Preparatory School to the Winter College on Optics: Advanced Optical Techniques for Bio-imaging

The Abdus Salam International Centre for Theoretical Physics

Principles of Microscopy I: Point Spread Function and Resolution

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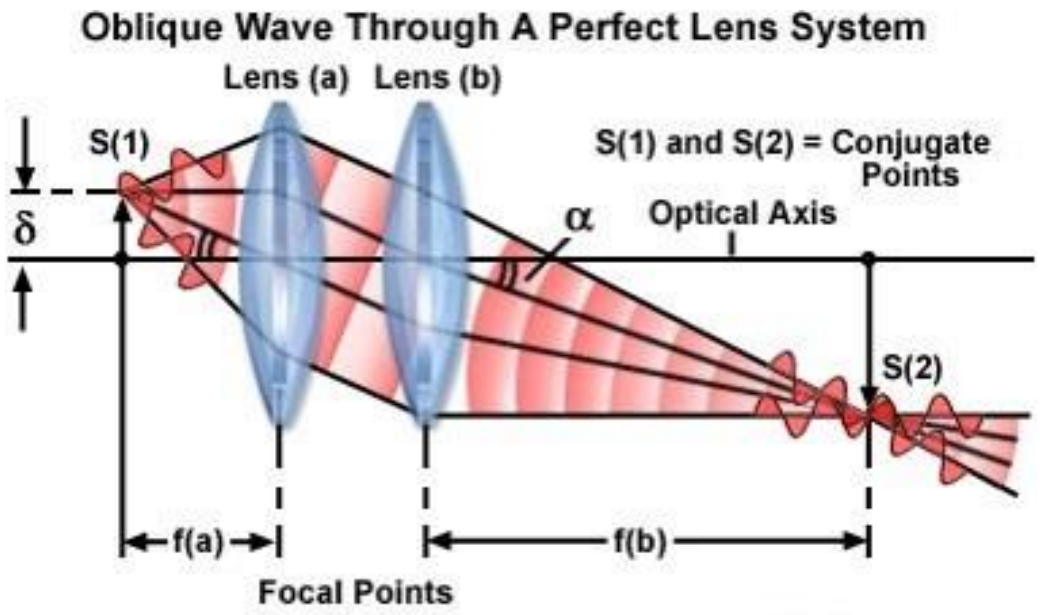
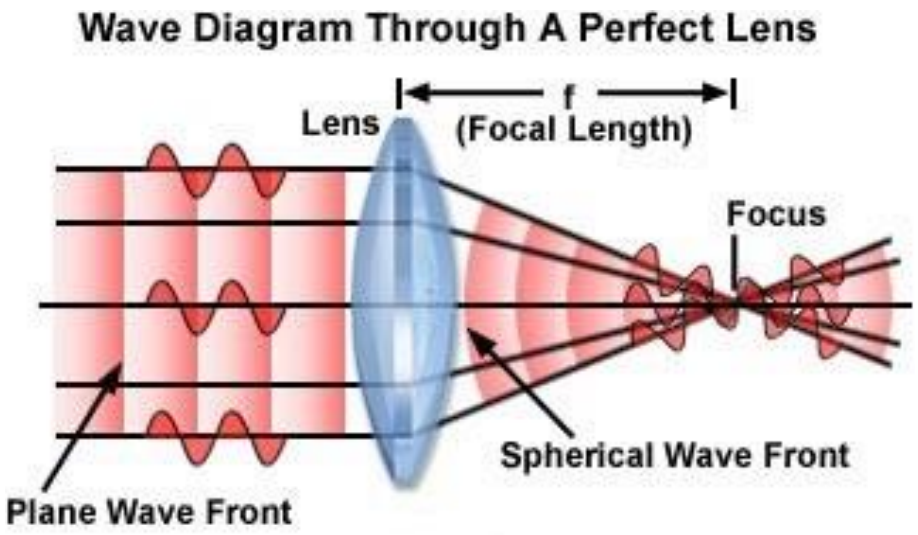
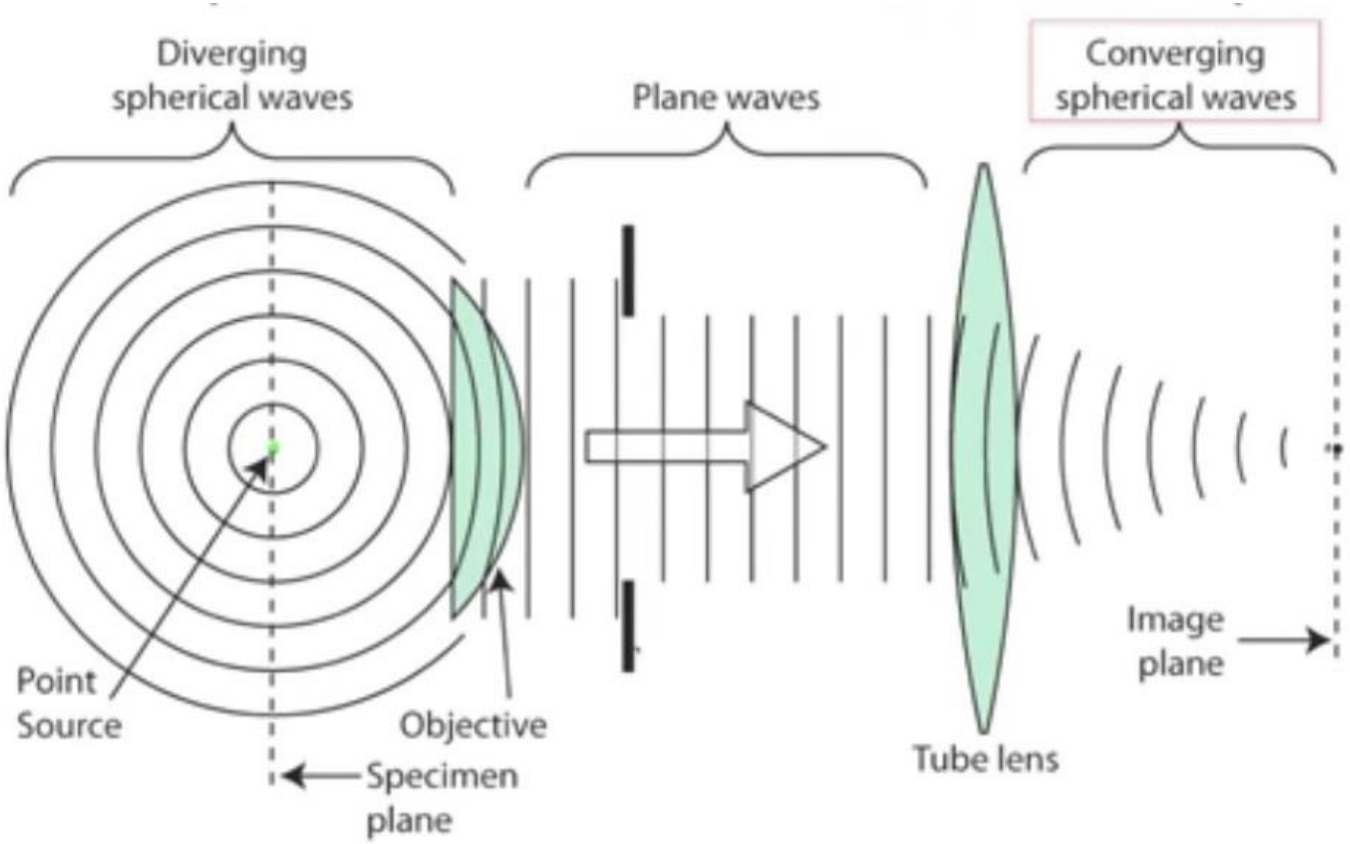
Wave optics view of the effect of the microscope lenses



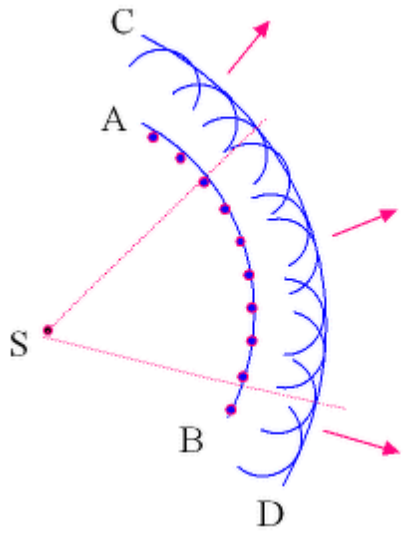


Wave optics view of the effect of the microscope lenses



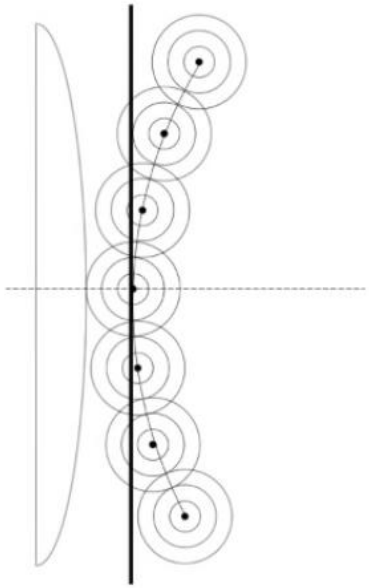
For a point object in a focal plane, what is the distribution of light at and near image plane (PSF)?

Huygens wavelet approach



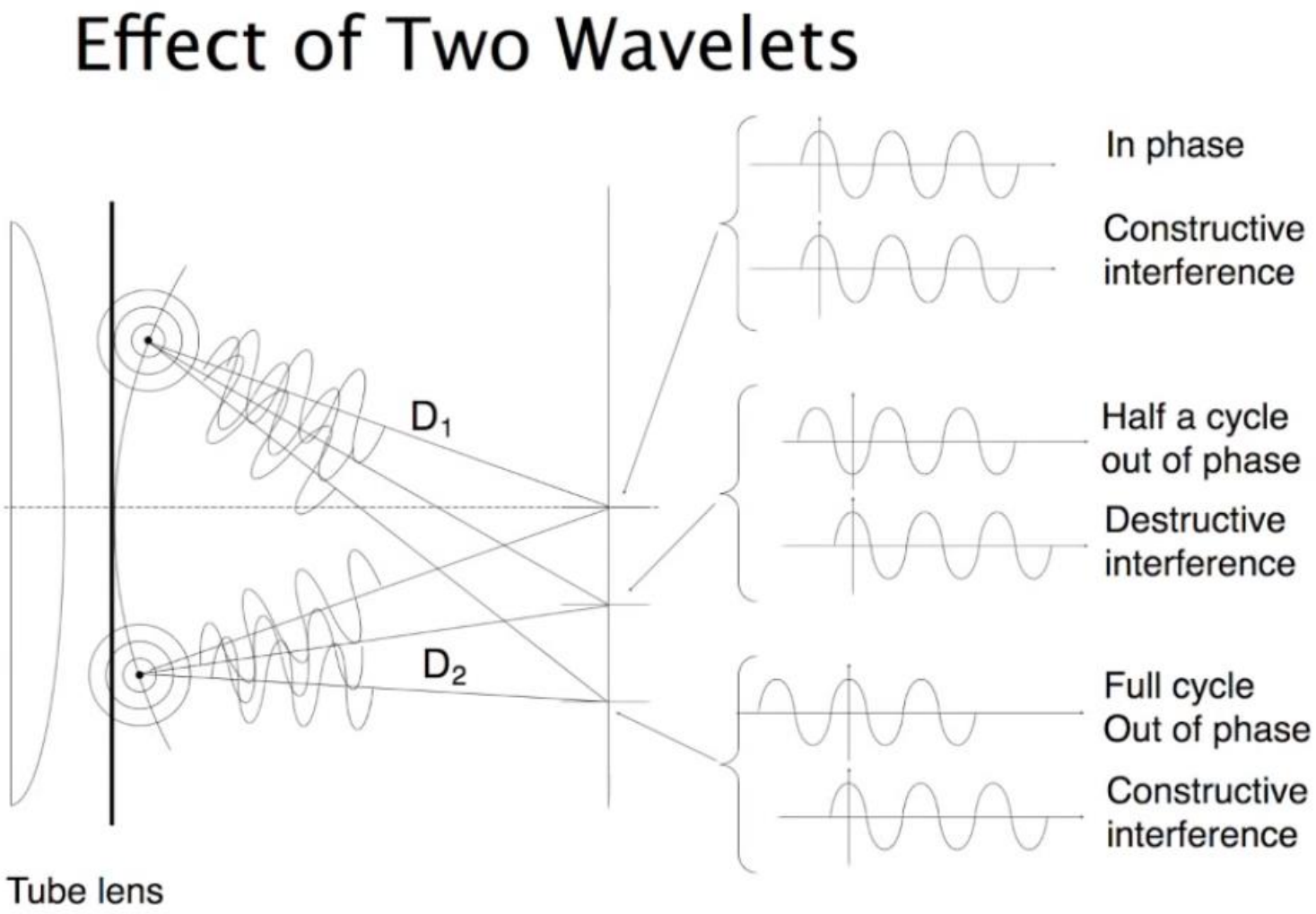
Huygens' Principle:
Each wavefront is the envelope of the wavelets. Each point on a wavefront acts as an independent source to generate wavelets for the next wavefront. AB and CD are two wavefronts.

