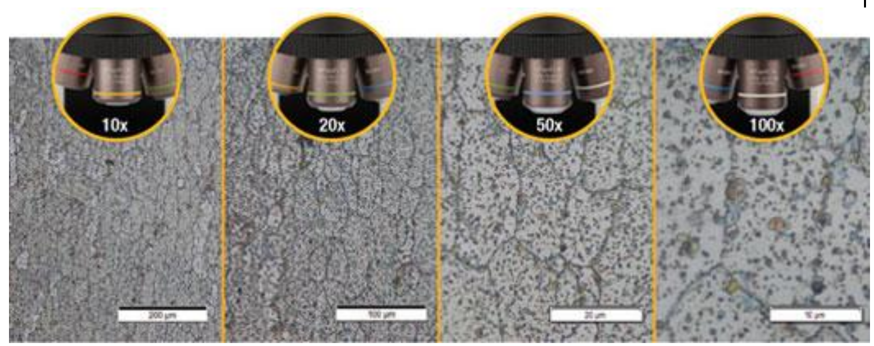
# CCAM's Selection of

Zeiss Microscope Objectives

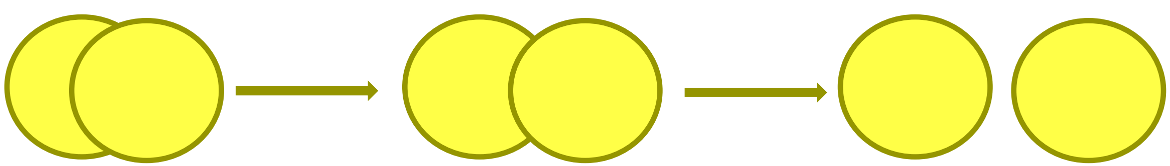
# Things to consider when selecting an objective




1. Magnification

   Image scale

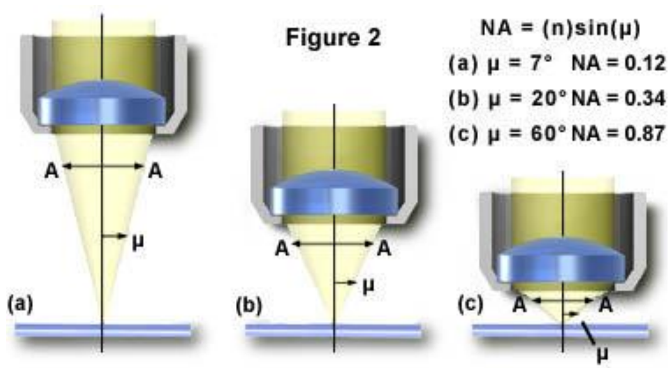
2. Resolution
   - The minimum separation distance between two points that are clearly resolved.
   - The resolution of an objective is limited due to diffraction and the nature of light
   - Defined by Abbe's formula
   - $d = \lambda / 2NA$ ($\lambda$ = wavelength of light used, NA = the numerical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

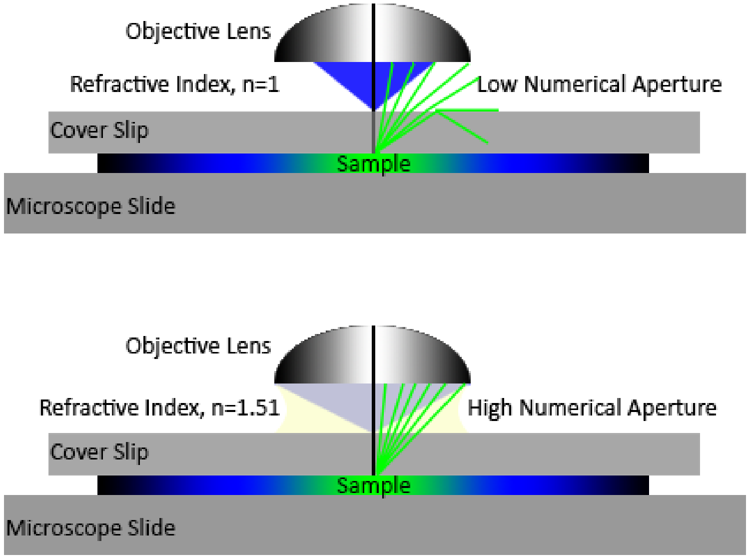
n = refractive index of medium between front lens element and cover slip.

