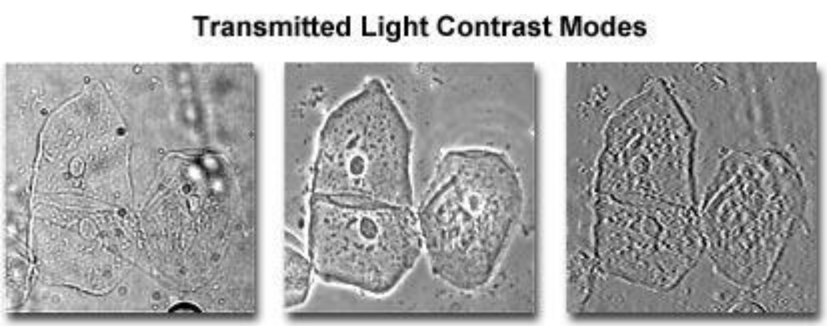


https://www.micro-shop.zeiss.com/

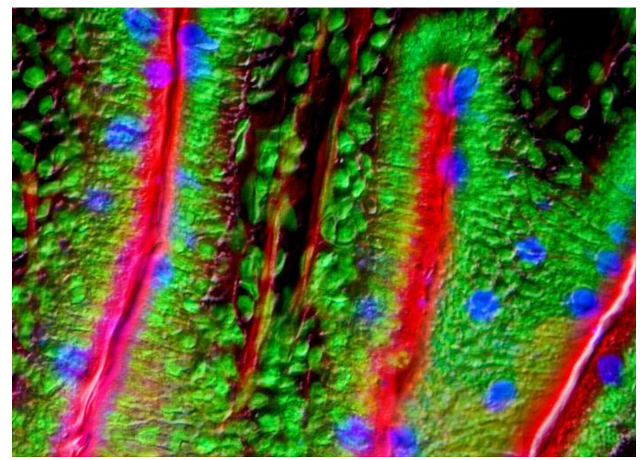
Things to consider when selecting an objective

8. Contrast Method/Application

   Objectives are customized to be used for particular imaging techniques; bright-field, fluorescence, differential interference contrast (DIC), phase contrast.



Bright-field   Phase contrast   DIC



Fluorescence

(http://www.olympusmicro.com/primer/techniques/contrast.html)

(https://www.microscopyu.com/galleries/fluorescence)

Objective Class

Objective	Spherical Aberration	Chromatic Aberration	Field Curvature
Achromat	1 color	2 colors	No
Plan-Achromat	1 color	2 colors	Yes
Fluorite	2-3 colors	2-3 colors	No
Plan-Fluorite	3-4 colors	2-4 colors	Yes
Plan-Apochromat	3-4 colors	4-5 colors	Yes

Note: Plan (flat-field) objectives provide a corrected flat field. An uncorrected lens may provide only 10-12mm of flatness while a plan objective can provide a flat field across 18-26mm.

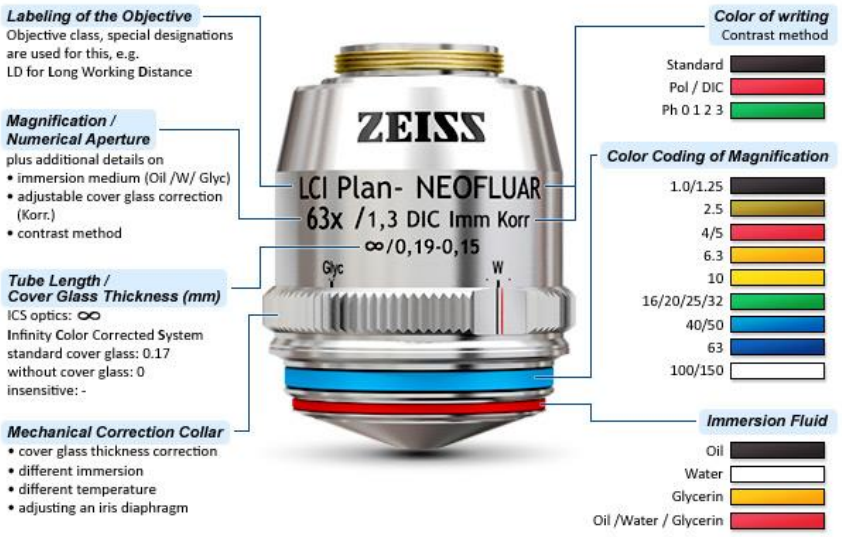
Objective Class (cont.)

Objective	Features
Plan-Apochromat	Best chromatic correction, flatness of field and the highest numerical apertures.
C-Apochromat	Adjustable correction collar to correct for differences in refractive indices and preparation thickness, water immersion variety is useful for aqueous specimens.
Fluar	High numerical apertures, high transmission of visible spectrum to near UV wavelengths, objective of choice for weak fluorescent signals.
Plan-Neofluar	Chromatic correction, high resolving power, flatness of field.
C-Achroplan	Corrected for axial chromatic aberration in two wavelengths, blue and red, flatness of field and adjustable correction collar.