Huygens wavelet approach

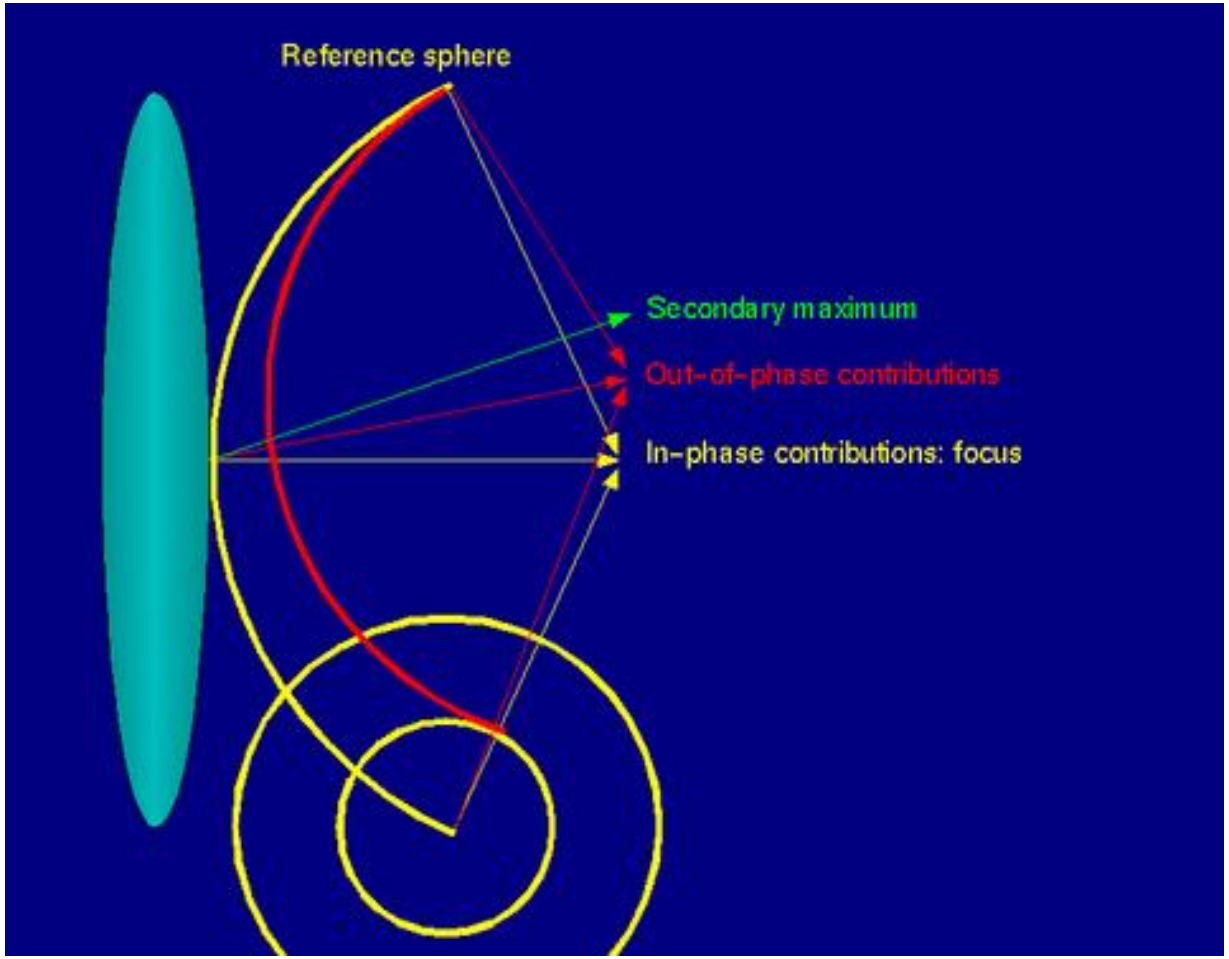


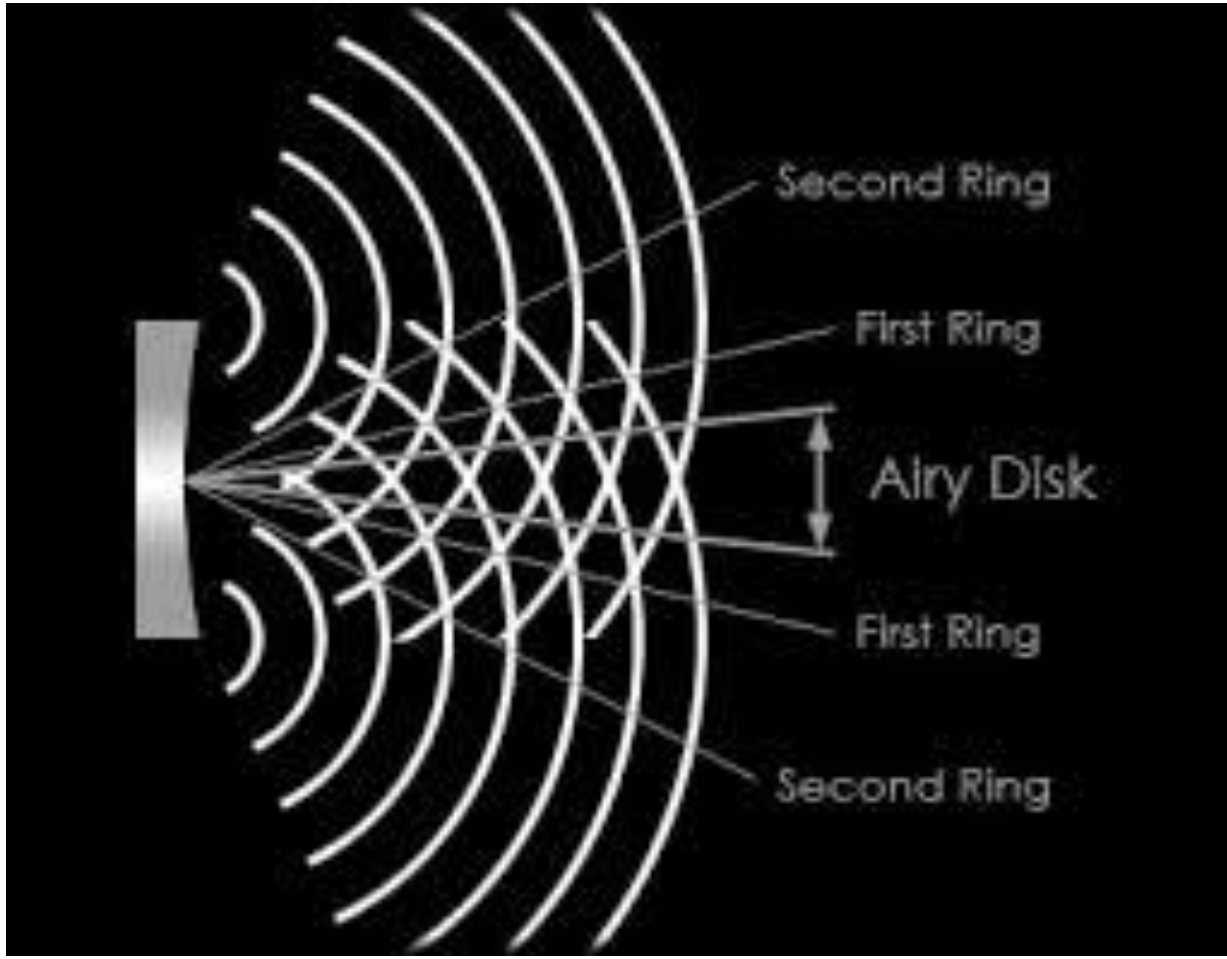
- Each of the infinite points in the emerging wavefront acts like a point source
- Each point emits a wavelet
- All wavelets from the same wave front are mutually coherents
- That is, they oscillate synchrony
- Therefore, they interfere wich each other in a predictable way

