Charles
Chevalier's
Horizontal
Microscope
(circa 1834)

Visual Microscopy Workshop Series

Resolution

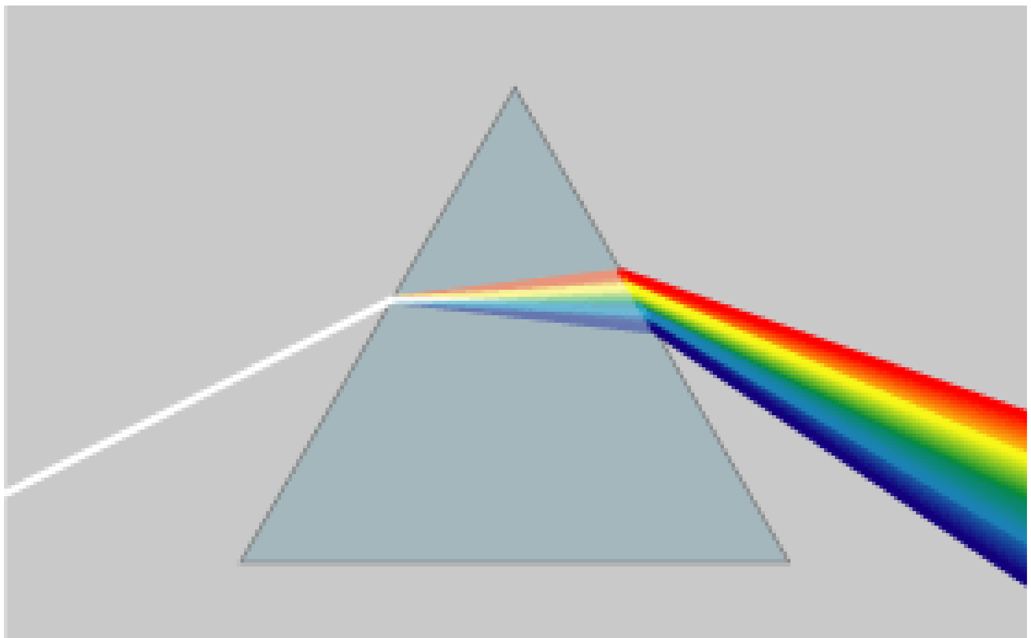
- know the limit and how to achieve it -

