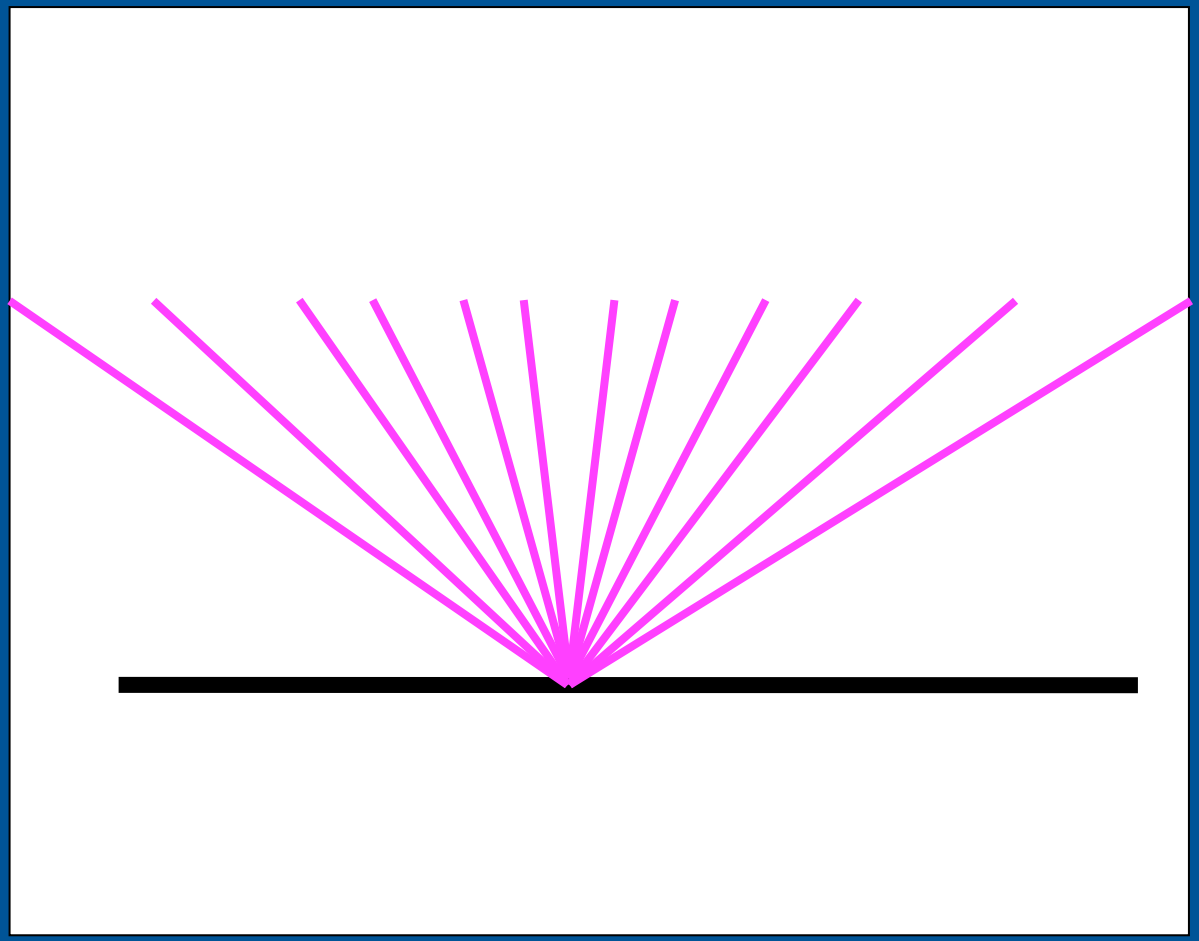
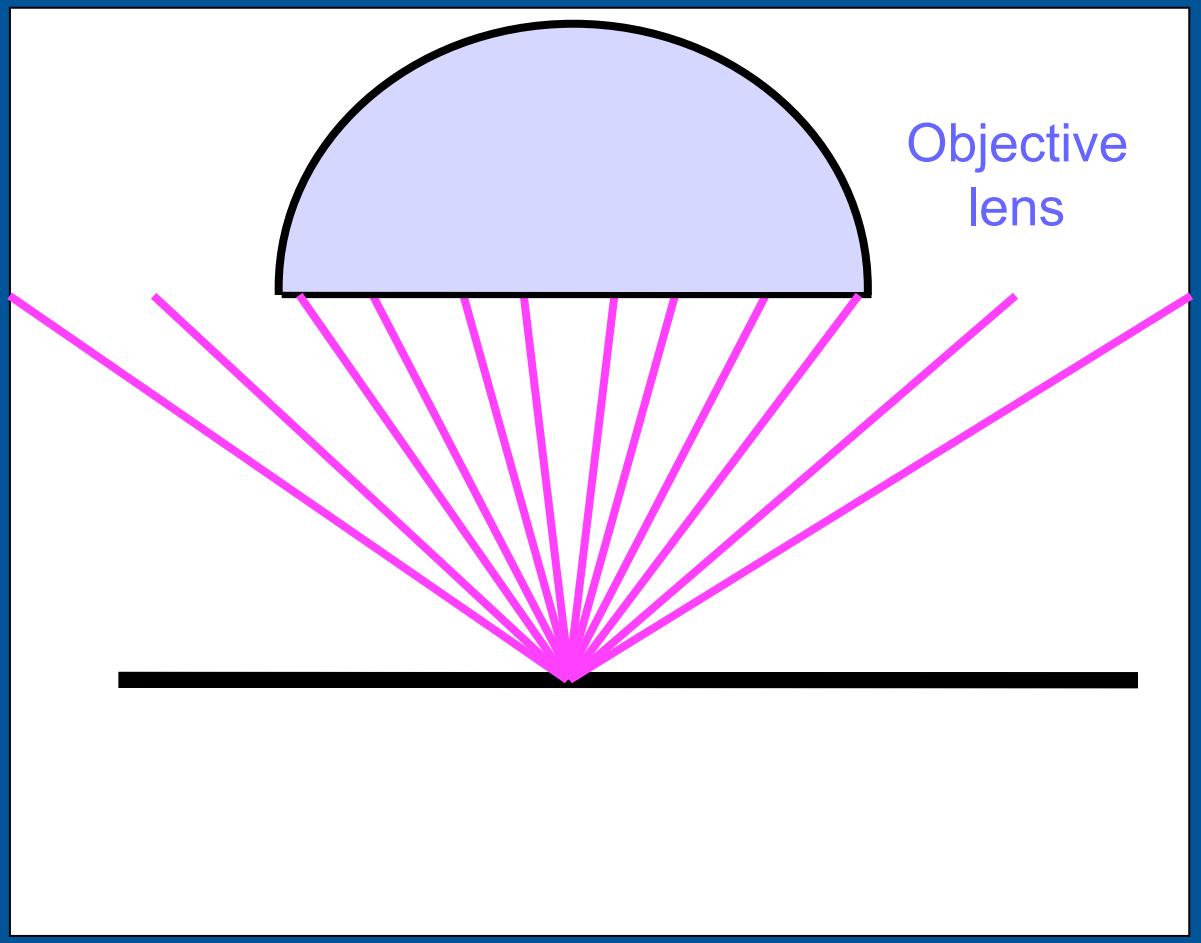
The importance of Aperture in the Microscope