Interference of wavelets

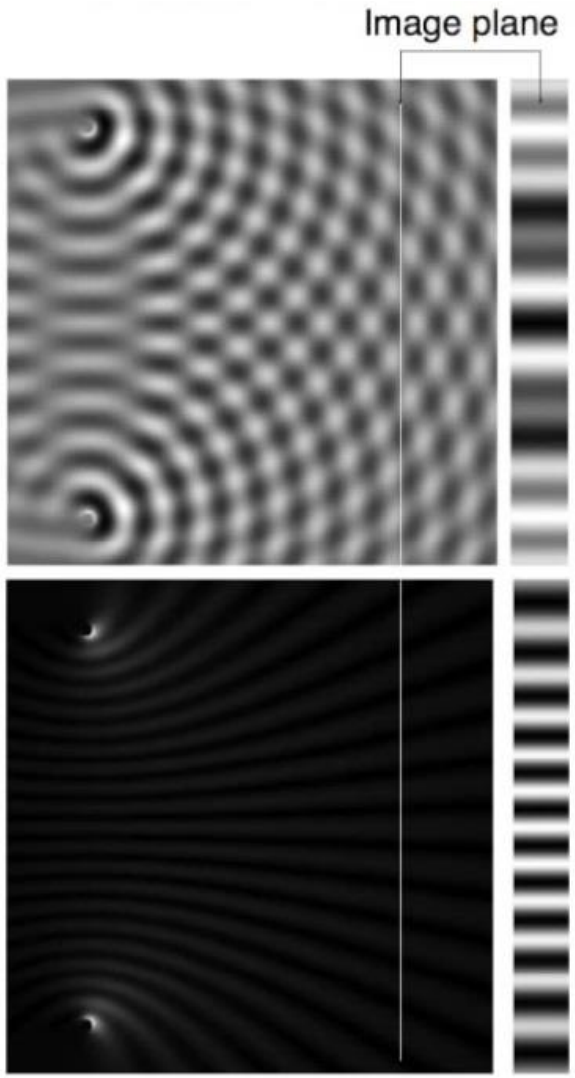


Effect of two wavelets



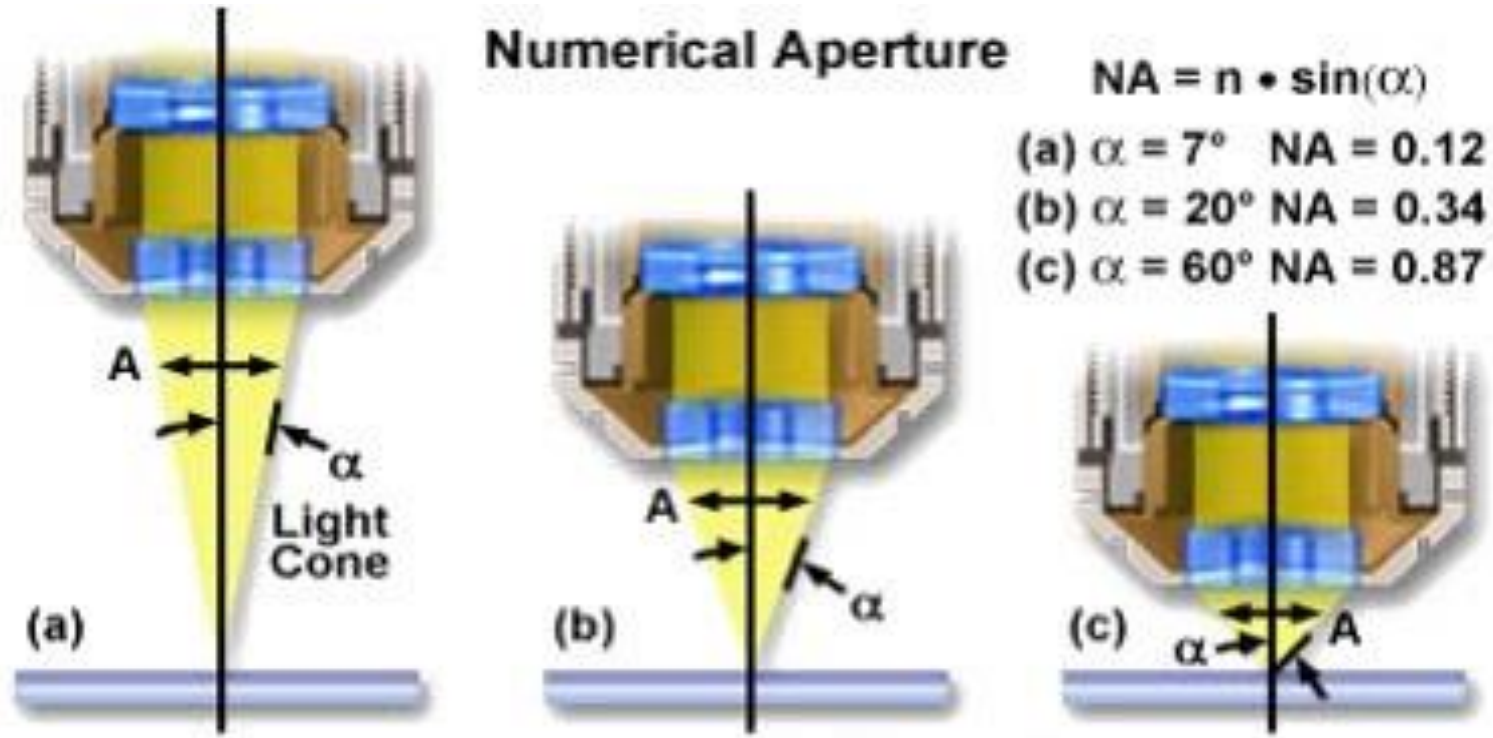


Interference from two wavelets



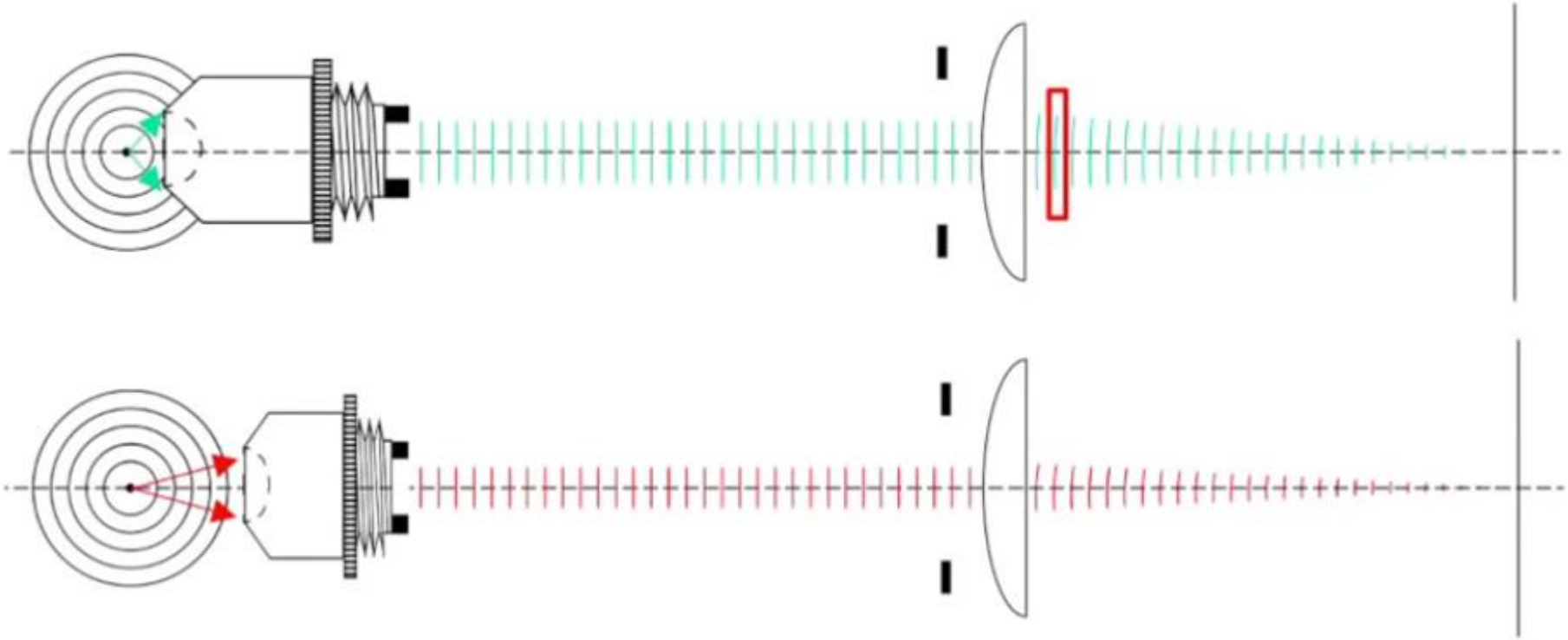
- At an instant in time (top)
  - Mesh of dark and bright lines
- Detectors (including the eye) collect intensity
- Intensity is the addition of a full cycle squared
- The detected interference pattern has dark and bright lines
- Dark areas = destructive interference
- Bright areas = constructive interference
- Nothing to signify where the image plane is

Numerical aperture and resolution



Numerical aperture and Resolution

High Numerical Aperture: Larger plane and spherical waves

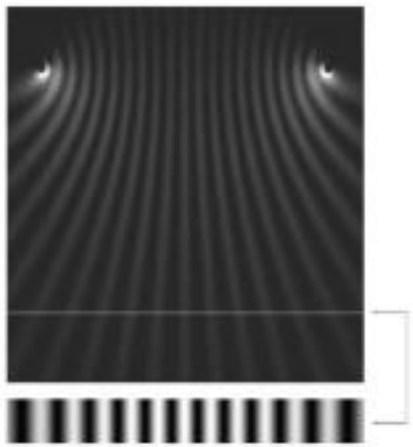


Low Numerical Aperture: Smaller plane and spherical waves

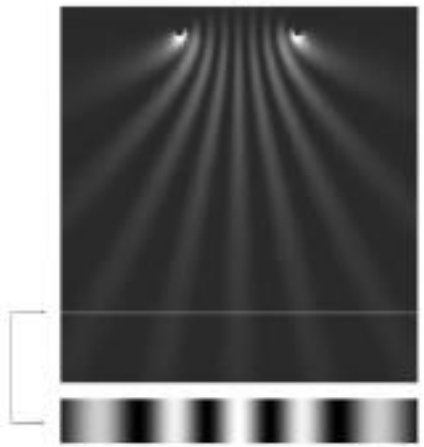
Numerical aperture and Resolution

The finest fringes determines the finest detail that can be found in an image. Their period is related to the objective's numerical aperture

Extreme wavelets widely separated



Extreme wavelets closer together



High NA

Gives a short fringe period

Gives narrow fringes

Low NA

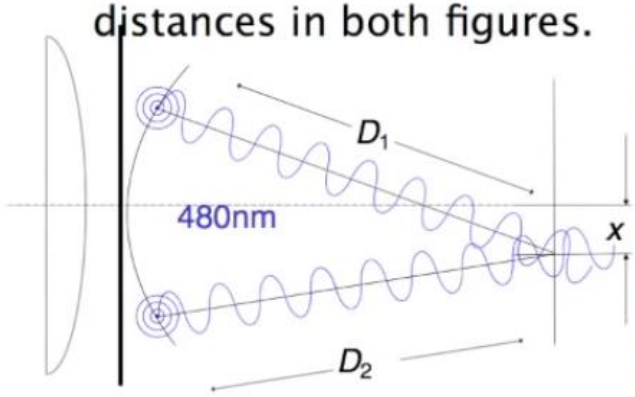
Long fringe period

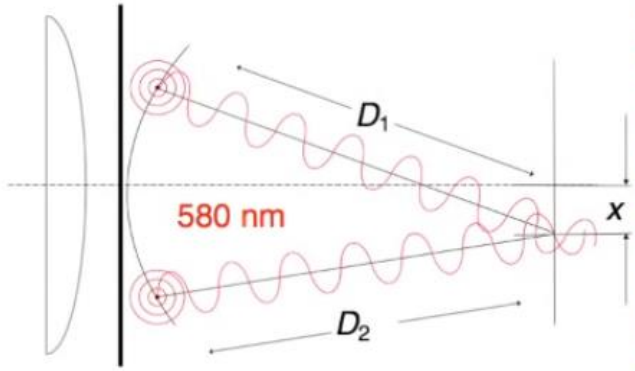
Wide fringes

Wavelength and Resolution

The finest fringes sets the finest details in an image. Their period and width is also related to wavelength

D_1 , D_2 and x are the same distances in both figures.





Shorter wavelength (blue)