$\mu$ = ½ the angular aperture (A)

# Things to consider when selecting an objective

3. Numerical Aperture/Refractive index (cont.)

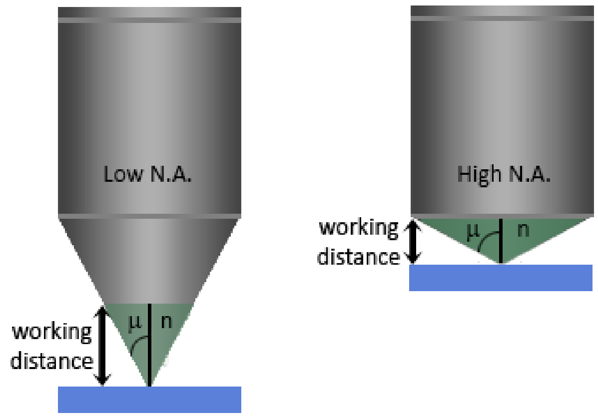
- The refractive index is the limiting factor in achieving numerical apertures greater than 1.0.
- To obtain a higher numerical aperture, a medium with a higher refractive index must be used.
- Highly corrected lenses are designed with higher numerical apertures.



# 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.



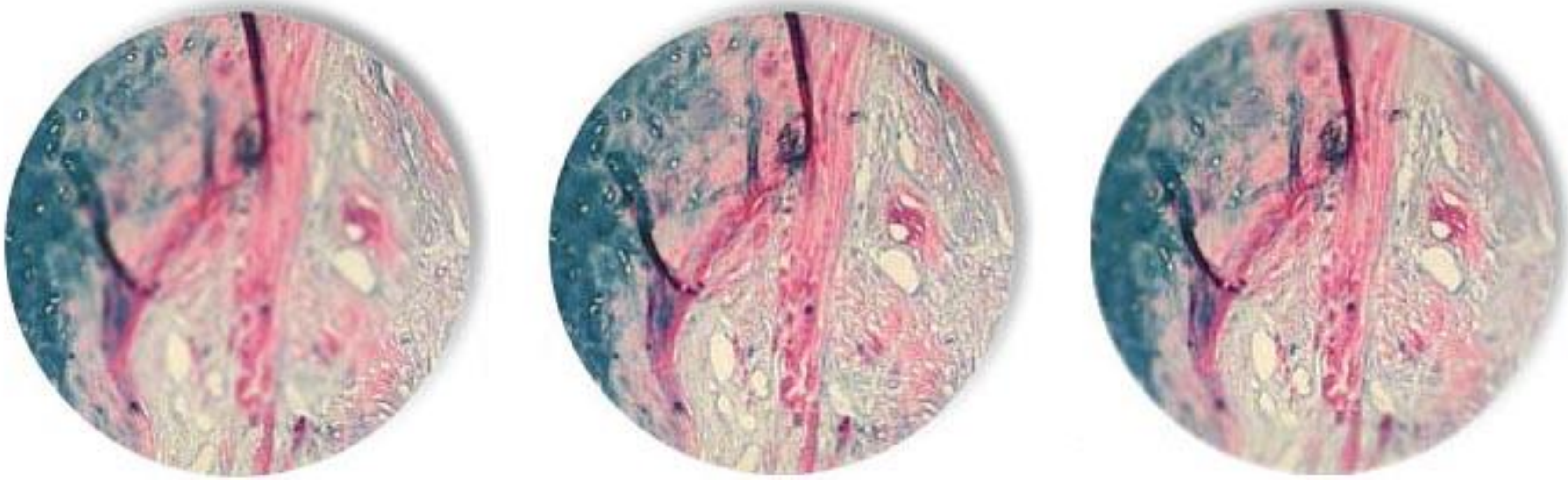

- Note the working distance is reduced with the increase in numerical aperture and magnification.