Consider that every ray leaving the object carries some information about fine detail in the object



The importance of Aperture in the Microscope

Consider that every ray leaving the object carries some information about fine detail in the object



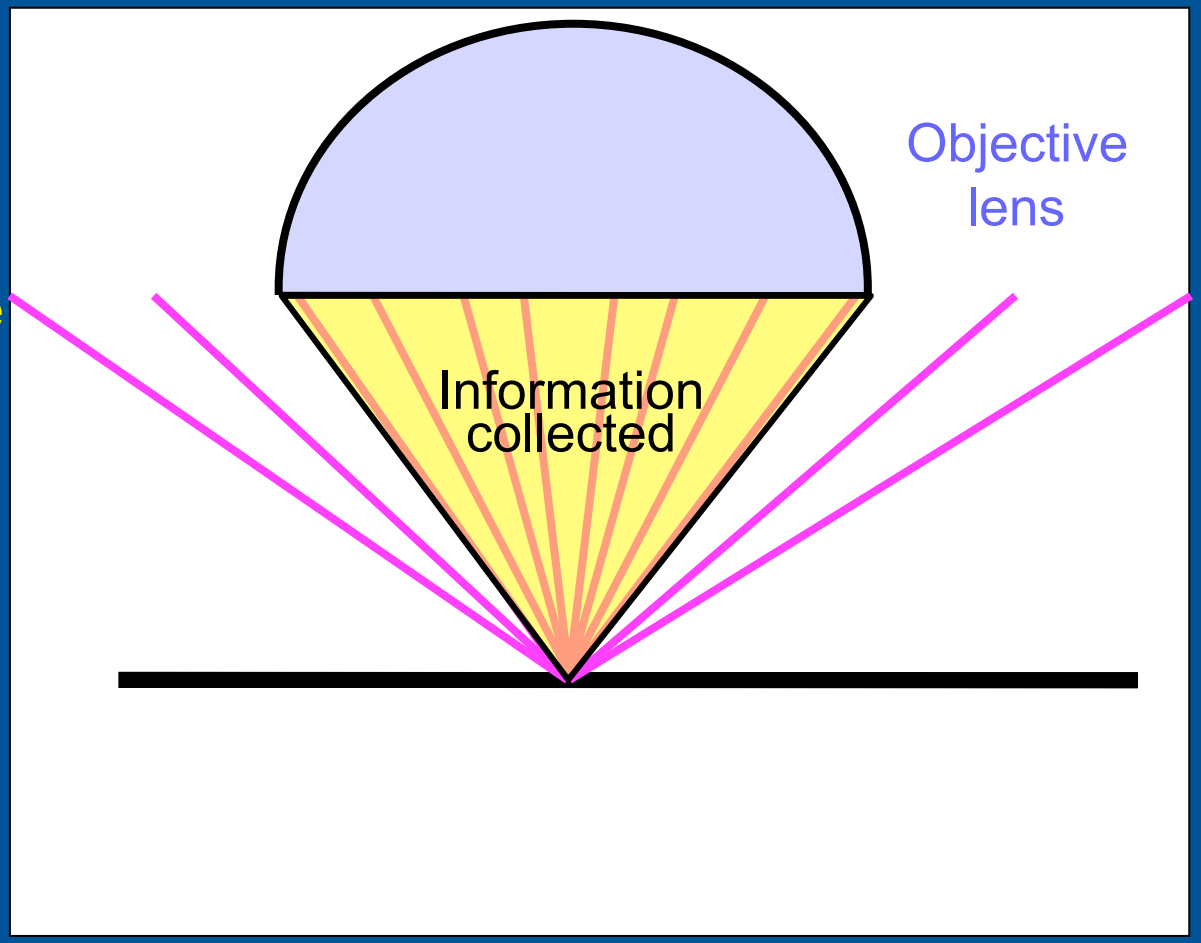
The importance of Aperture in the Microscope

Consider that every ray leaving the object carries some information about fine detail in the object

Some of these rays

– and only *some* information –

will be collected by the objective



The importance of Aperture in the Microscope

Consider that every ray leaving the object carries some information about fine detail in the object