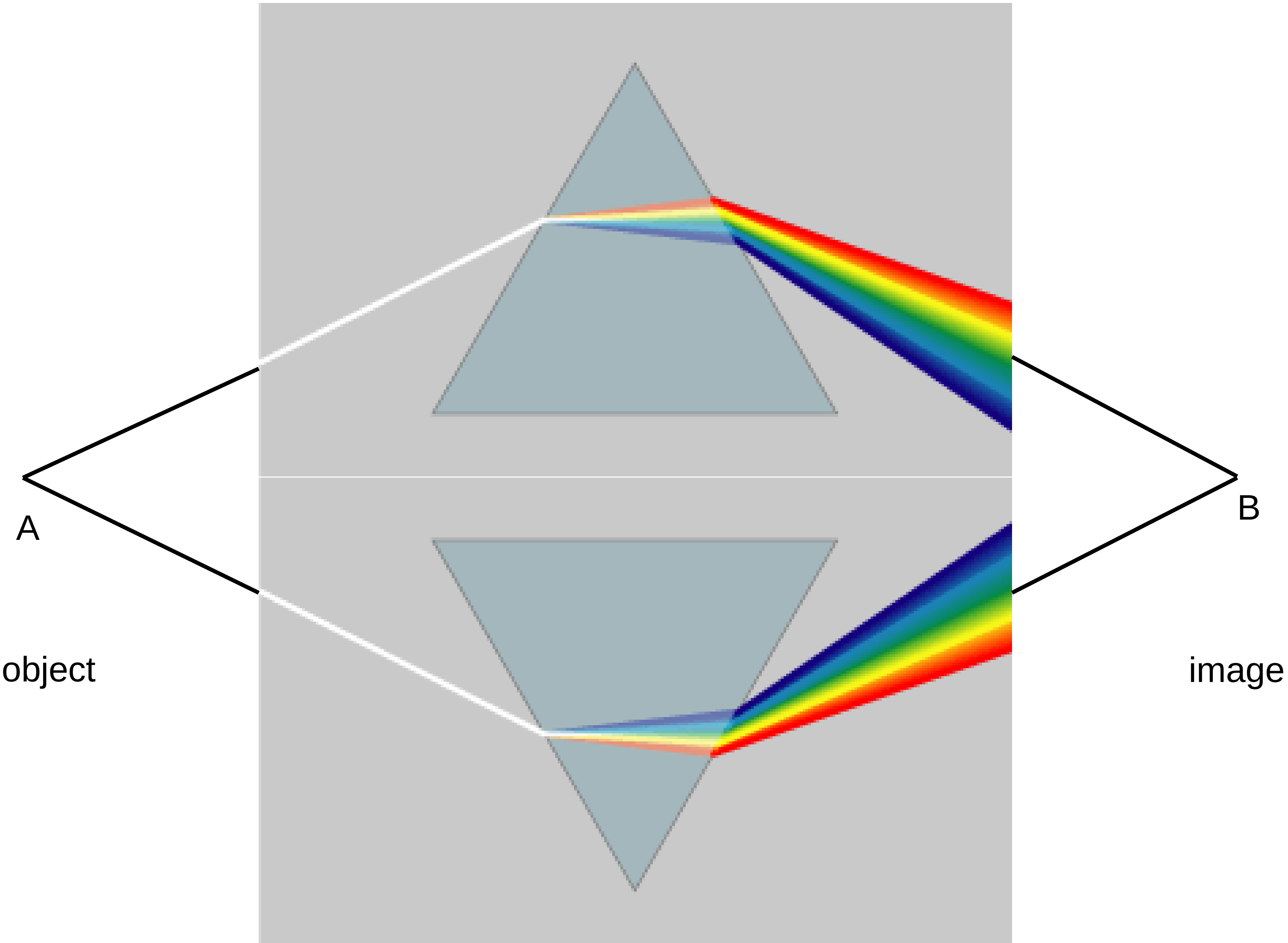
Lai Ding

BWH NeuroTechnology Studio

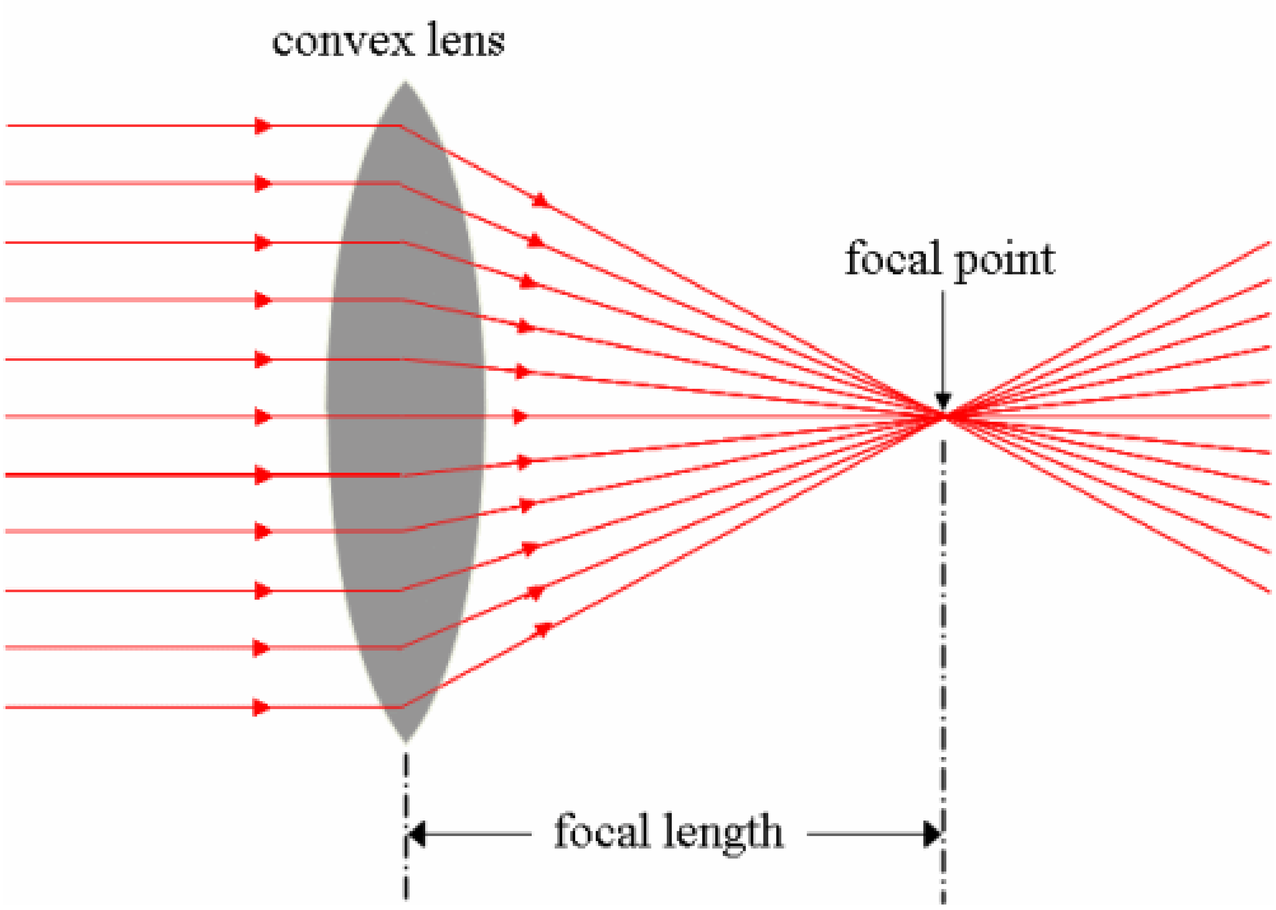
Optical Imaging System: use visible light and glass lens to form image