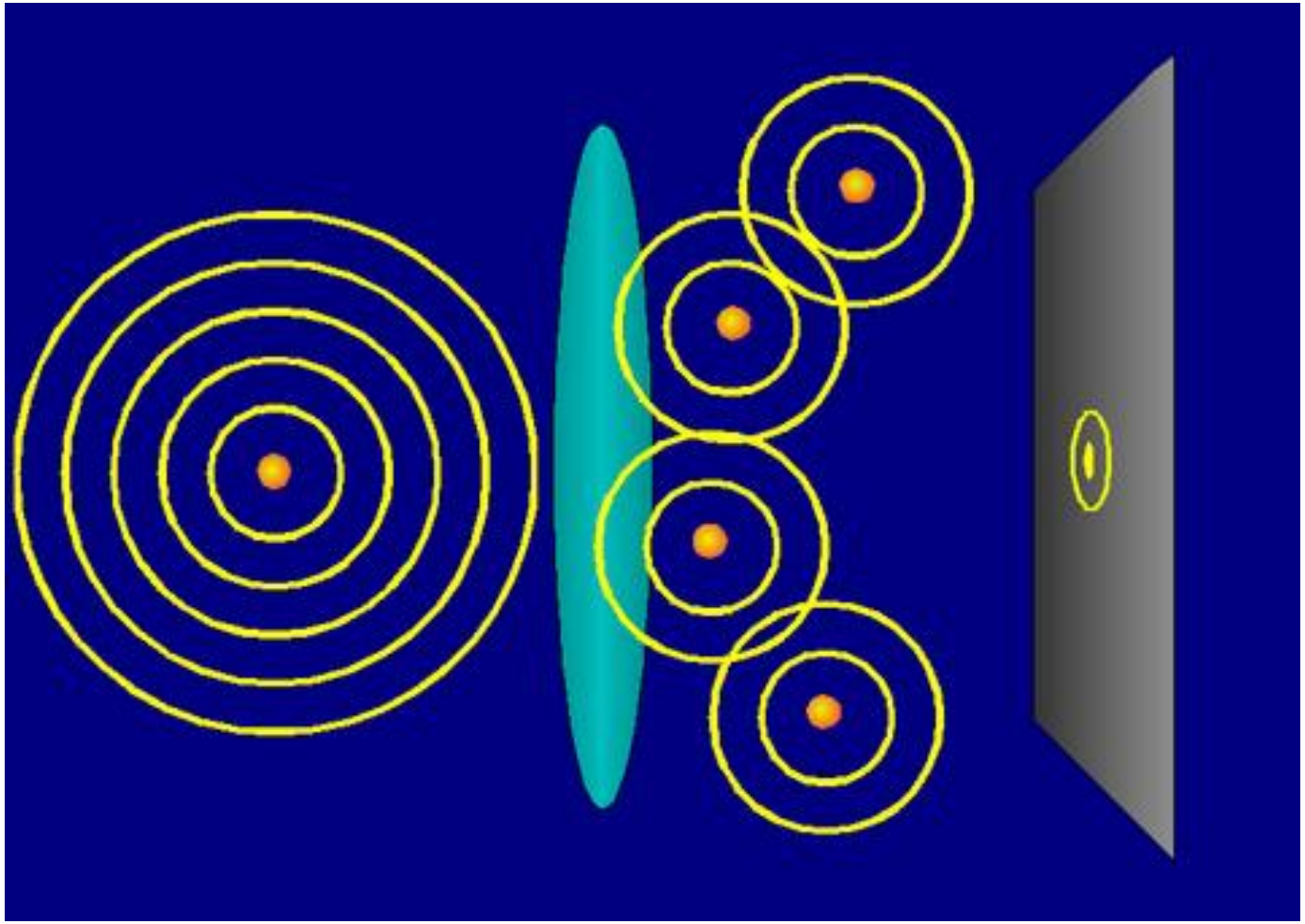
More cycles per μm of D_1 or D_2

So there is a larger phase difference for the same absolute distance

Gives rise to a shorter fringe period and narrower fringes

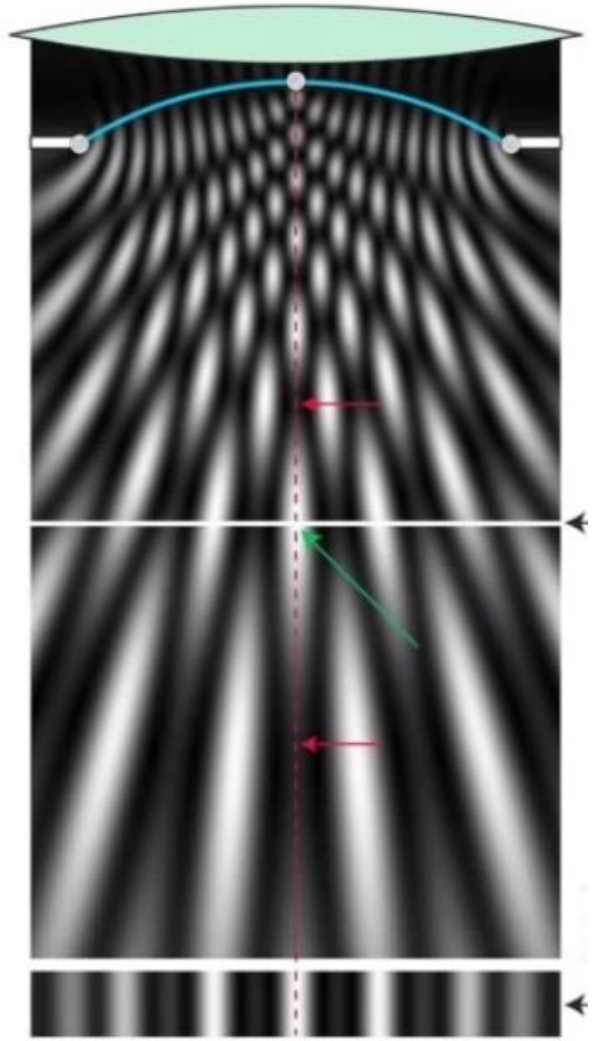
Converse for Longer wavelength (red)

Effect of multiple wavelets



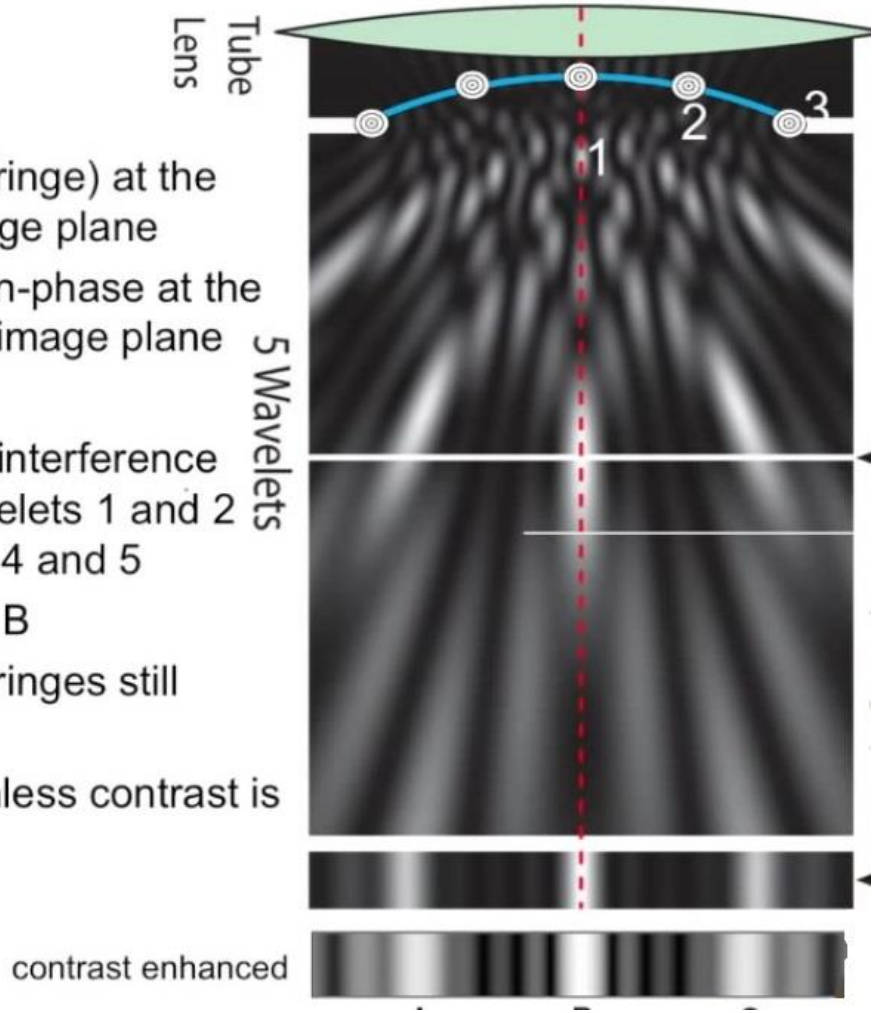
Effect of three wavelets

- Two wavelets at the rim of the lens
- One wavelet at the center of the lens
- Three wavelets are in-phase at the center of the image plane
- Recorded intensity
  - "Bar" pattern turns into "islands" of light
  - At the image plane, center fringes are brighter
    - All wavelets in phase



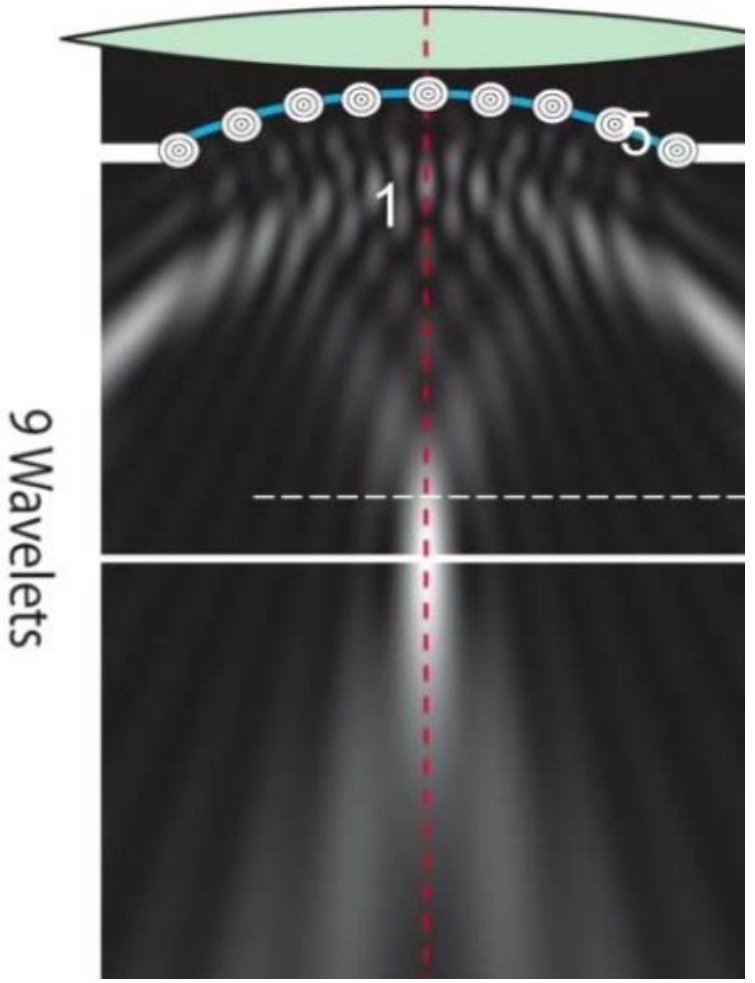
Effect of five wavelets

- Brightest band (fringe) at the center of the image plane
  - All wavelets in-phase at the center of the image plane
- Fringes A and C
  - Constructive interference between wavelets 1 and 2 and between 4 and 5
  - Dimmer than B
- Other (dimmer) fringes still present
  - Not visible unless contrast is enhanced