Some of these rays

– and *some* information –

will be collected by the objective

and some rays

– and some information –

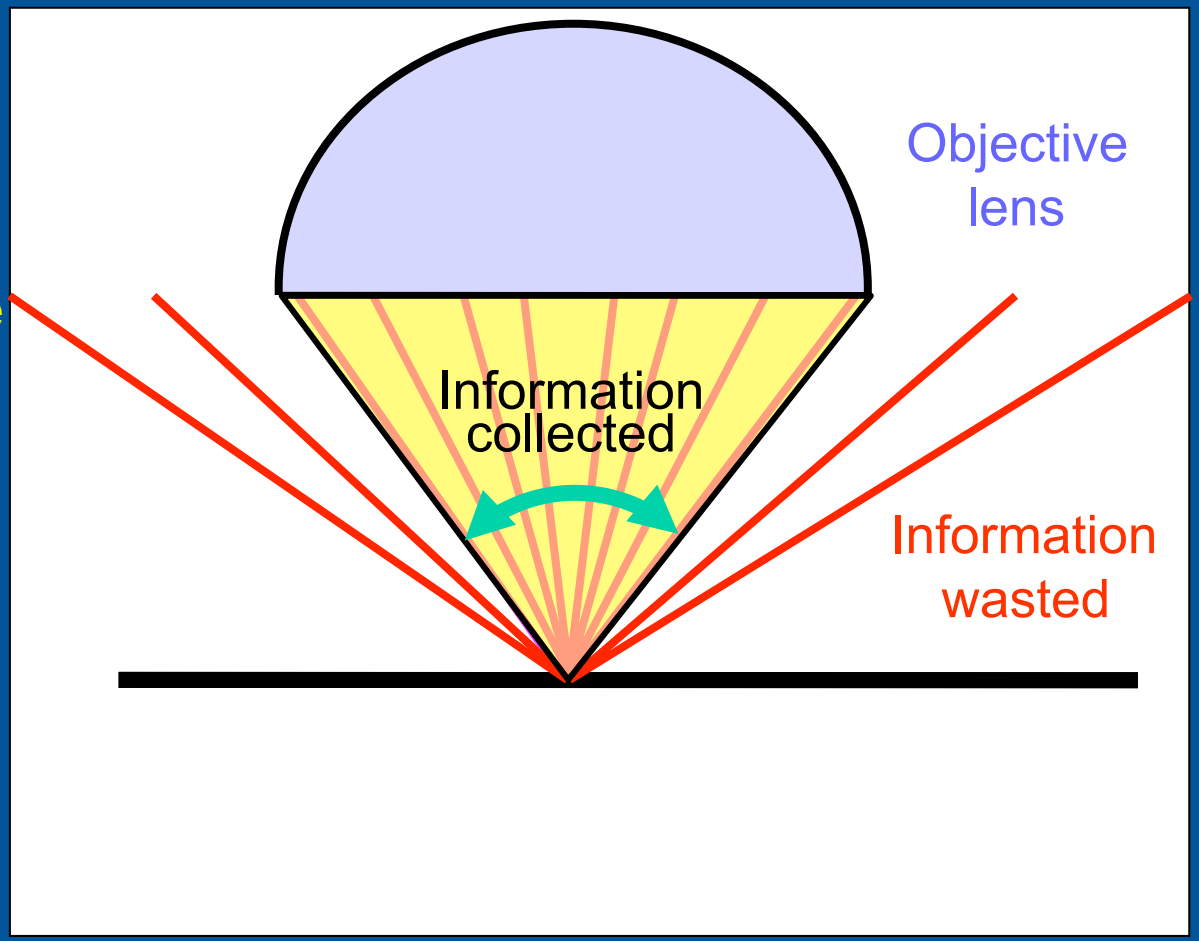
will *NOT* be collected,