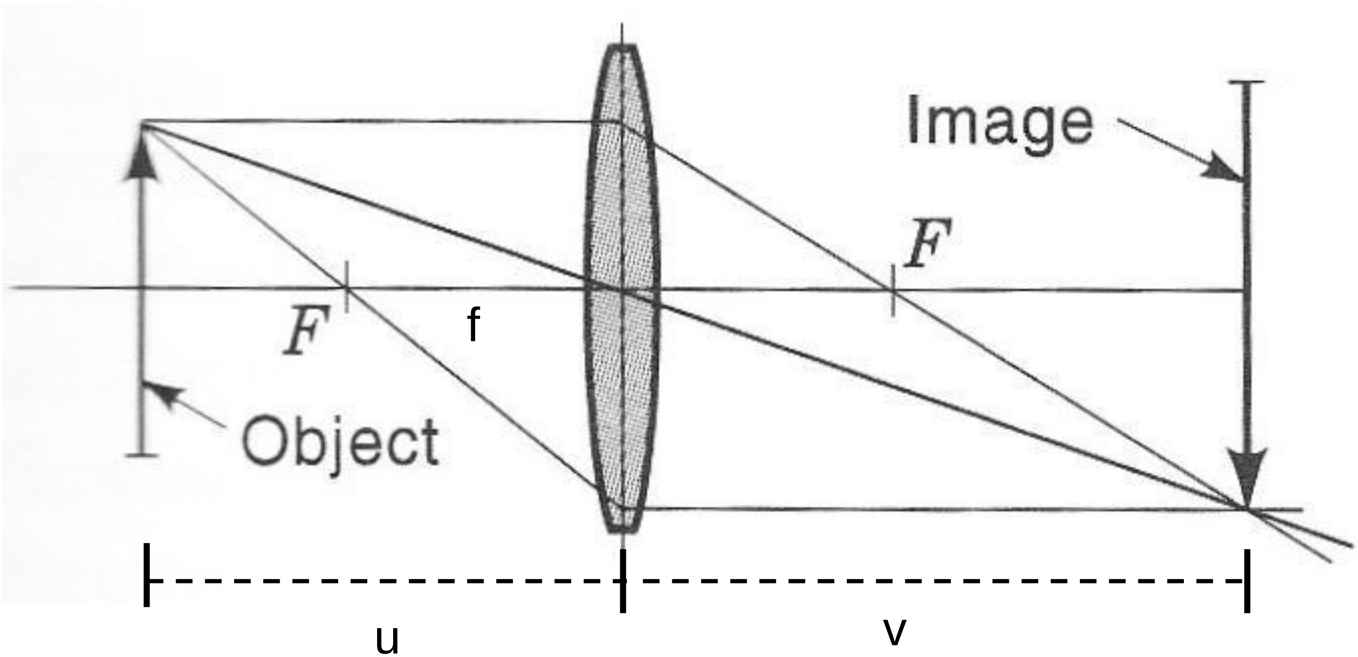
Why a glass lens can form image?




A
B
object
image

Thin lens image formation



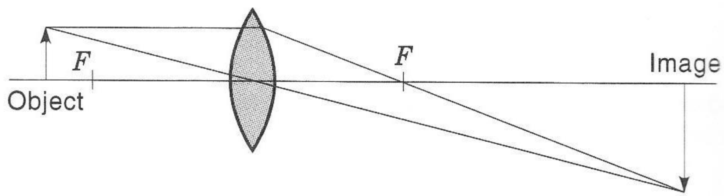


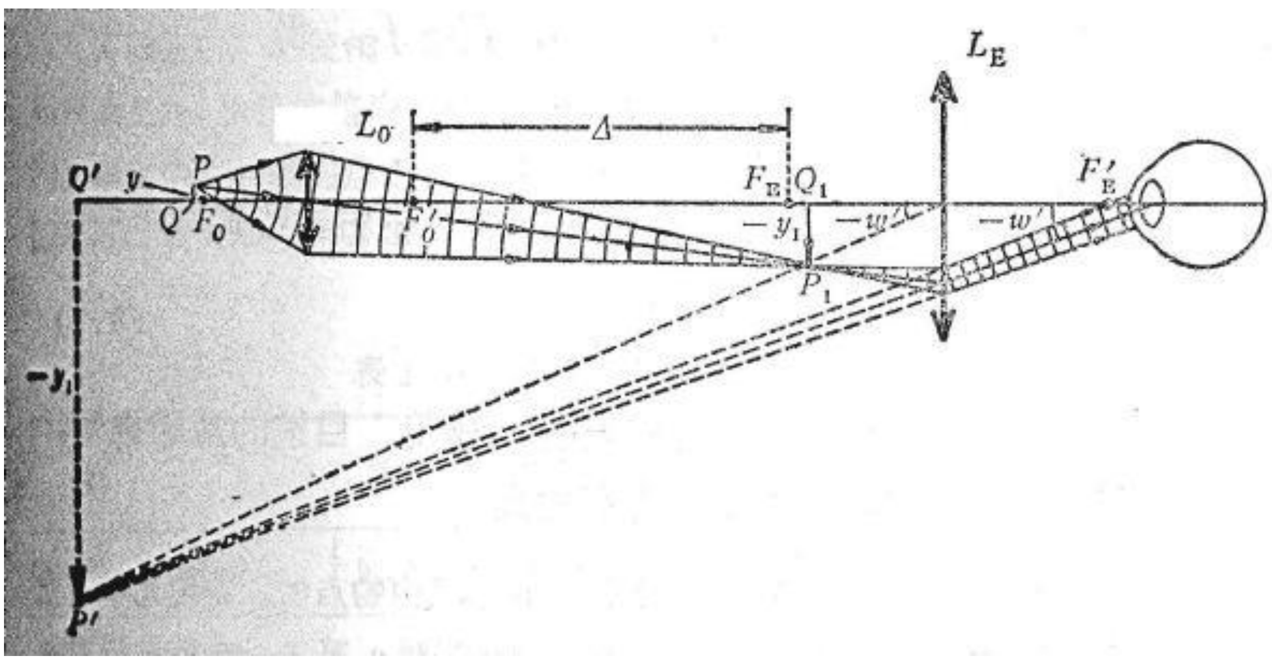
$$\frac{1}{v} + \frac{1}{u} = \frac{1}{f}$$