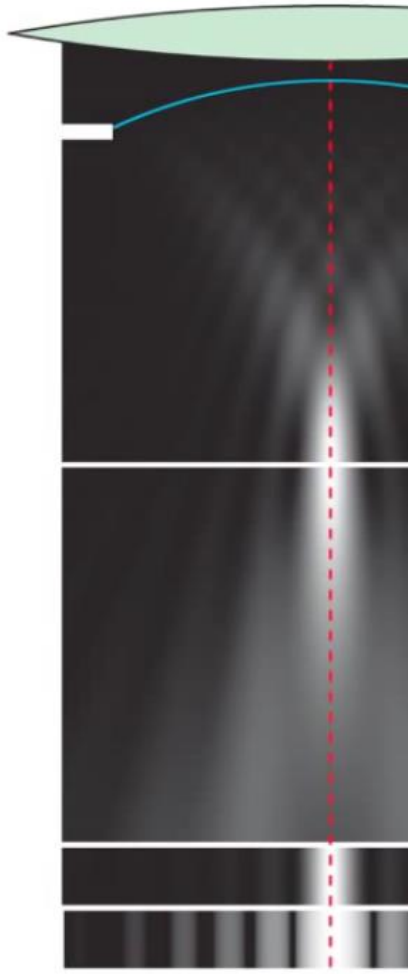
Effect of nine wavelets

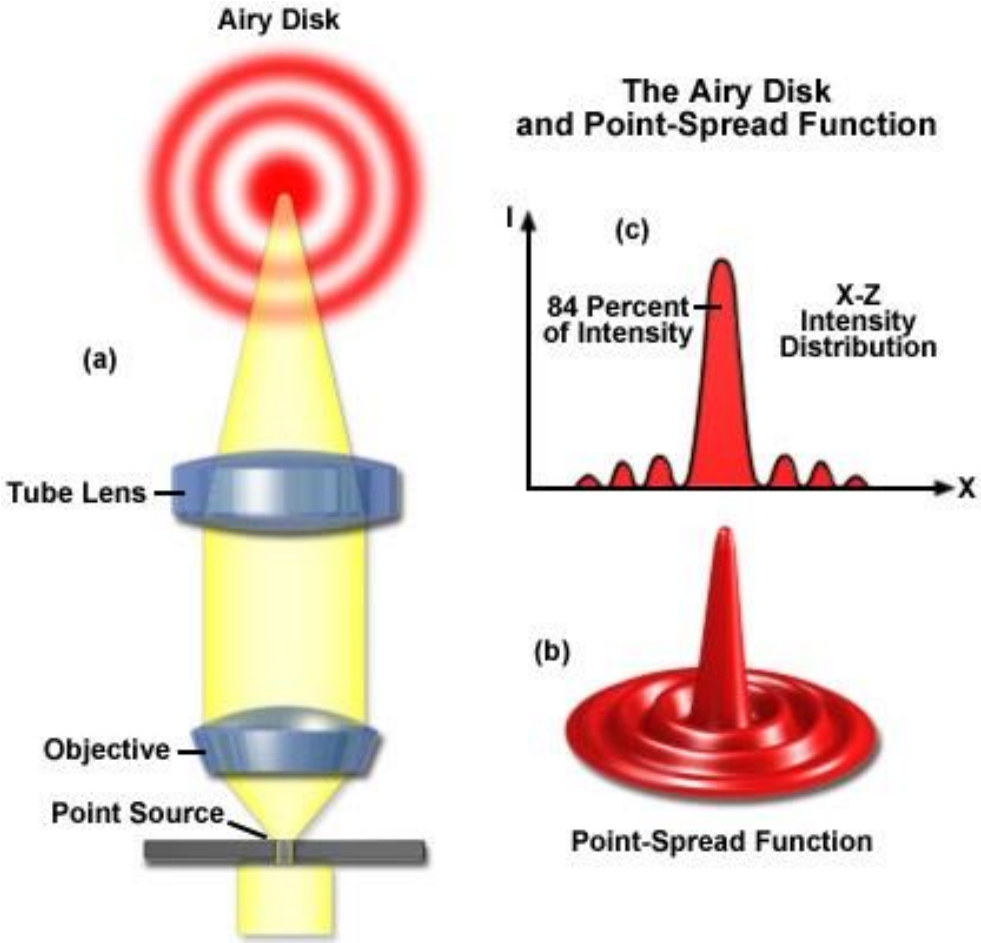
- Light concentrated at the center
  - All wavelets in-phase at the center of the image plane
- Single main peak visible
- Other (dimmer) fringes
  - Not visible unless contrast is enhanced
  - Fringes due to constructive interference between pairs of adjacent wavelets are dim
- Most light is within a cone



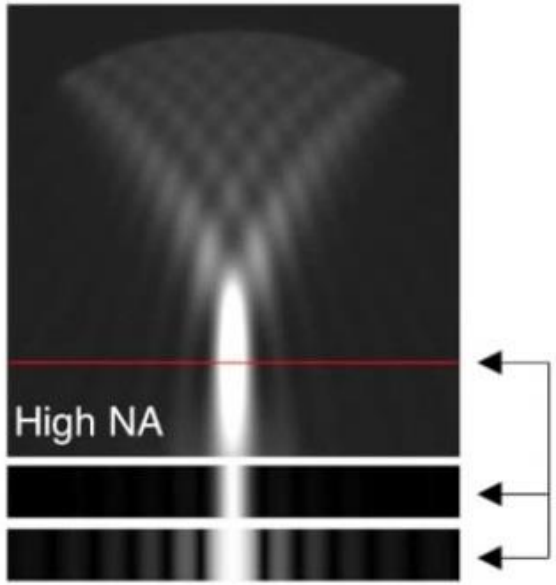
Effect of all the wavelets: the PSF

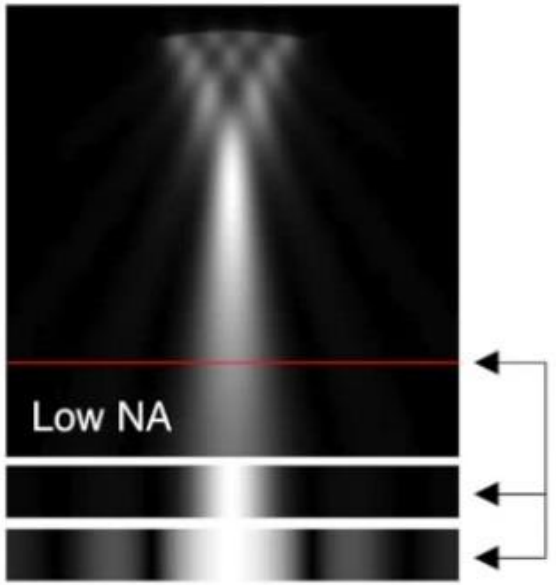
- All wavelets in phase at the center of the image plane
  - Light is concentrated in the middle of the image plane
  - Main peak is brightest by far
- Other fringes (or "side lobes")
  - Dimmer than main peak
  - Intensity progressively decreases away from the center
- Most of the light is within a cone (the hourglass shape mentioned earlier)
- Width of main peak set by the extreme pair of wavelets at the rim of the lens





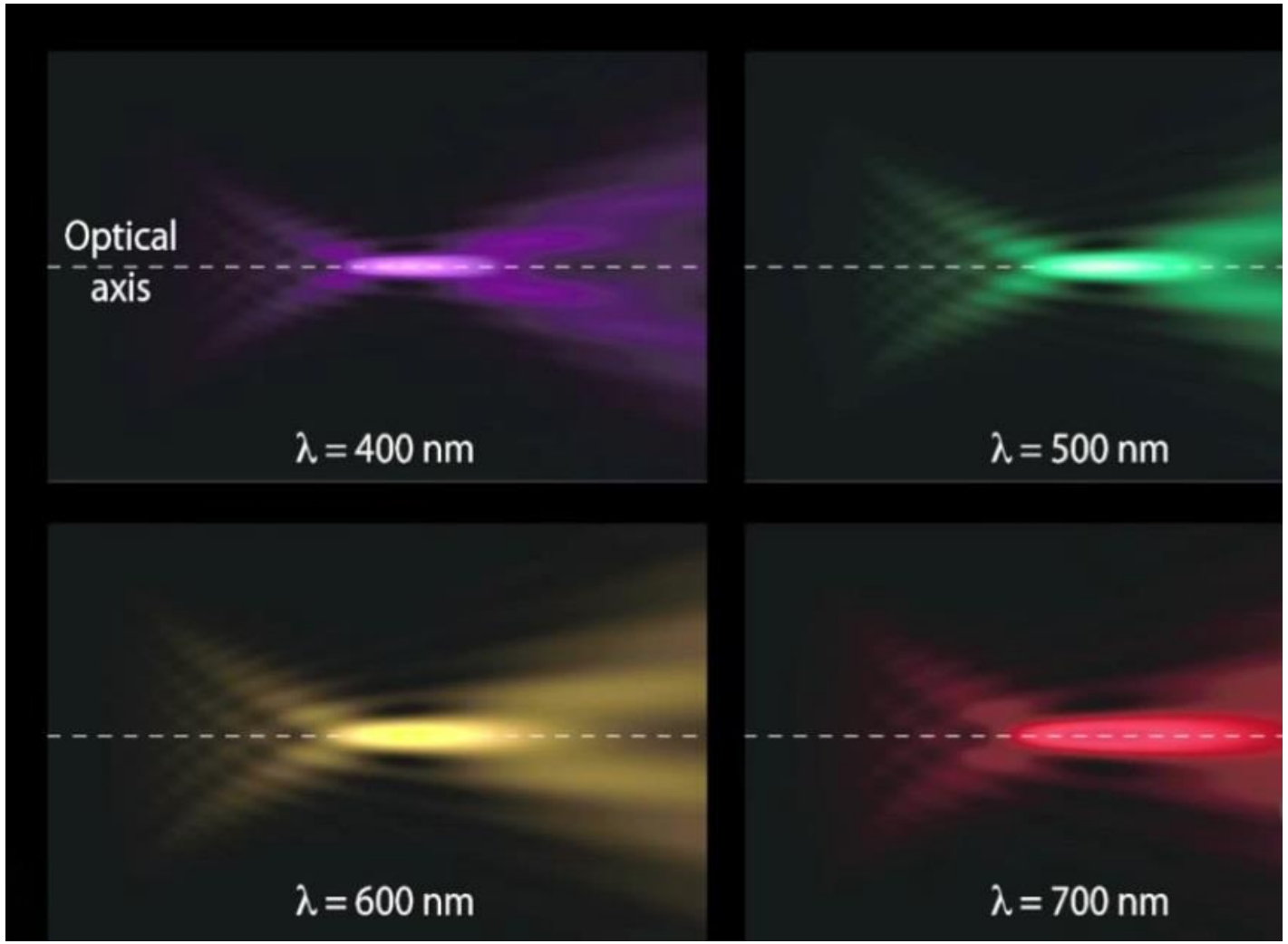
Effect of the numerical aperture on fringes



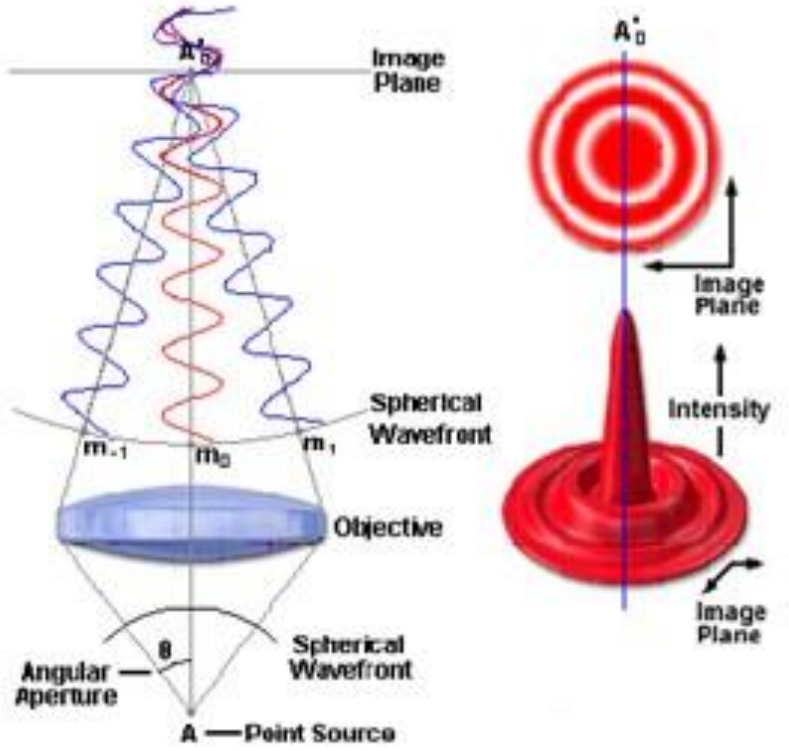


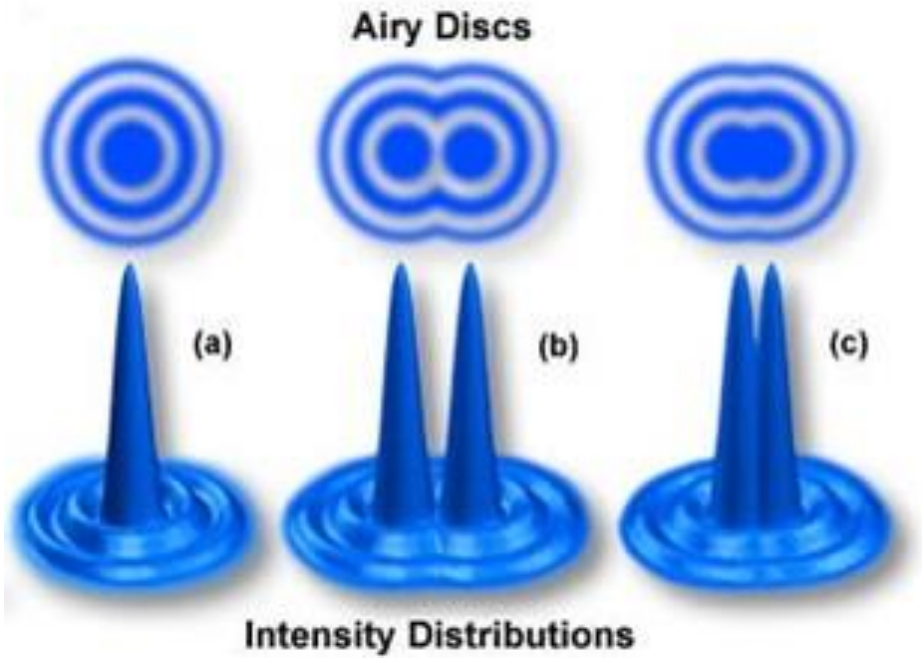
- High NA
  - Wider separation between wavelets possible
  - As a result small central peak
- Low NA
  - Only narrow separation between wavelets
  - Broad central peak