# 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

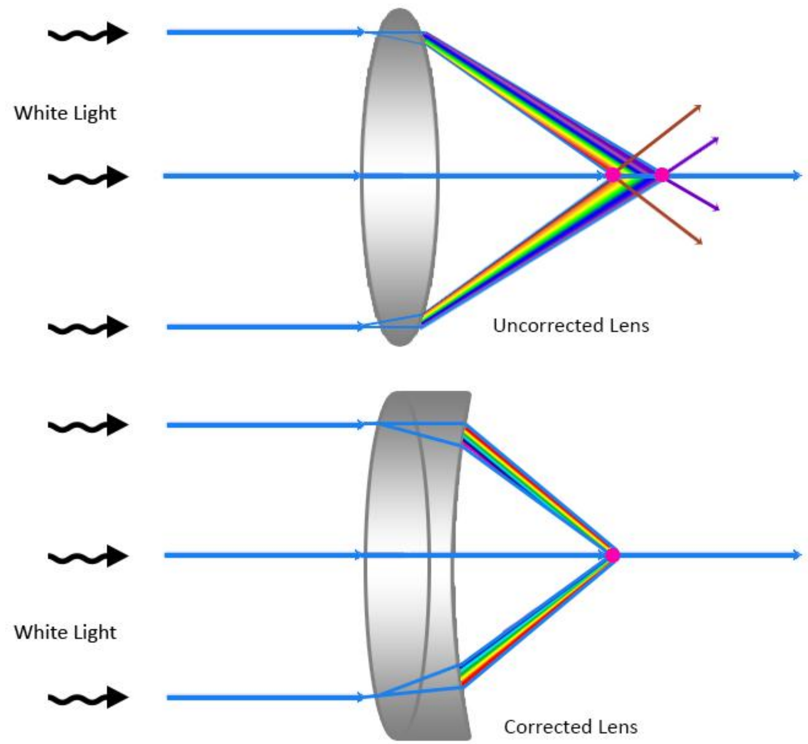
https://micro.magnet.fsu.edu/primer/anatomy/fieldcurvature.html

# Things to consider when selecting an objective

6. Chromatic aberration

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

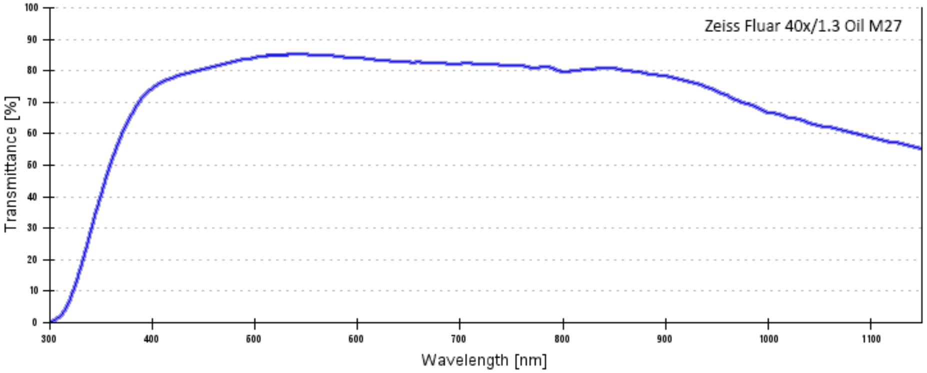
- Achromatic – focuses two wavelengths of light, e.g. red and blue
- Apochromatic – focuses three wavelength of light, e.g. red, green and blue