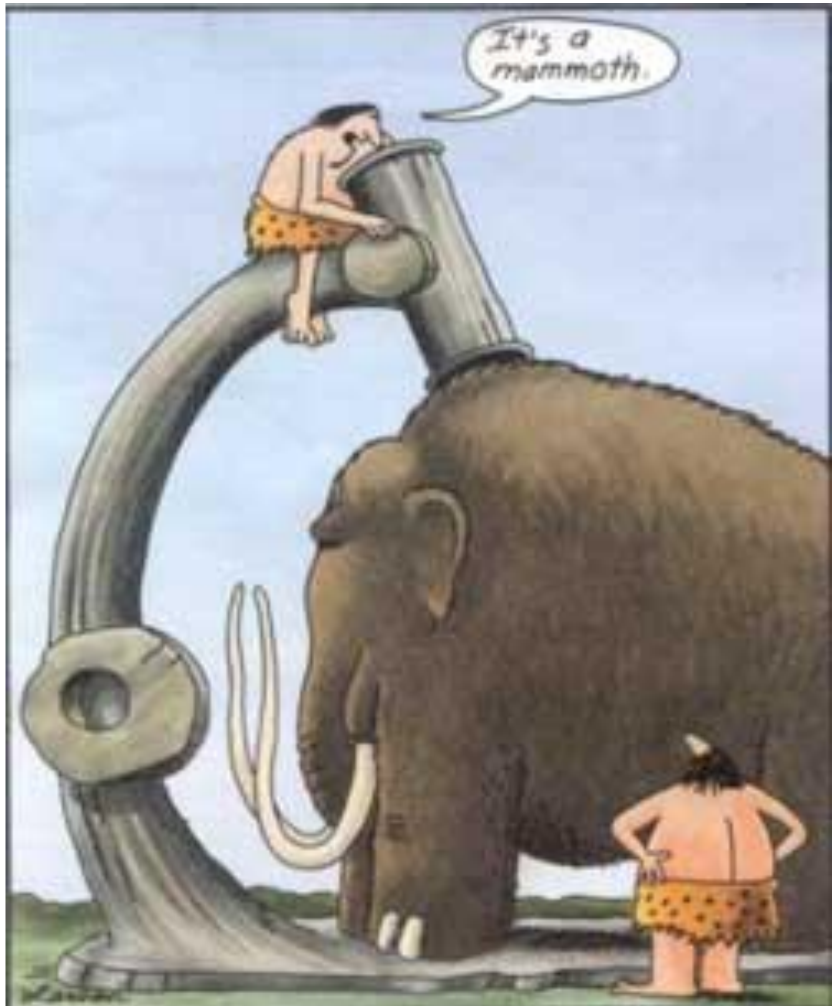
$$\text{Magnification } M = \frac{v}{u} = \frac{f}{u - f}$$

Want higher magnification? Move object closer to F

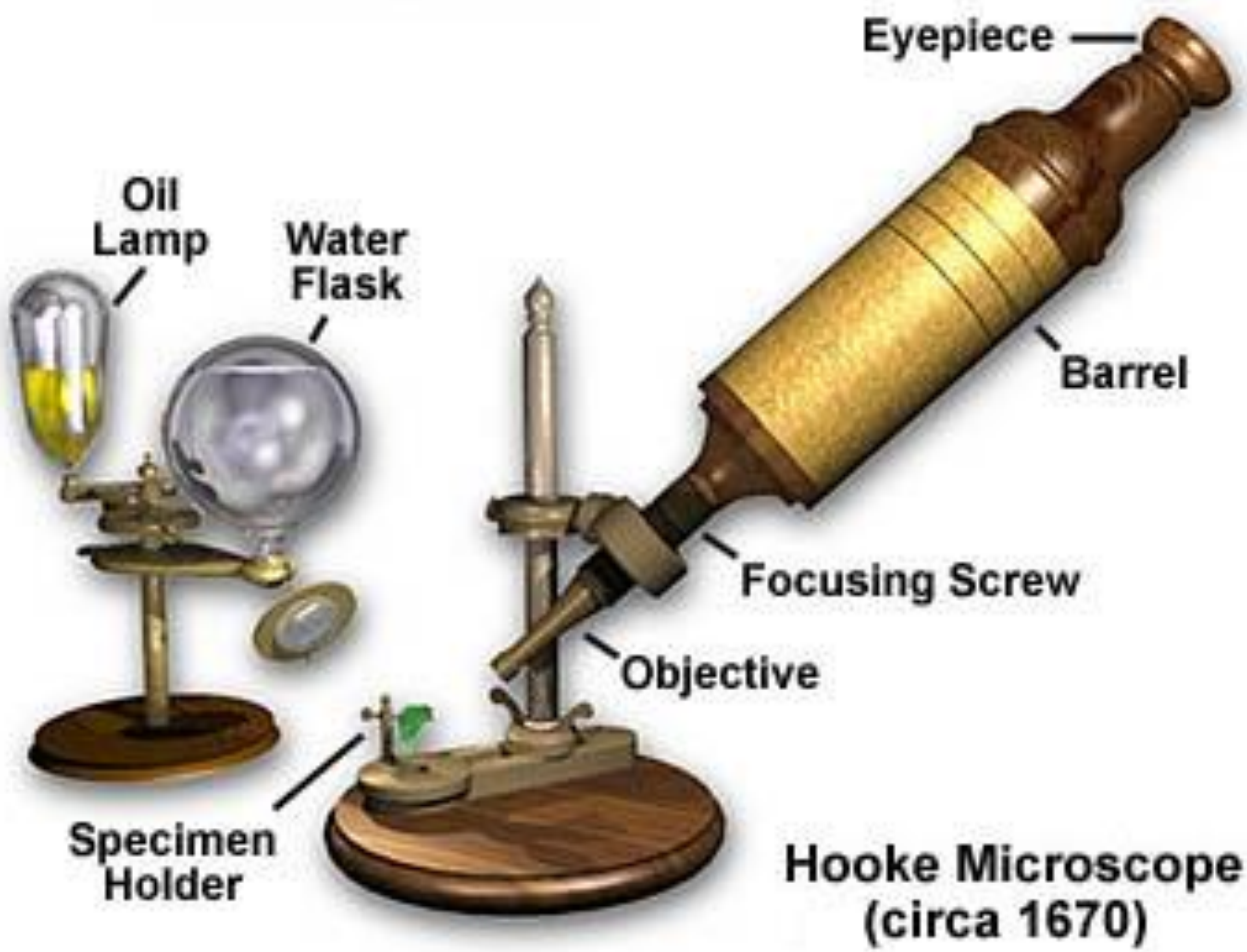
Microscope







Early microscopes


Eyepiece
Oil Lamp
Water Flask
Barrel
Focusing Screw
Objective
Specimen Holder
Hooke Microscope (circa 1670)