and will be wasted

Resolution will therefore depend on the angular *aperture*

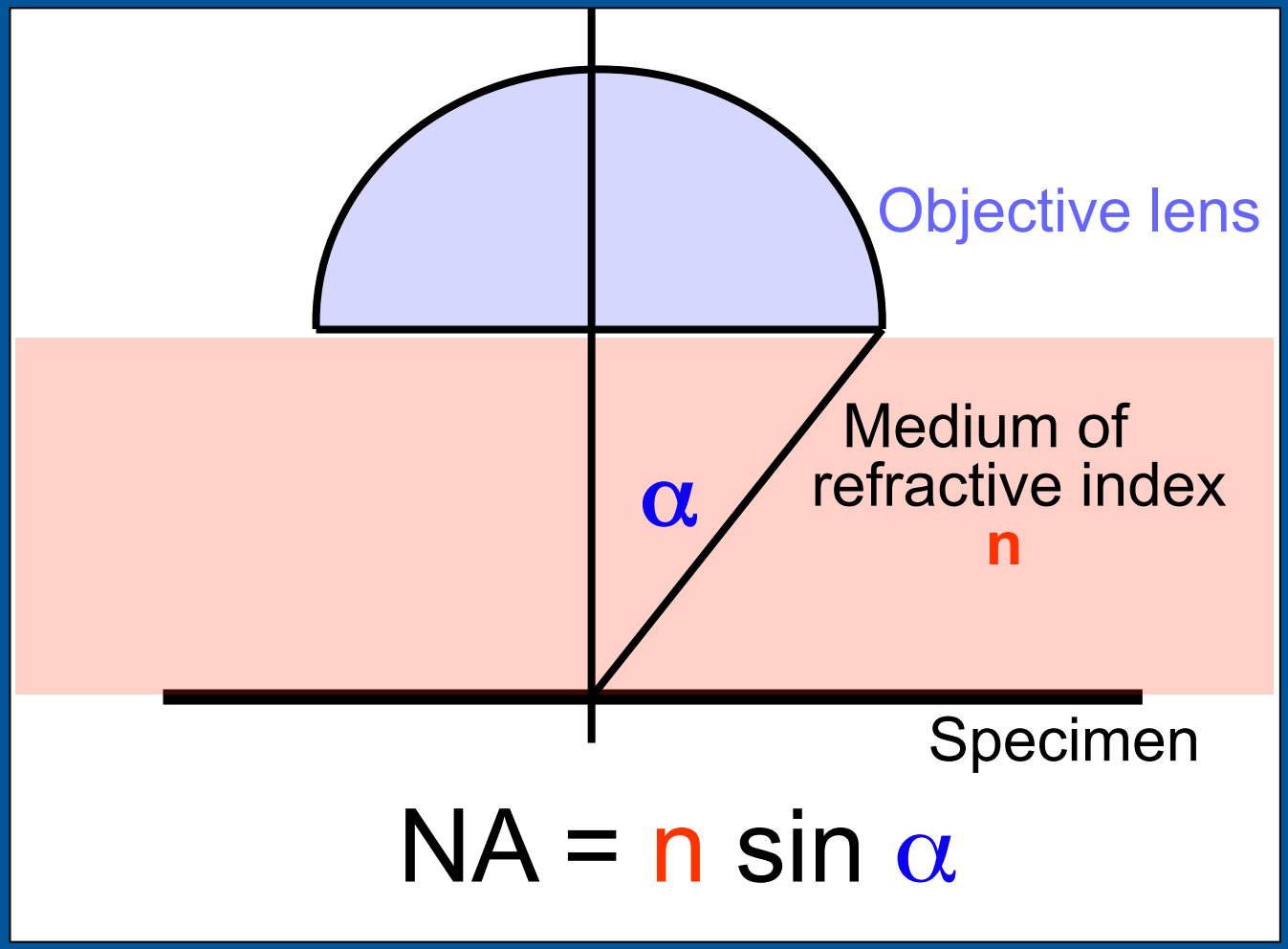
of the objective -

the larger the imaging aperture the higher the resolution