PSF is smaller for shorter wavelength light

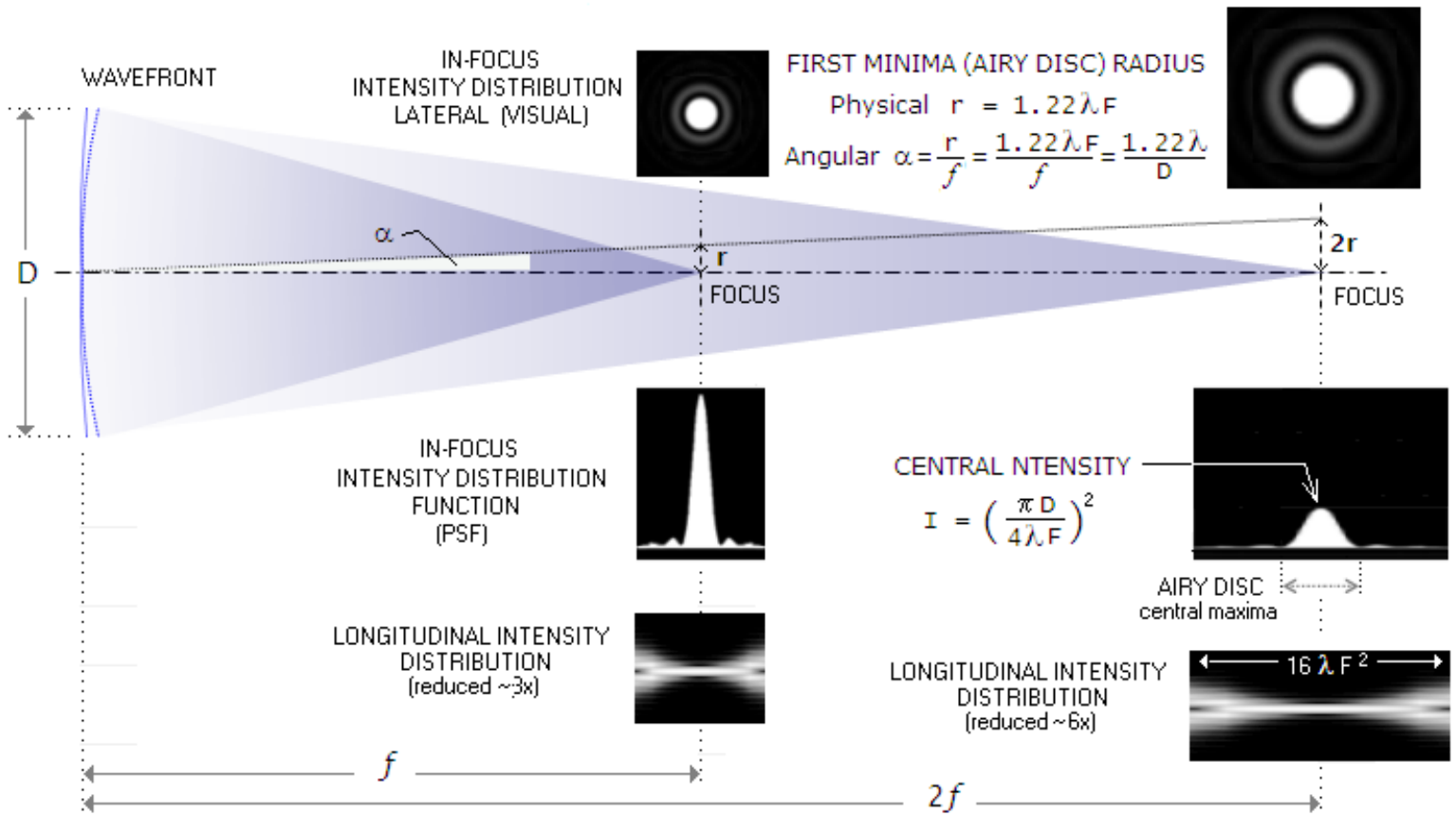


Effect of multiple wavelets: Airy discs





Airy discs parameters



$$\Delta x_{\text{Airy}} \sim \frac{b\lambda}{D}, \lambda = \frac{2\pi}{\omega}.$$

PSF light distribution near the image plane (xy and xz)

Looking down on the in-focus plane (XY)

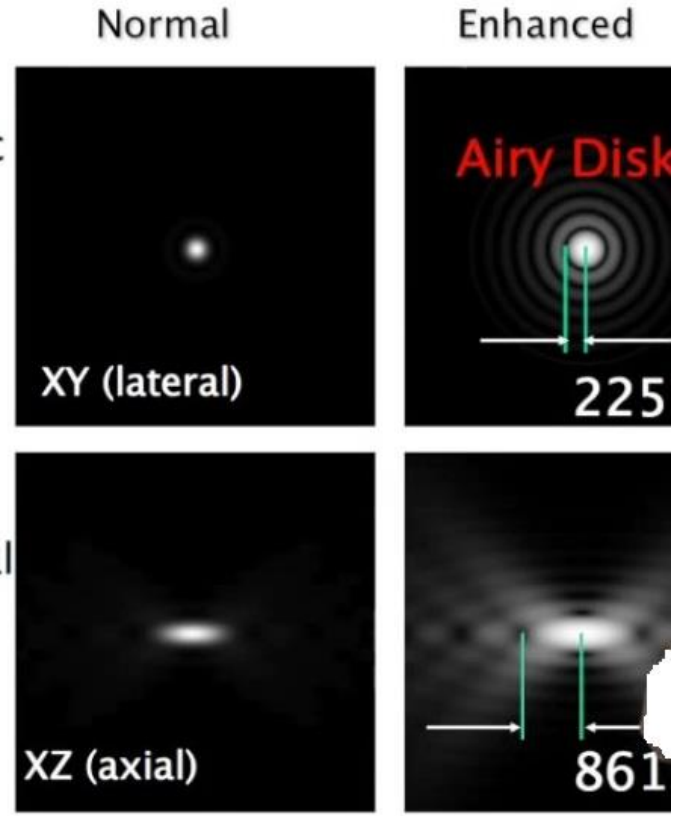
- PSF has center "Airy" disk
- PSF has a series of concentric rings
- Larger rings have progressively lower intensity
  - 1st dark ring radius
    - $0.61\lambda/NA$

On an xz section

- More spread along the optical axis (z axis)
  - First dark island at
    - $2n\lambda/NA^2$
- Most light within two cones

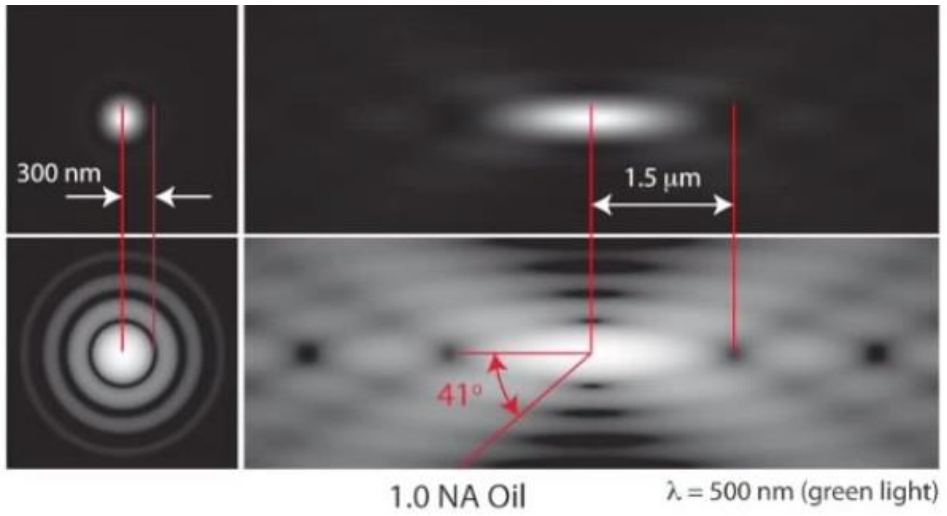
Sizes shown

- 1.4 NA, 480 nm wavelength

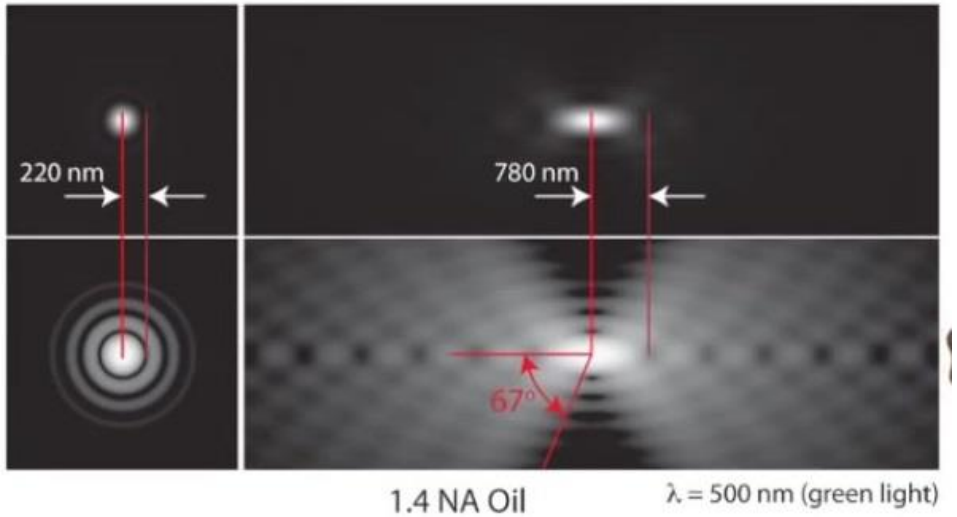


Numerical aperture´s influence on PSF: bigger effect on axial than lateral spread