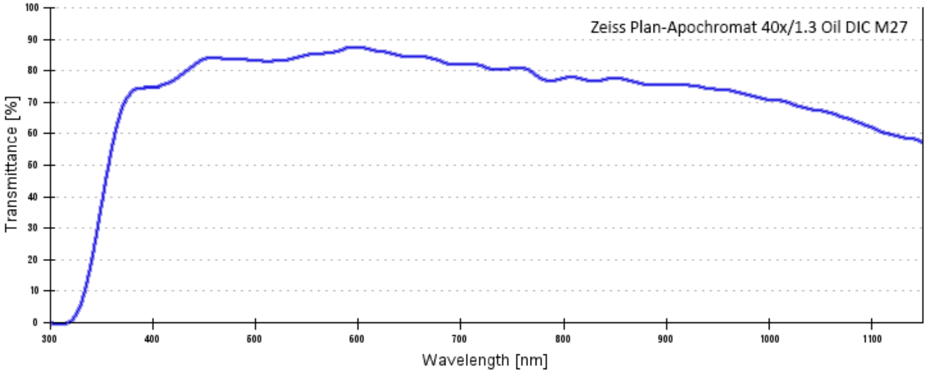


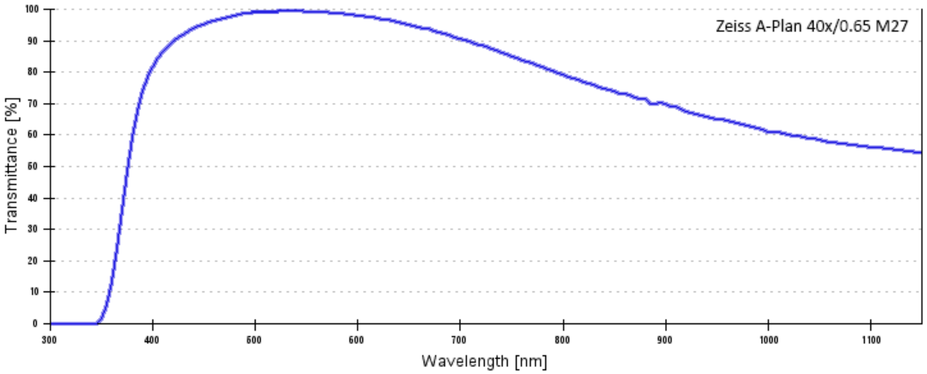
# Things to consider when selecting an objective

7. Light Transmission

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



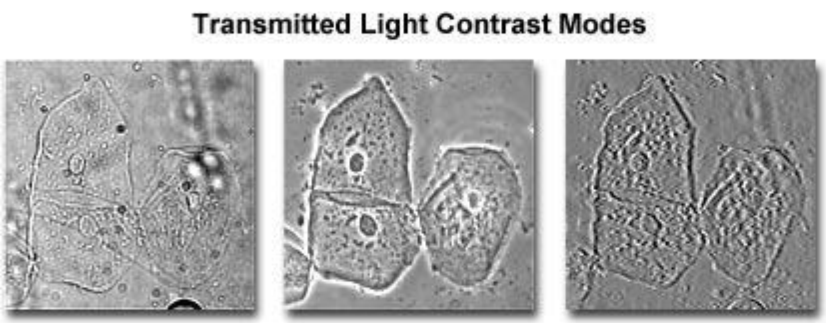




https://www.micro-shop.zeiss.com/

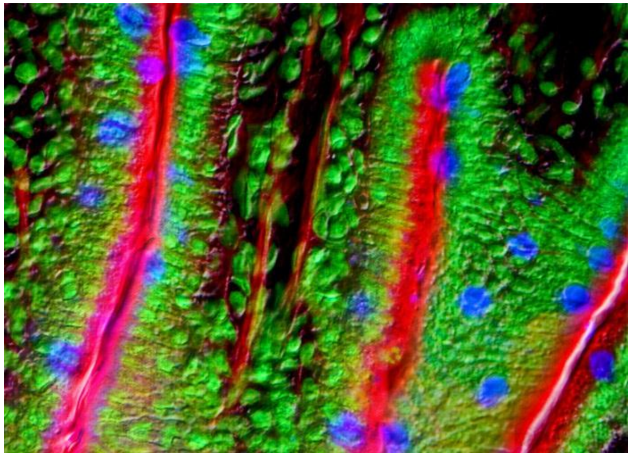
# Things to consider when selecting an objective

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.