Numerical Aperture

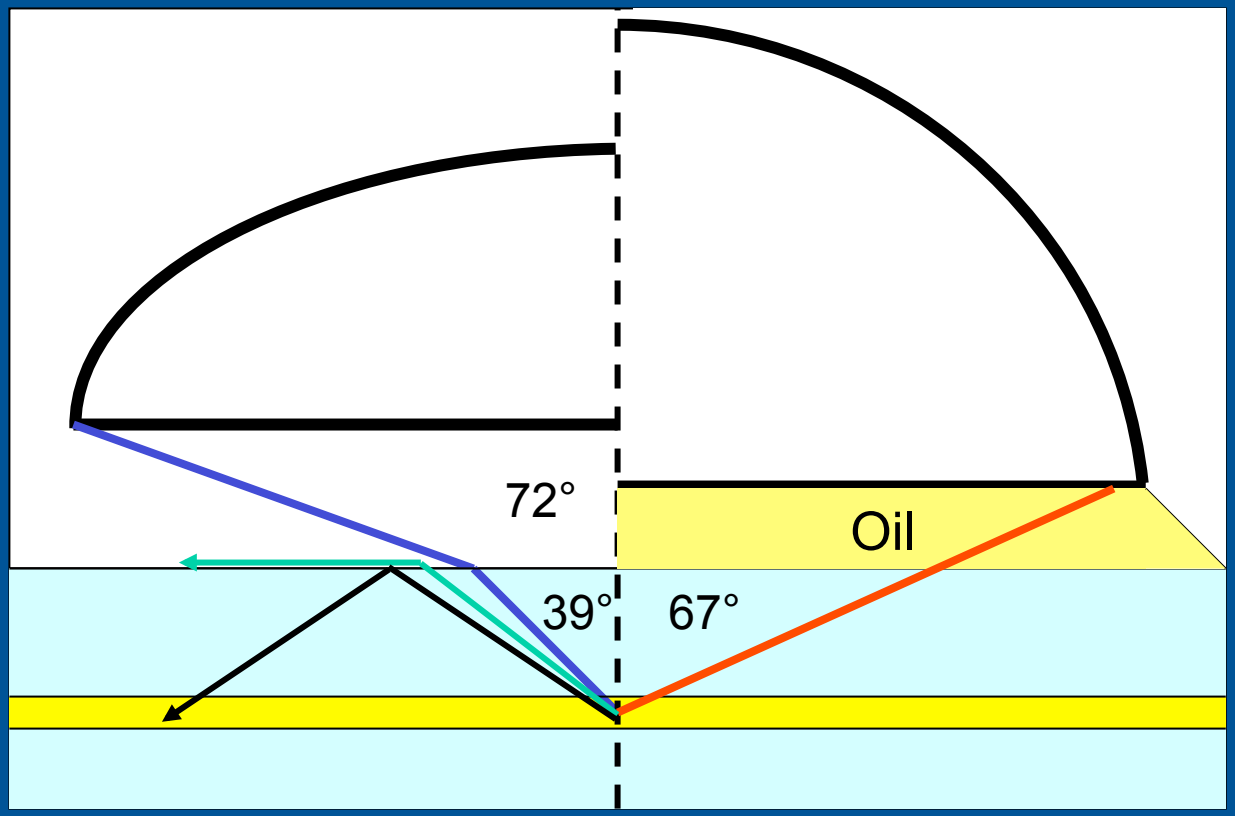
NA

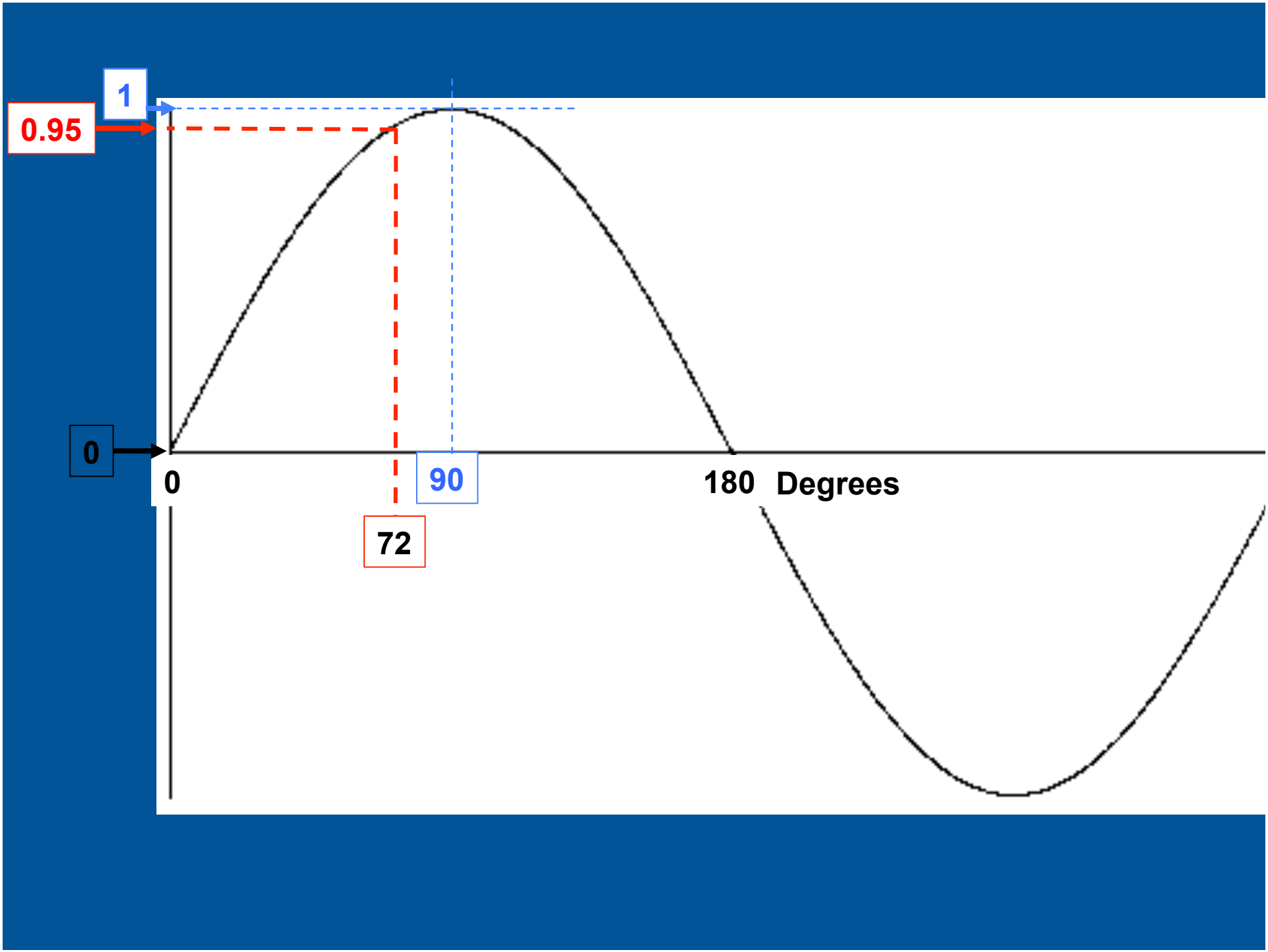


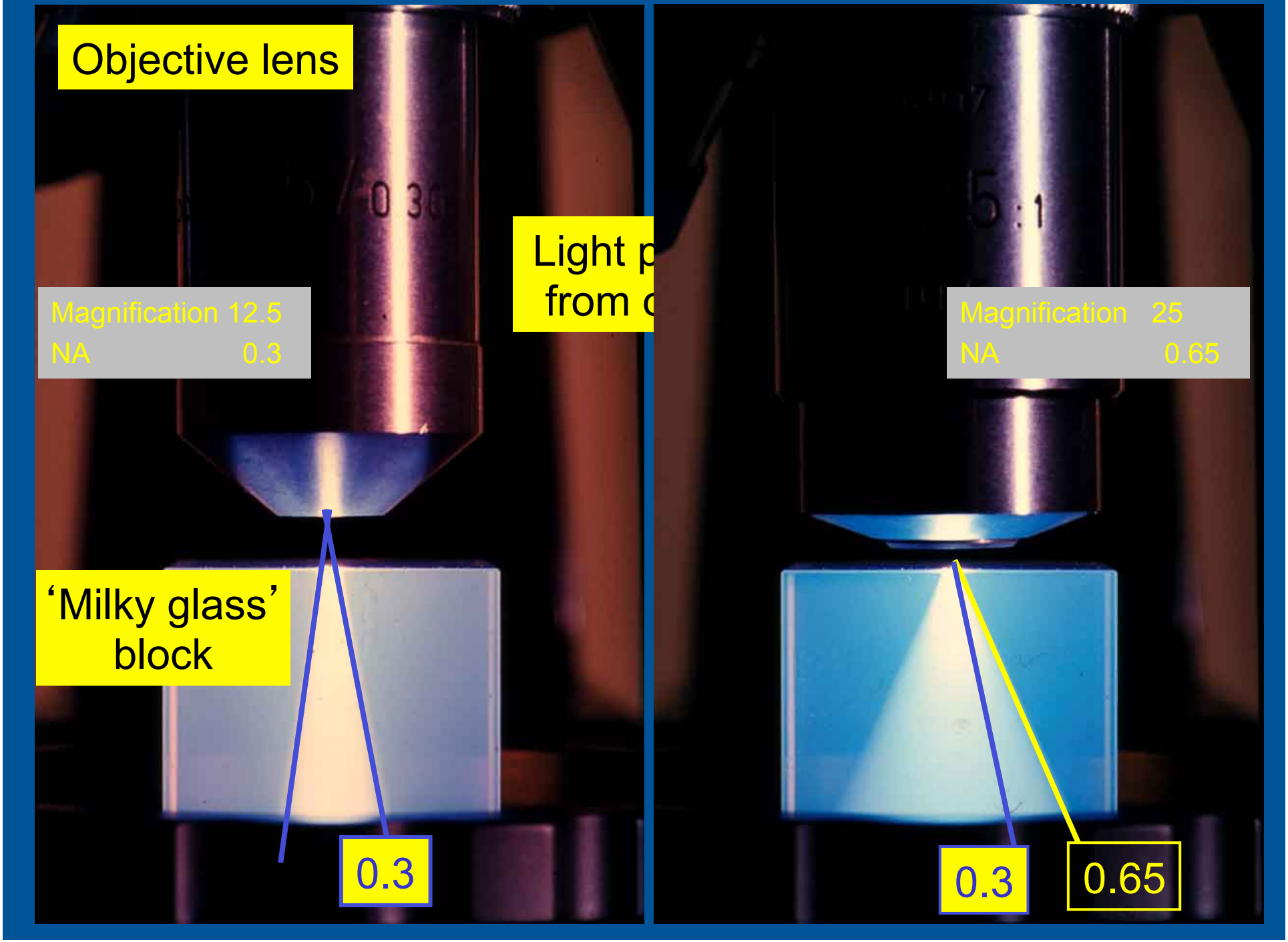
$$NA = n \sin \alpha$$