NA 1.0
Lateral
Slightly larger spot
Axial
Much longer spot
Narrower cone



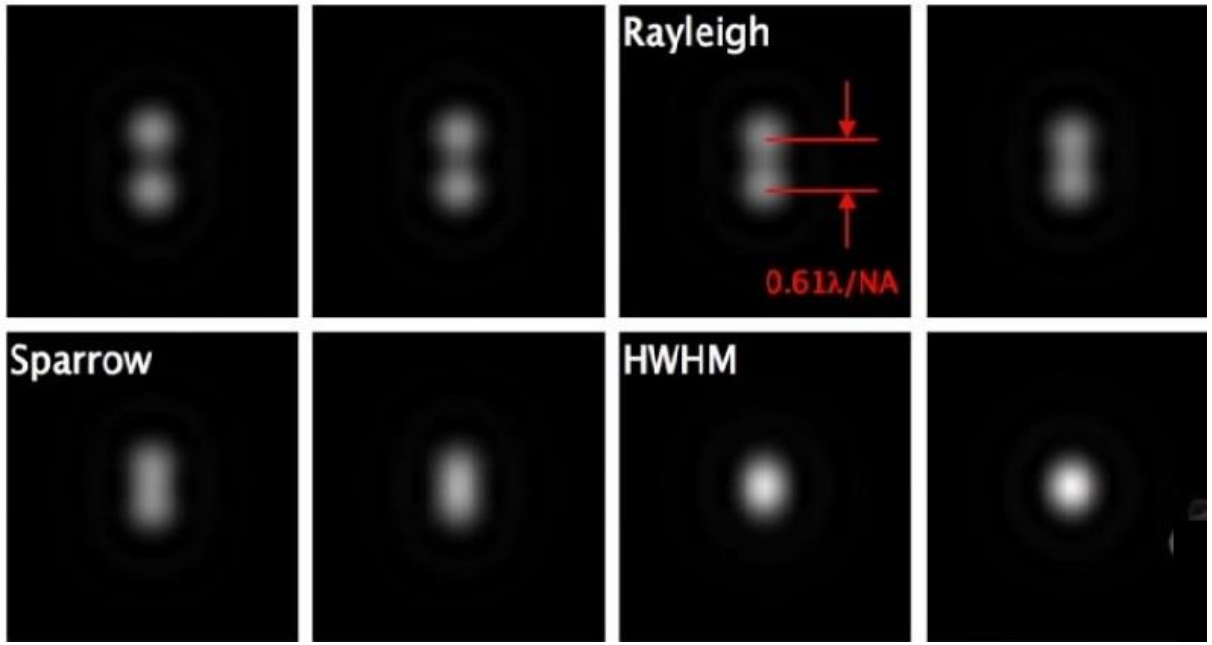
NA 1.4
Lateral
Compact spot
Axial
Long spot
Wide cone



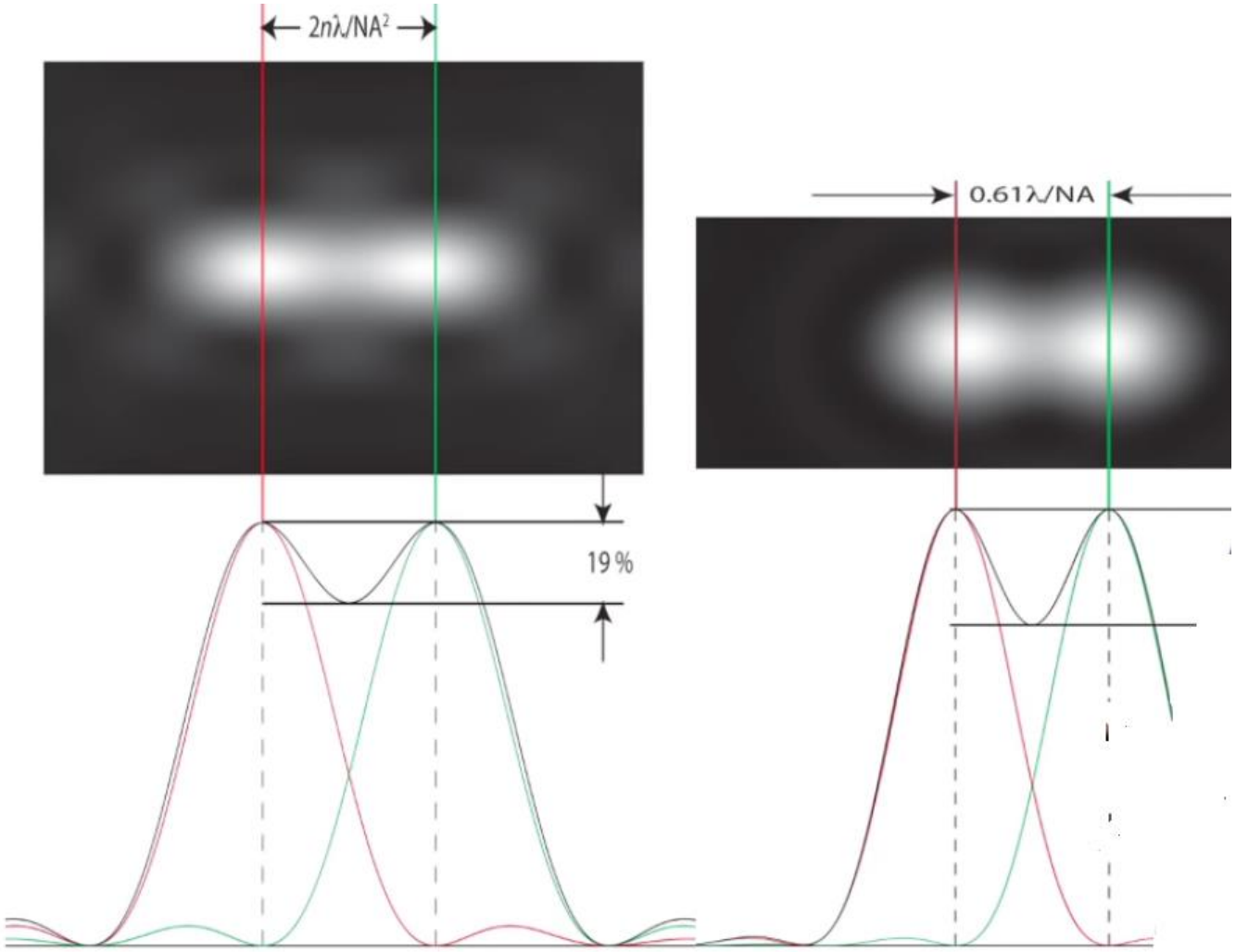
Resolution in the light microscope

How close can two equal point sources be and still be seen as two points?

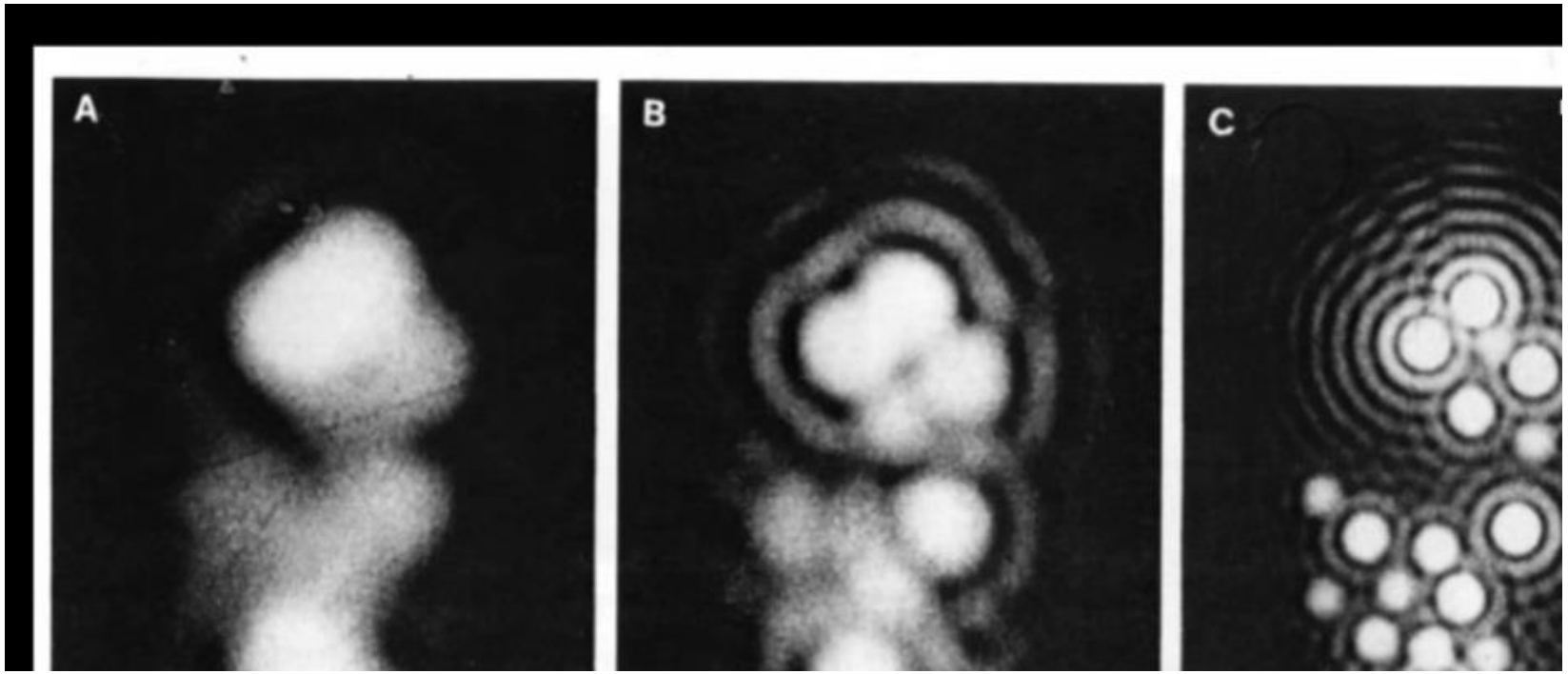
No generally-accepted criterion but most microscopists use the Rayleigh criterion



Rayleigh criterion (lateral and depht)



The higher the NA the smaller the Rayleigh criterion and the better the resolution