Looking at the Objective;
A Wealth of Information

Information that is labeled on the barrel of the objective:

- Type
- Magnification/Numerical Aperture
- Tube Length/Cover Glass Thickness
- Color Coded
    - Contrast
    - Method of Contrast
    - Magnification
    - Immersion fluid



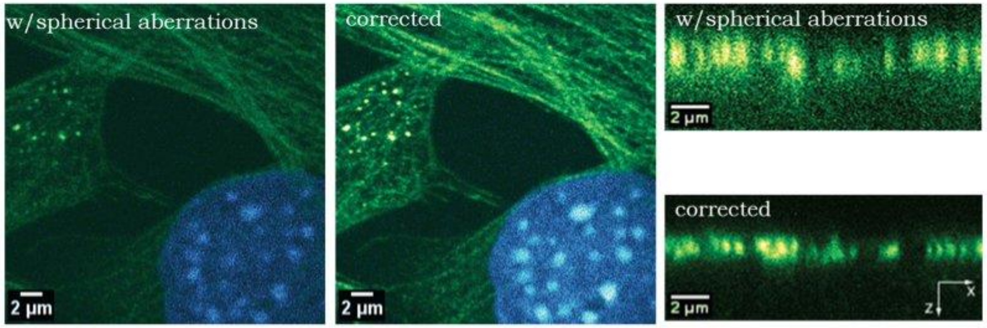
http://zeiss-campus.magnet.fsu.edu/tutorials/basics/objectivecolorcoding/indexflash.html

Preparation Notes

- The CCAM Zeiss objectives are all designed to be used with a 0.17mm/#1.5 glass cover slip (standard variation of 0.16 – 0.19 mm).
- Avoid Spherical Aberration

  Spherical aberration focuses axial and peripheral rays to different points, it blurs the image of a point source of light leading to reduced image contrast and sharpness and eliminates much of the fluorescence of an object.

  - Use immersion and mounting medias of similar refractive indices; i.e. do not image using an oil immersion objective (n=1.52) for a specimen mounted in a watery solution (n=1..33)
  - Spherical aberration increases with sample depth therefore it is best to position the specimen directly under the cover glass



http://www.dcsc.tudelft.nl/Education/ThesisProposals/proposal-5714.html

CCAM Objectives on Zeiss LSM 780

Objective	Numerical Aperture	Working Distance (mm)	Confocal XY resolution (µm) (λ = 488 nm	Optimal Pixel Size (µm)	Confocal Z Resolution (µm) (λ = 488 nm)	Optimal Slice Spacing (µm)
C-Apochromat 10x W	0.45	1.8	.43	.22	4.5	2.25
Plan-Apochromat 20x Air	0.80	.55	.24	.12	1.07	0.53
C-Achroplan 32x W	0.85	1.1	.23	.11	1.26	.63
Plan-Apochromat 40x W	1.2	.28	.16	.08	.63	.32
Plan-Apochromat 40x Oil DIC	1.3	.21	.15	.08	.61	.31
Plan-Apochromat 63x Oil DIC	1.4	.19	.14	.07	.53	.26

XY res = $.6 \cdot \lambda/NA$ (widefield)
For widefield PH > 5 A.U.
XY res = $.4 \cdot \lambda/NA$ (confocal)
Pixel size = XY res/2 (Nyquist criterion)

(Resolution based on Rayleigh criteria)

Z resolution = $2\lambda \cdot n/NA^2$ (widefield)
Z resolution = $1.4 \cdot \lambda \cdot n/NA^2$ (confocal)
Slice spacing = Z res/2 (Nyquist criterion)

(Resolution based on Rayleigh criteria)

M = magnification PH = pinhole
AU = Airy Units n = ref. index
Air = 1.0 Water = 1.337 Oil = 1.518
Brightness = $\alpha NA^4/M^2$

CCAM Objectives on Zeiss LSM 880

Objective	Numerical Aperture	Working Distance (mm)	Confocal XY resolution (µm) (λ = 488 nm	Optimal Pixel Size (µm)	Confocal Z Resolution (µm) (λ = 488 nm)	Optimal Slice Spacing (µm)
EC Plan-Neofluar 10x Air	0.30	5.2	.65	.32	7.60	3.80
Plan-Apochromat 20x Air	0.80	.55	.24	.12	1.07	0.53
LD LCI Plan-Apochromat 25x Variable Immersion	0.80	.57	.24	.12	Water = 1.43 Silicon Oil = 1.50 Glycerin = 1.57 Oil = 1.62	Water = .71 Silicon Oil = .75 Glycerin = .78 Oil = .81
Plan-Apochromat 40x W	1.2	.28	.16	.08	.63	.32
Fluar 40x Oil	1.3	.21	.15	.08	.61	.31
Plan-Apochromat 63x Oil DIC	1.4	.19	.14	.07	.53	.26

XY res = $.6 \cdot \lambda/NA$ (widefield)
For widefield PH > 5 A.U.
XY res = $.4 \cdot \lambda/NA$ (confocal)
Pixel size = XY res/2 (Nyquist criterion)

(Resolution based on Rayleigh criteria)

Z resolution = $2 \lambda \cdot n/NA^2$ (widefield)
Z resolution = $1.4 \cdot \lambda \cdot n/NA^2$ (confocal)
Slice spacing = Z res/2 (Nyquist criterion)

(Resolution based on Rayleigh criteria)

M = magnification PH = pinhole
AU = Airy Units n = ref. index
Air = 1.0 Water = 1.337 Oil = 1.518
Silicone Oil = 1.404 Gylcerin = 1.470
Brightness = $\alpha NA^4/M^2$