Numerical Aperture

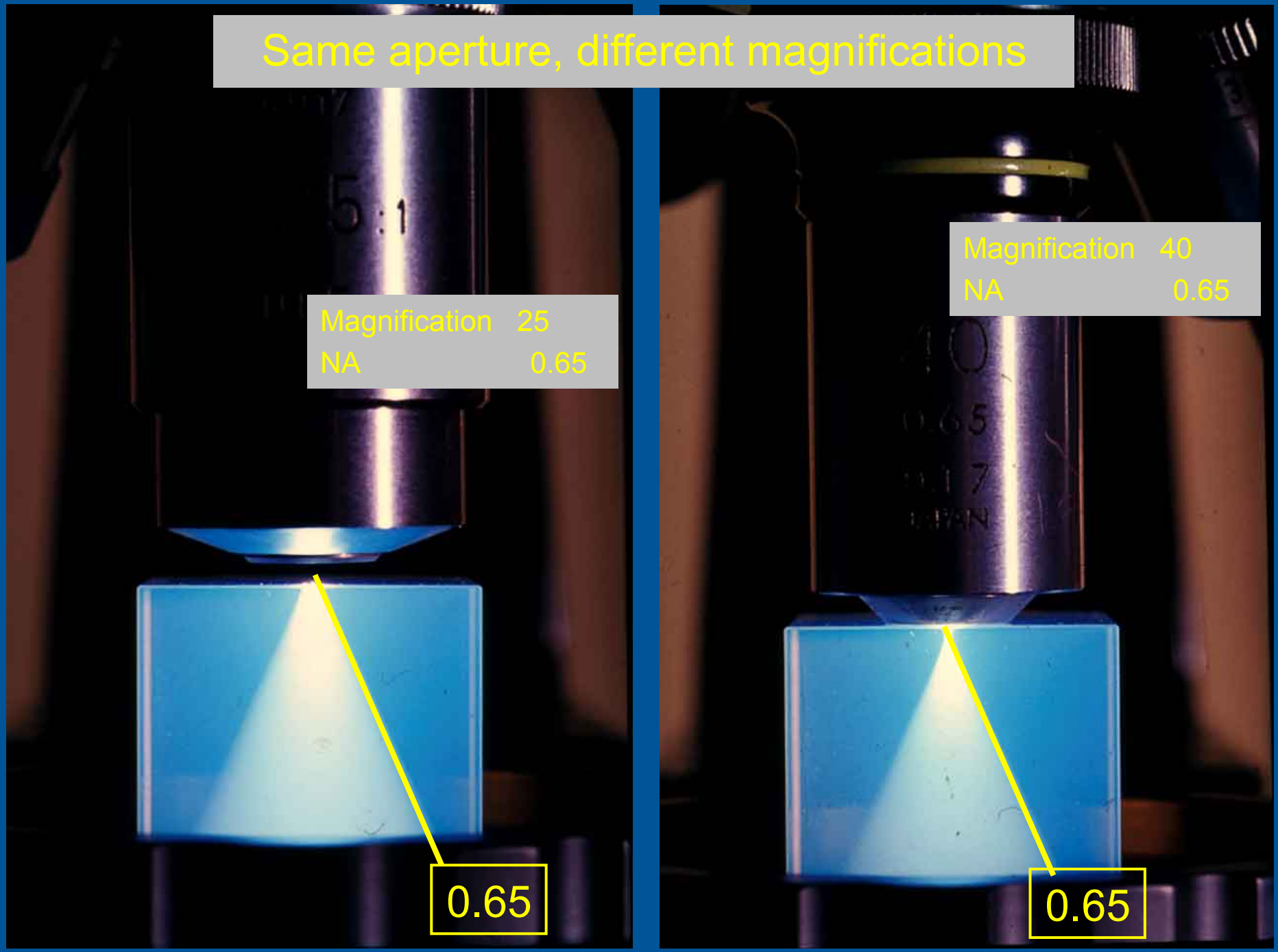
Dry Objective	Immersion Objective
NA = 1 x sin 72°	NA = 1.515 x sin 67°
= 1 x 0.95	= 1.515 x 0.92
= 0.95	= 1.4