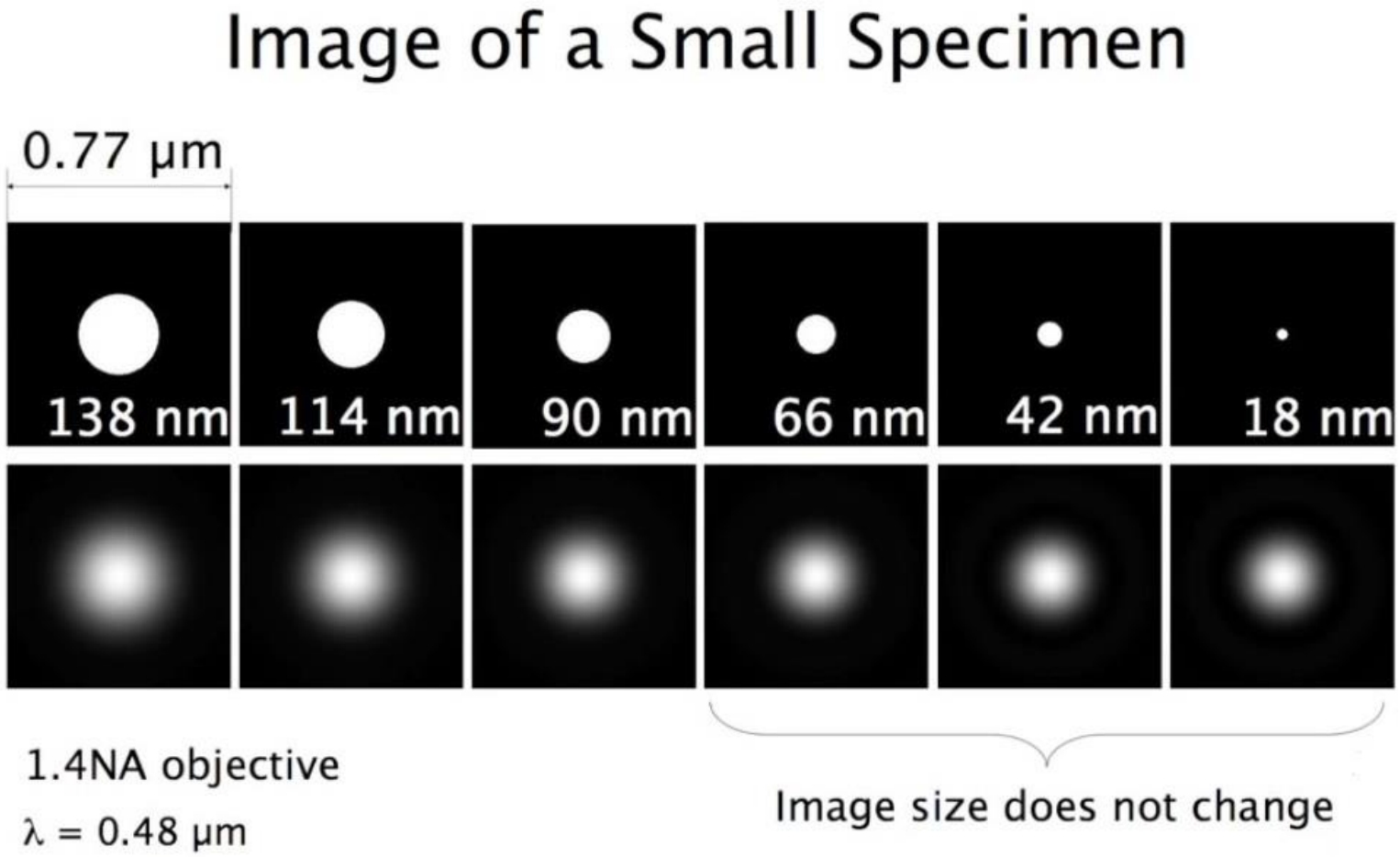


Low NA

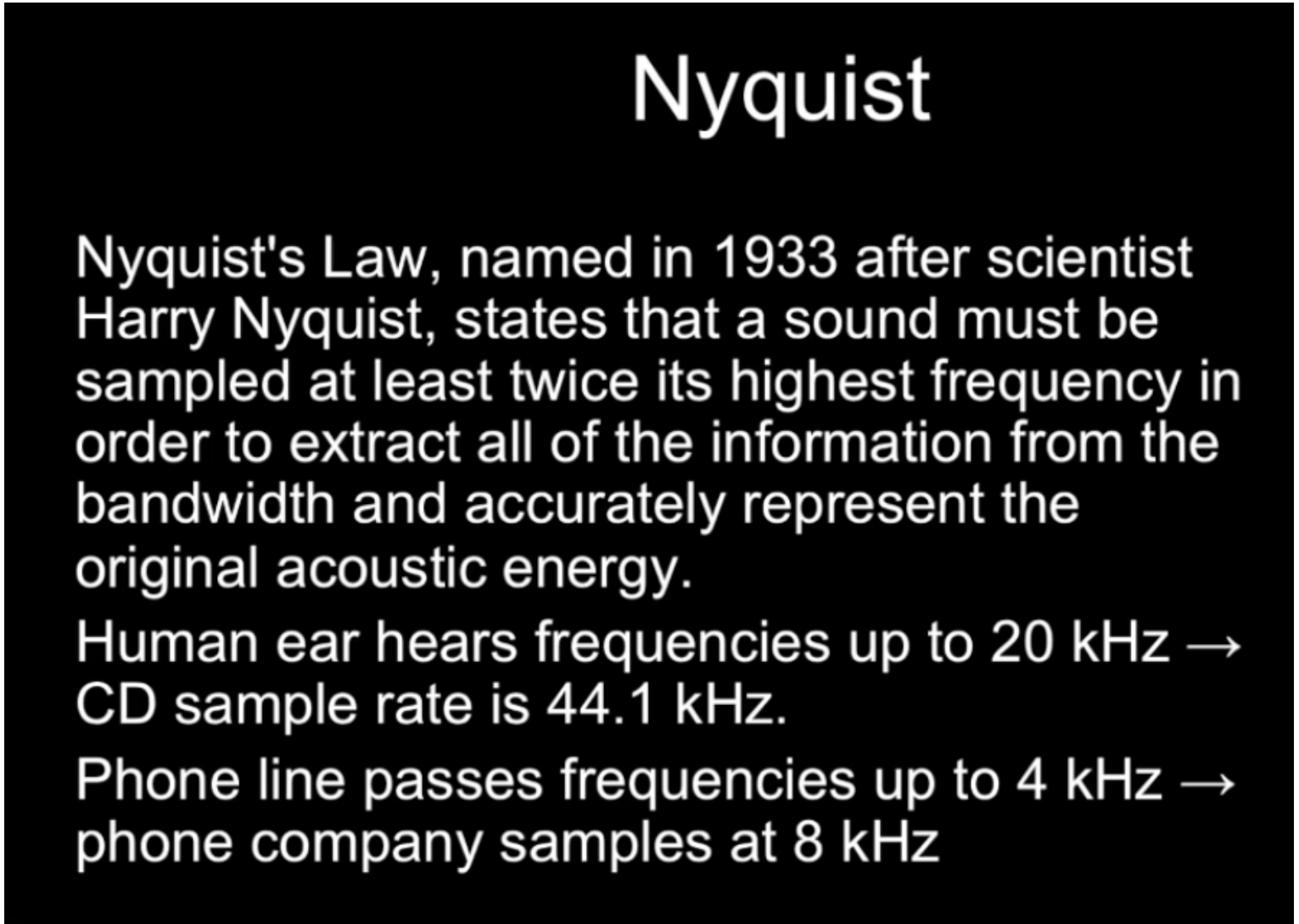
Medium NA

High NA

Resolution limit



Resolution: the sampling theorem



Resolution: the sampling theorem

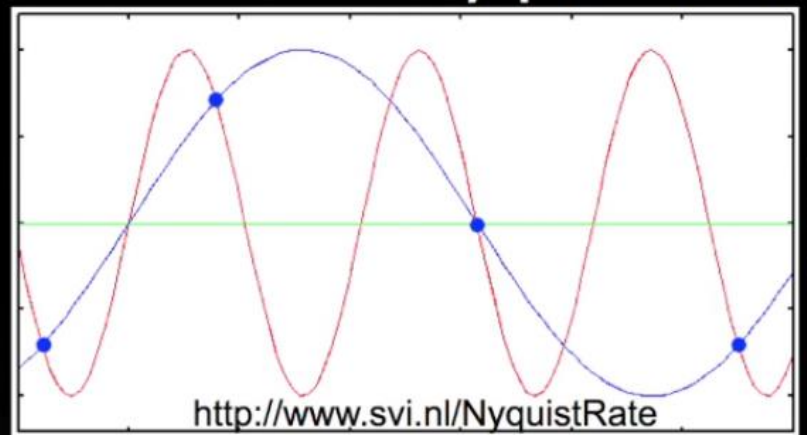
The sampling theorem:

A continuous function can be completely represented by a set of equally spaced samples, if the samples occur at more than twice the frequency of the highest frequency component of the function

To capture a function with maximum frequency F, sample it at frequency

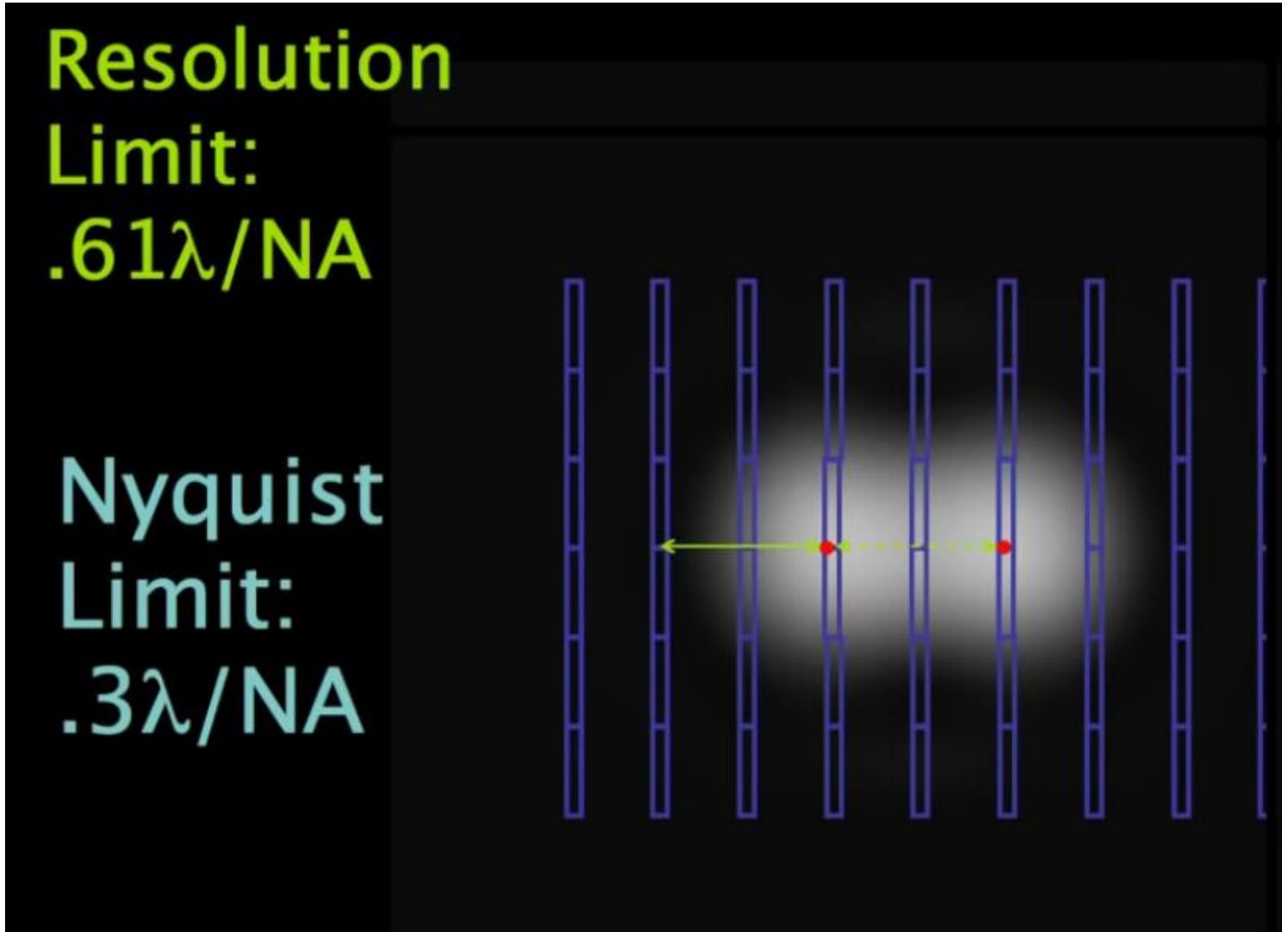
$N = 2F$.

N is called the Nyquist limit



Blue dots sample once per wavelength and under-sample the actual frequency (in red)

Resolution limit: the sampling theorem



Example:

- CCD has 12x12 μm^2 pixels (square)
- Using 60 X 1.4 NA 480 nm light, resolution limit (.61λ/NA) = .220 μm
- 60X magnification yields diffraction limited spots (.22 X 60) = 13.2 μm which is the distance of resolution limit on CCD face plate
- So what do you do?
- Mag changer to zoom by ~2X (or 100x objective)
- Resolution limit is now 26.4 μms on CCD which is > twice the sampling resolution of 12 μms

But if you need more resolution, what then?

- Shorter λ
- Higher index immersion liquid
- Confocal improve resolution sqrt 2
- If you can study single spots (i.e.., single molecules or particles in a sparse field)– can find their center at arbitrarily high resolution
- Multicolor resolution not limited by diffraction
- SUPER RESOLUTION (Optical Nanoscopy)

Thanks

I would like to thank Professor Jeff Lichtman (Harvard University) (iBiology.org microscopy course and educator resources) for his useful figures, lectures and remarks which we used for the preparation of the lecture