Robert Hooke
1635-1703

MICROGRAPHIA:

OR SOME

Physiological Descriptions

OF

MINUTE BODIES

MADE BY

MAGNIFYING GLASSES

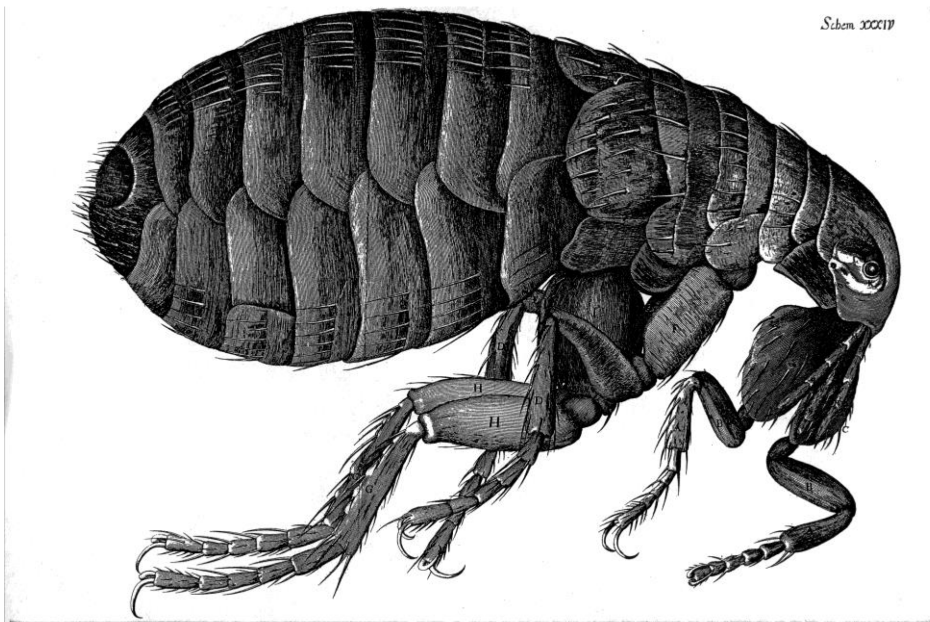
WITH

OBSERVATIONS and INQUIRIES thereupon.

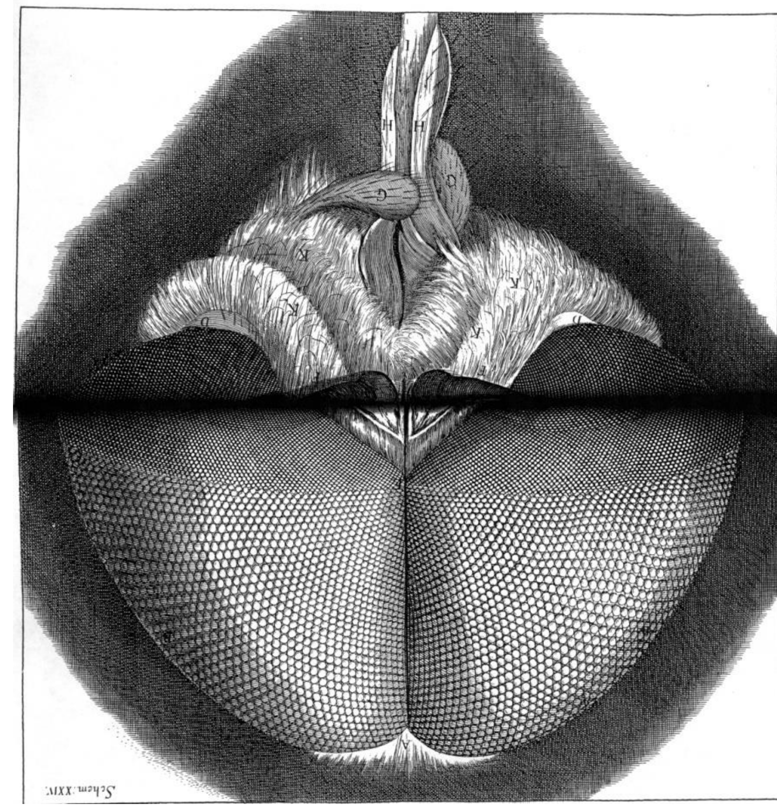
By *R. HOOKE*, Fellow of the ROYAL SOCIETY.

Non possis oculo quantum contendere Linceus,
Non tamen idcirco contemnas Lippus inungi. Horat. Ep. lib. 1.