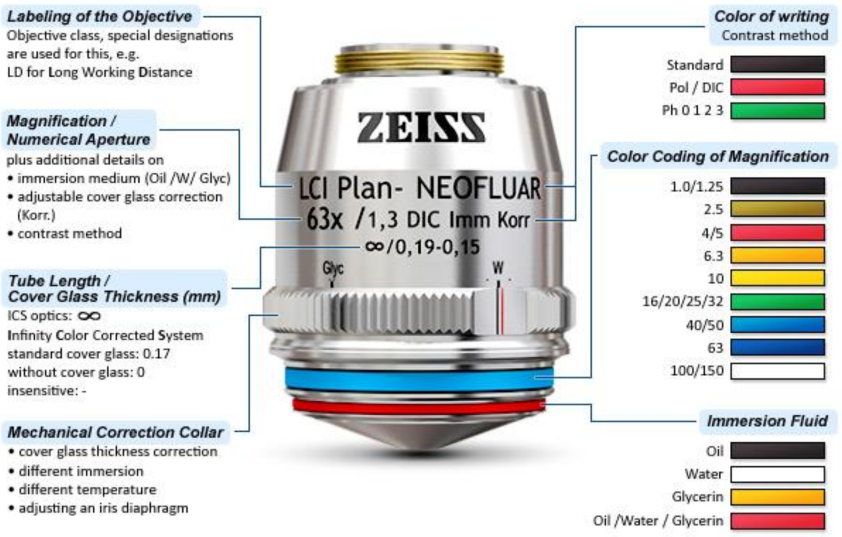
# Objective Class (cont.)

| 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



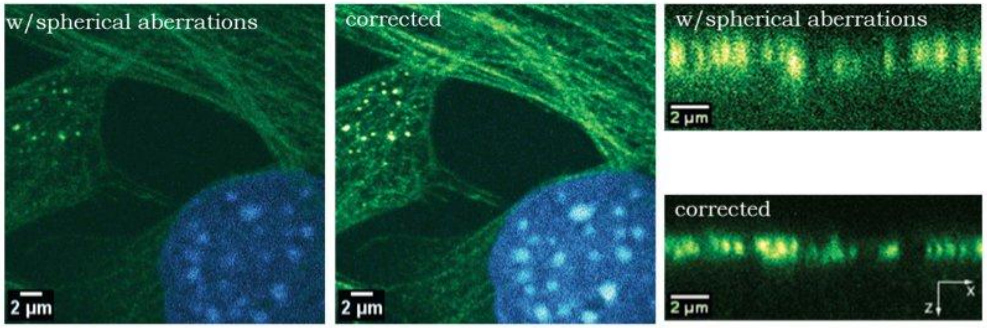

http://zeiss-campus.magnet.fsu.edu/tutorials/basics/objectivecolorcoding/indexflash.html

# 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) ($\lambda_{em}$ = 488 nm | Optimal Pixel Size (μm) | Confocal Z Resolution (μm) ($\lambda_{exc}$ = 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 · $\lambda_{exc}$/NA (widefield)
For widefield PH > 5 A.U.
**XY res = .4· $\lambda_{exc}$/NA (confocal)**
Pixel size = XY res/2 (Nyquist criterion)

(Resolution based on Rayleigh criteria)

Z resolution = 2·$\lambda_{em}$·n/NA$^2$ (widefield)
**Z resolution = 1.4·$\lambda_{em}$·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) ($\lambda_{em}$ = 488 nm | Optimal Pixel Size (µm) | Confocal Z Resolution (µm) ($\lambda_{exc}$ = 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<br>Silicon Oil = 1.50<br>Glycerin = 1.57<br>Oil = 1.62 | Water = .71<br>Silicon Oil = .75<br>Glycerin = .78<br>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 · $\lambda_{exc}$/NA (widefield)
For widefield PH > 5 A.U.
**XY res = .4· $\lambda_{exc}$/NA (confocal)**
Pixel size = XY res/2 (Nyquist criterion)

(Resolution based on Rayleigh criteria)

Z resolution = 2$\lambda_{em}$·n/NA$^2$ (widefield)
**Z resolution = 1.4·$\lambda_{em}$·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$^2$