1
0.95
0
0
90
180 Degrees
72

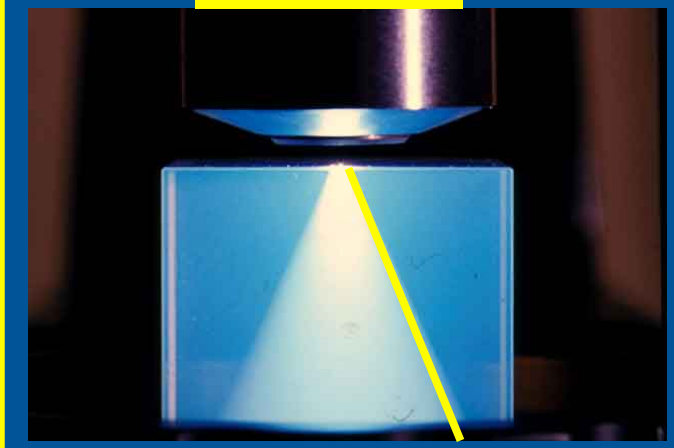

Objective lens
Light p
from c
Magnification 12.5
NA 0.3
‘Milky glass’ block
0.3
Magnification 25
NA 0.65
0.3
0.65

Same aperture, different magnifications

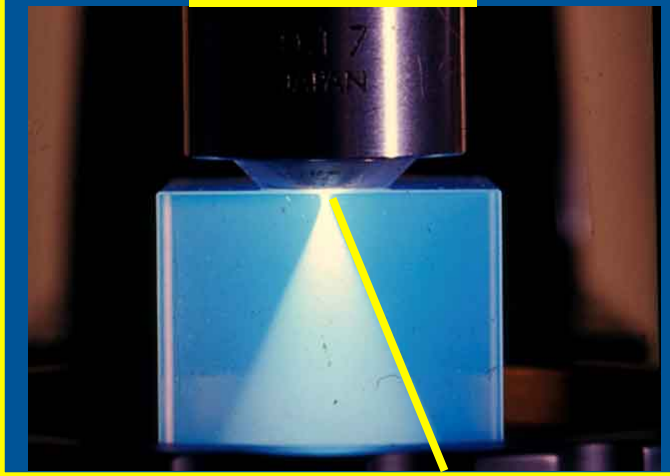


Same aperture
different magnifications

25 / 0.65

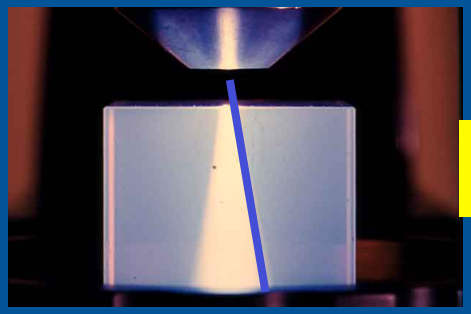


40 / 0.65

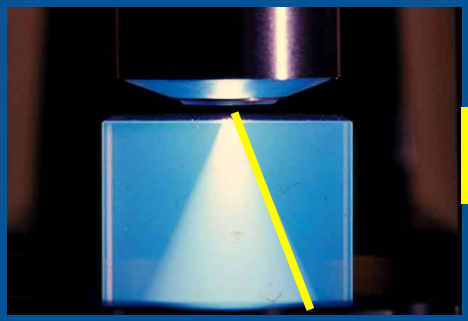


40 / 0.95

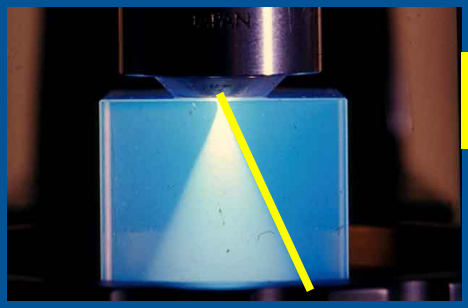
Same magnification
different apertures



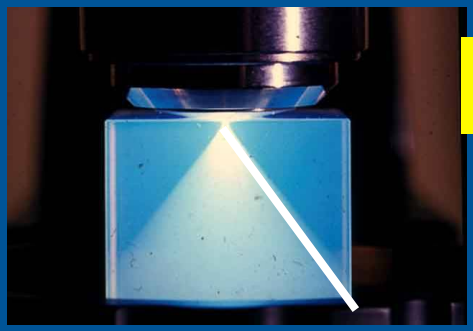
12.5 / 0.3



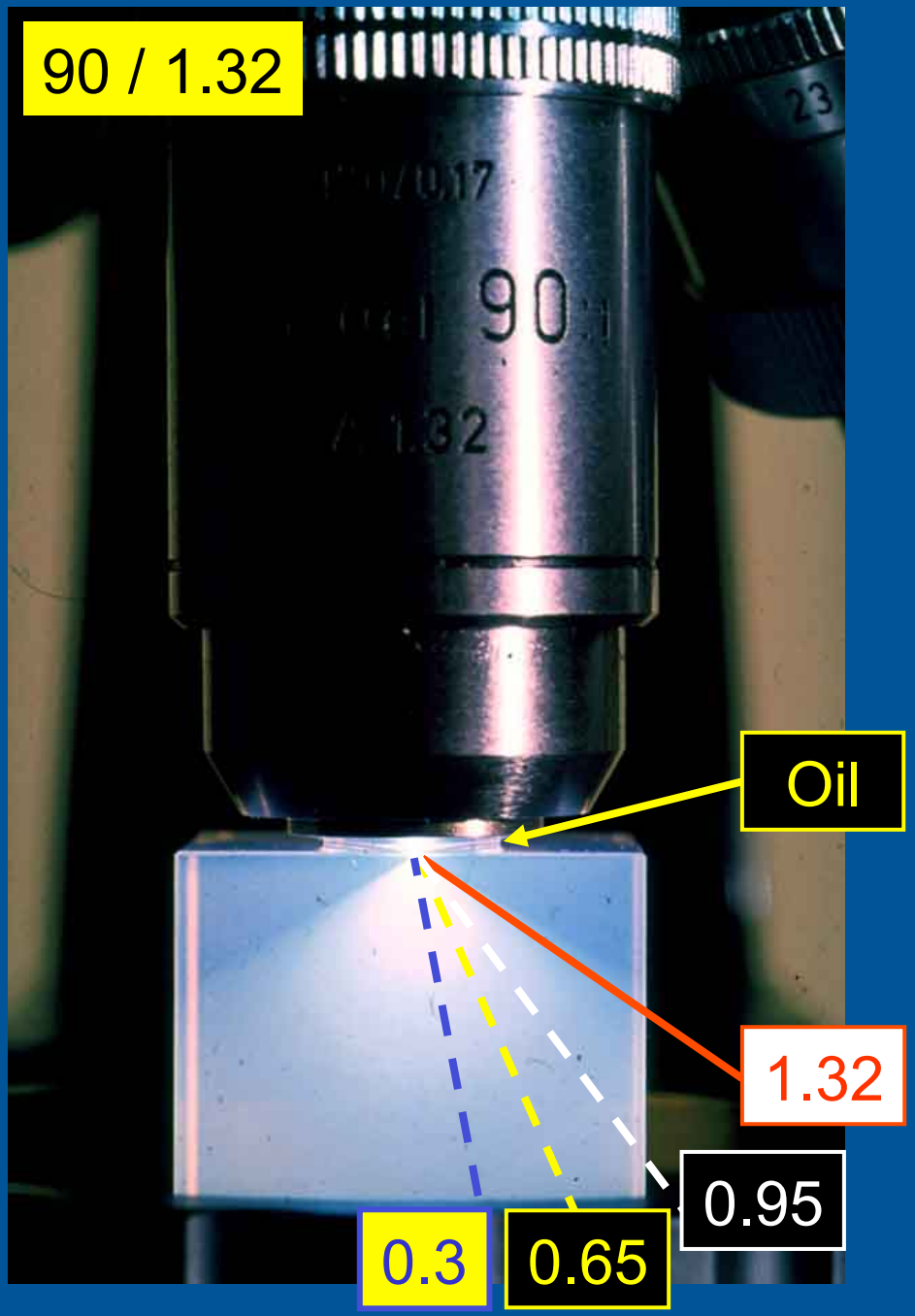
25 / 0.65



40 / 0.65

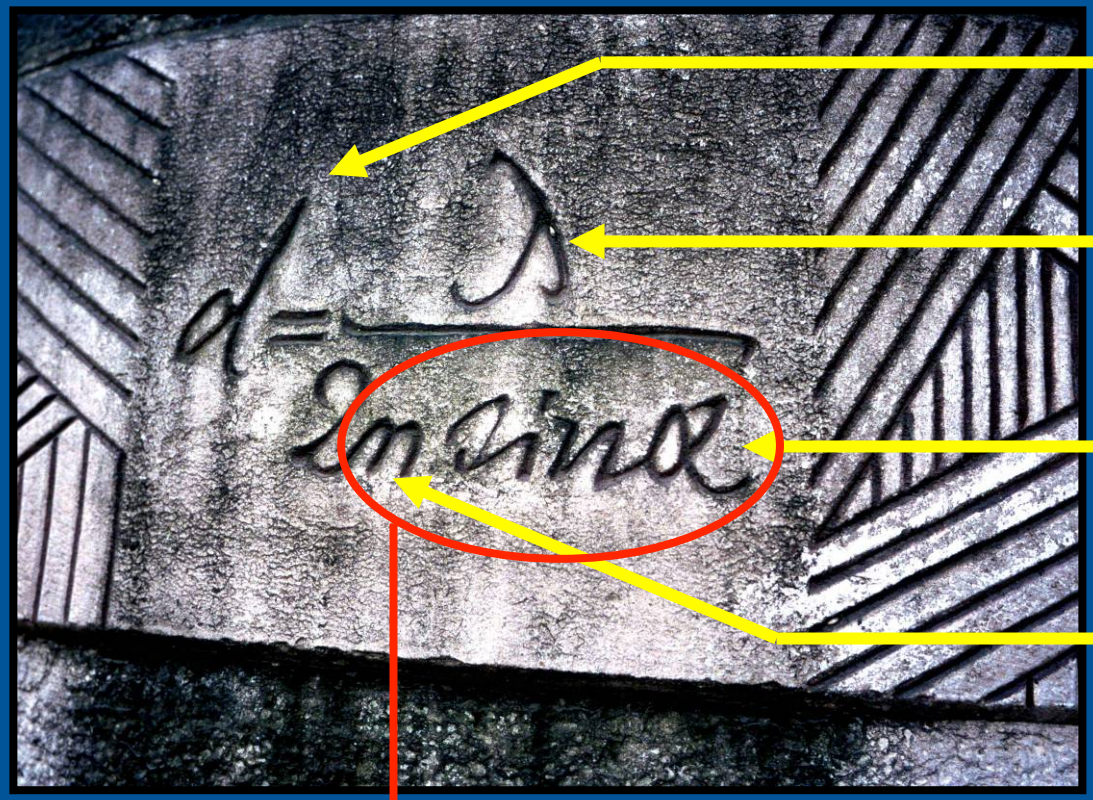


40 / 0.95



Why is Numerical Aperture Important?

Inscription on Ernst Abbe's memorial



d — Minimum resolved distance

λ — Wavelength of imaging radiation

α — Half-aperture angle

n — Refractive index of medium

Numerical Aperture

Minimum resolved distance is now commonly expressed as

$$d = 0.61\ \lambda / NA$$

$$d = \lambda / 2\, n. \sin \alpha$$

$$d = \lambda / 2\, NA$$

$$d = 0.5\, \lambda / NA$$

$$d = 0.61\, \lambda / NA$$

Why is Numerical Aperture Important?

- Resolution depends on NA
- Light transmission of objective
  depends on NA^2
- Depth of field of objective is (approximately)
  inversely proportional to NA^2