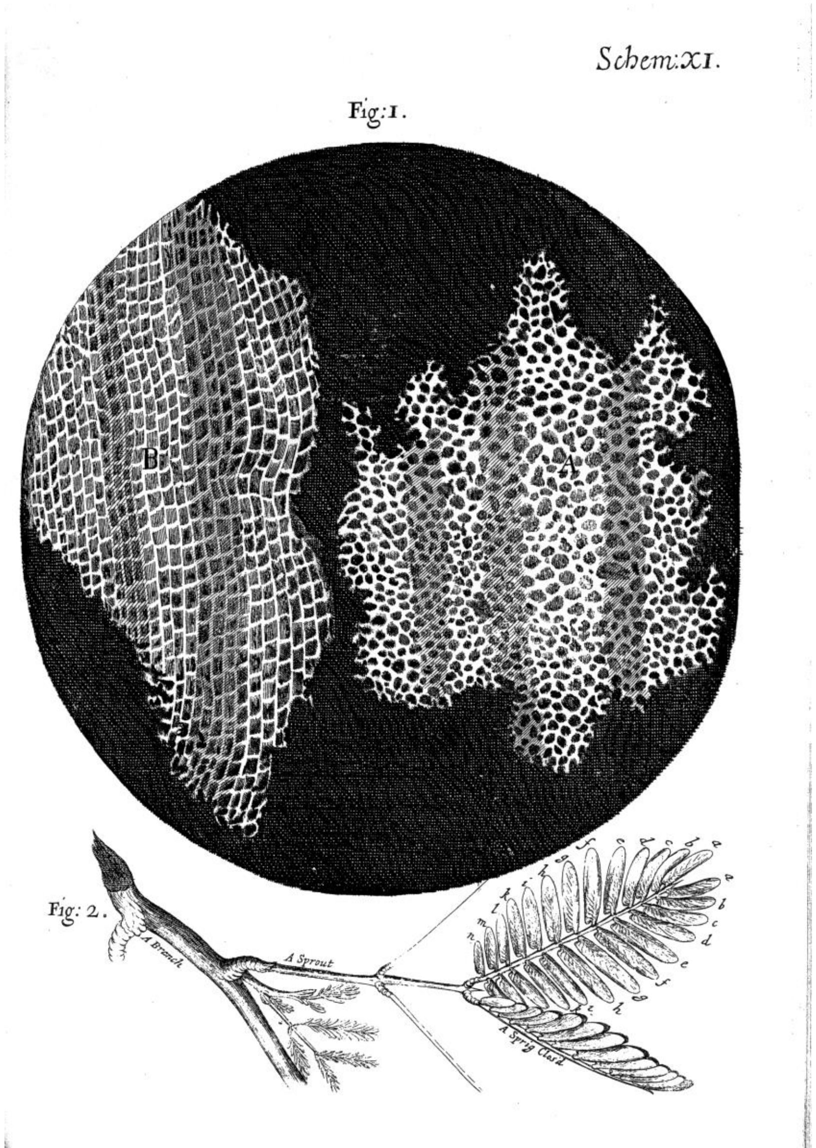
LONDON, Printed by *Jo. Martyn*, and *Ja. Allestry*, Printers to the ROYAL SOCIETY, and are to be sold at their Shop at the *Bell* in S. *Paul's* Church-yard. M DC LX V.



flea



fly eye



cork slice

Hooke named them “cell”

(reminds him of a monk's quarters)

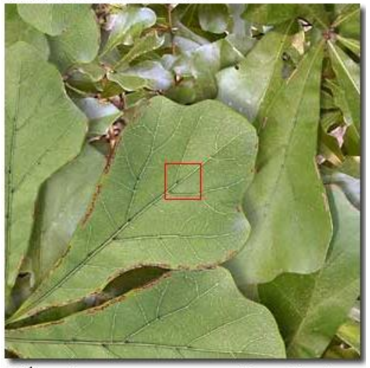
Leica DM 600

Mag 10x -1000x

objective 100x
eye piece 10x

Why not more?

Oak tree leaves at actual size.



10^{-1} meters 10 centimeters

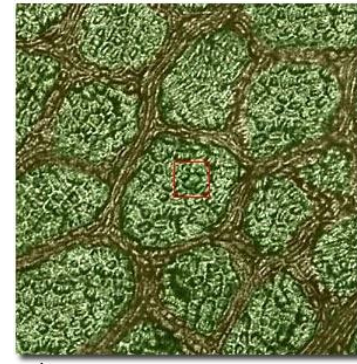
Surface of an Oak leaf magnified 10 times.

10^{-2} meters 1 centimeter

Surface of an Oak leaf magnified 100 times.

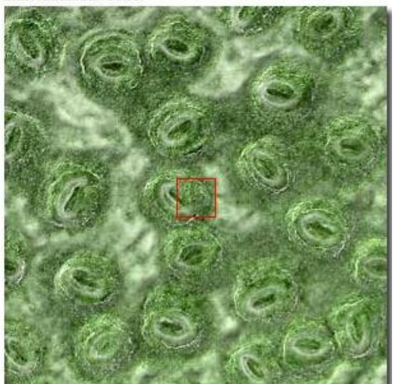
10^{-3} meters 1 millimeter

Cells on the leaf surface.



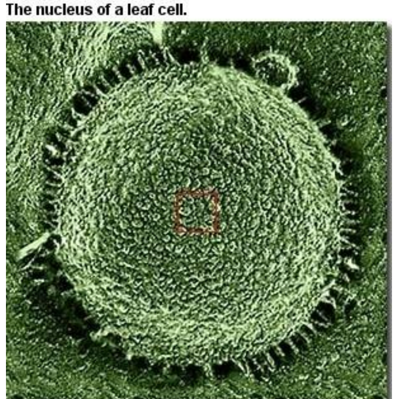
10^{-4} meters 100 micrometers

Individual leaf cells.



10^{-5} meters 10 micrometers

The nucleus of a leaf cell.

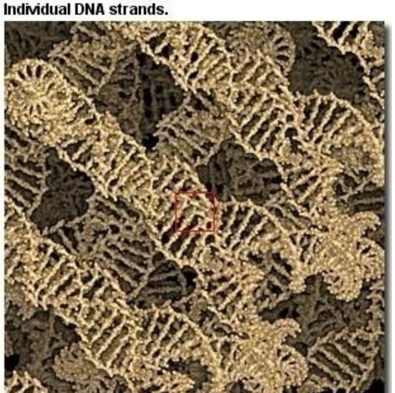


10^{-6} meters 1 micrometer

Chromatin in the leaf cell nucleus.

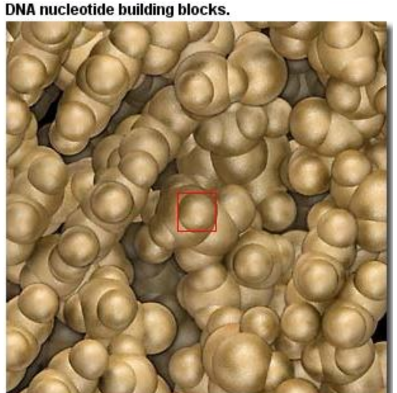
10^{-7} meters 100 nanometers

Individual DNA strands.



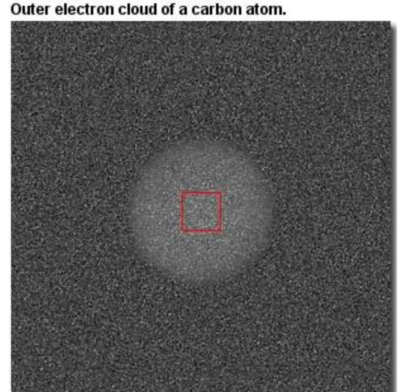
10^{-8} meters 10 nanometers

DNA nucleotide building blocks.

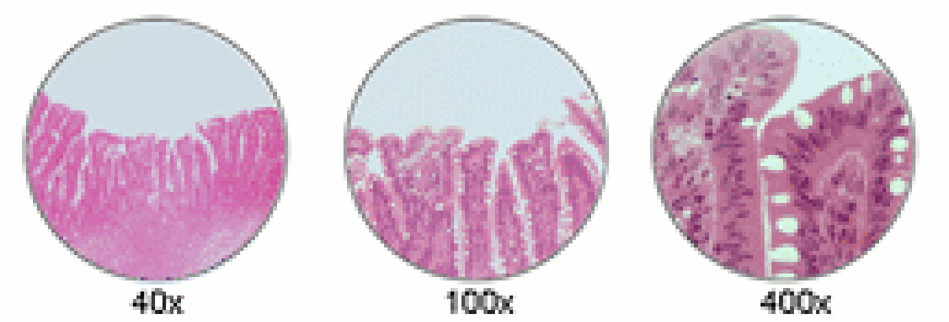


10^{-9} meters 1 nanometer

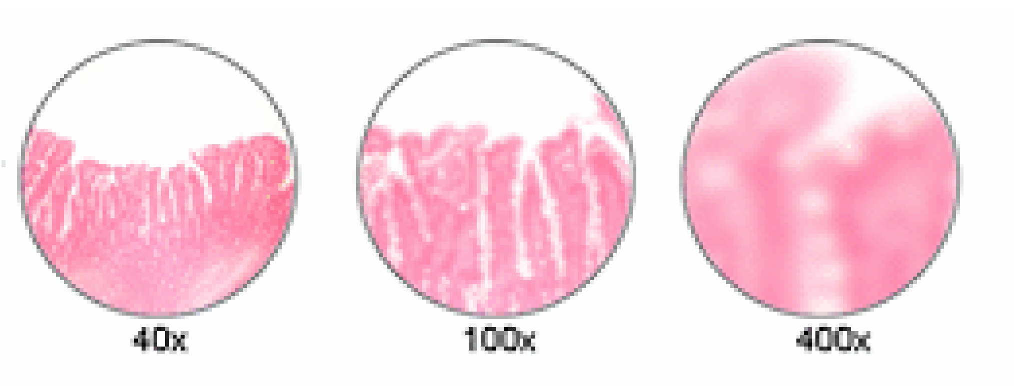
Outer electron cloud of a carbon atom.



10^{-10} meters 100 picometers



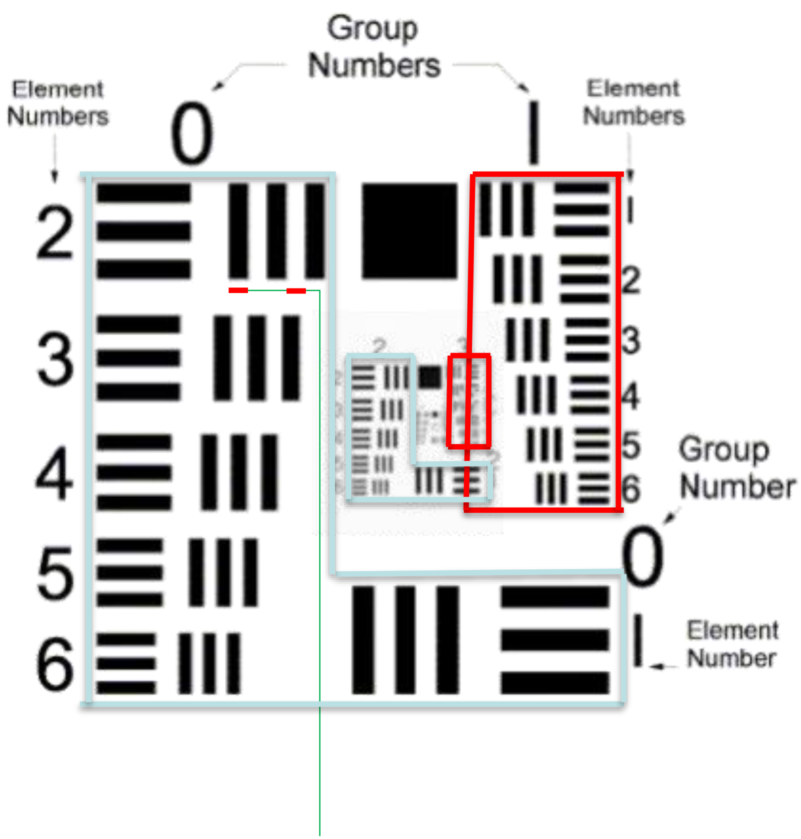
Useful magnification



Empty magnification

USAF resolution target slide

1951 USAF Resolution Target



Line Width (446.43um)

Group	Element	Linewidth (micron)
0	1	500.00
	2	446.43
	3	396.83
	4	354.61
	5	314.47
	6	284.09
Group 1… Group 6		
7	1	3.91
	2	3.47
	3	3.11
	4	2.76
	5	2.46
	6	2.19
8	1	1.95
	2	1.74
	3	1.55
	4	1.38
	5	1.23
	6	1.10
9	1	0.98
	2	0.87
	3	0.78

Live demo   Zeiss LSM710

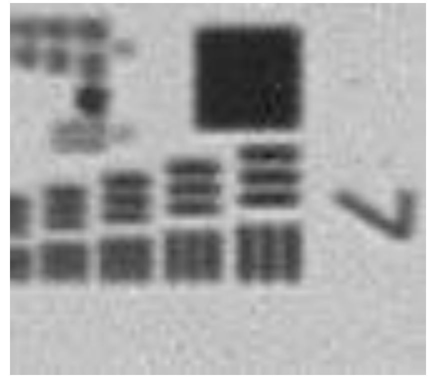
2.5X/0.12 Zeiss: empty magnification

On USAF slide

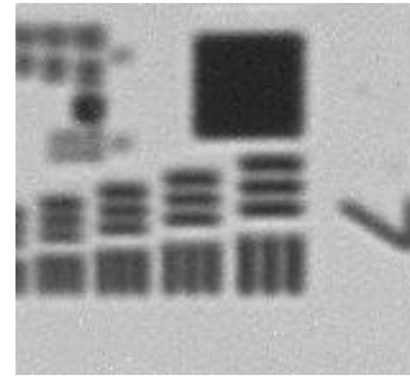
Zoom 1	5-6
Zoom 2	6-3
Zoom 4	7-2
Zoom 8	7-3
Zoom16	7-4
Zoom 32	7-4

1X

2X

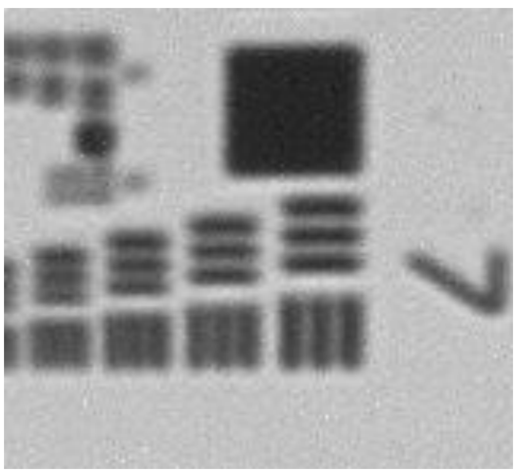


4X

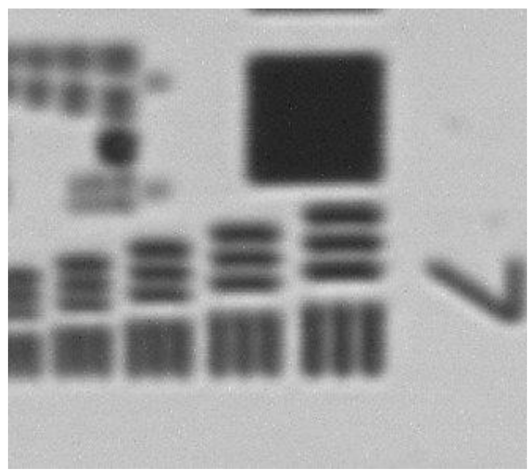


8X

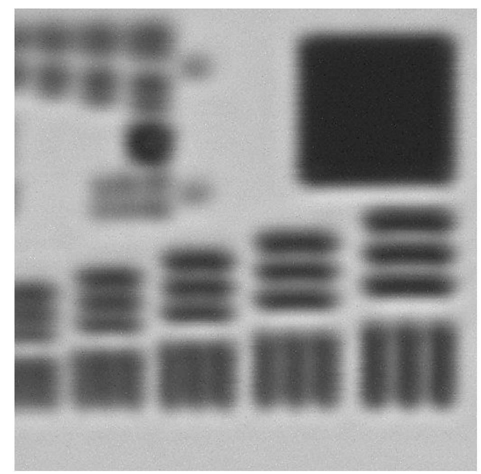
Useful Magnification



8X

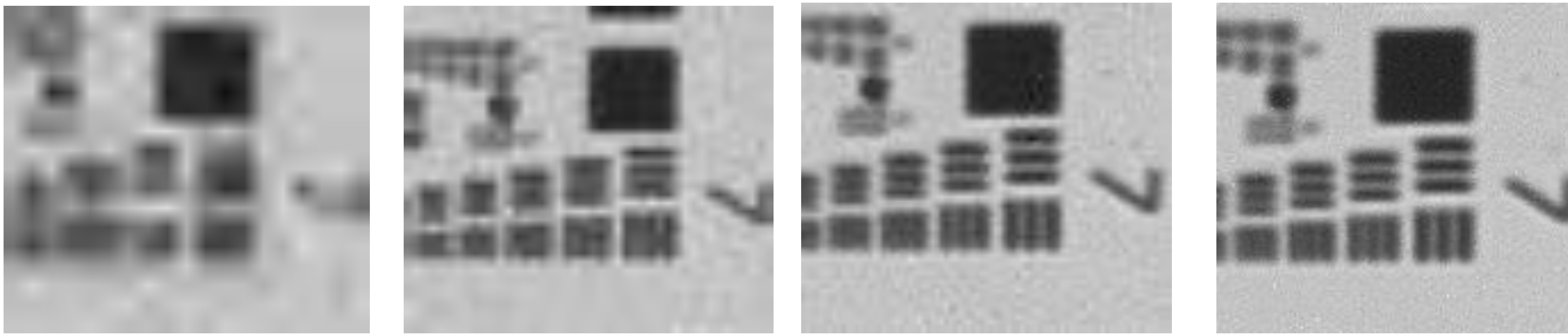


16X



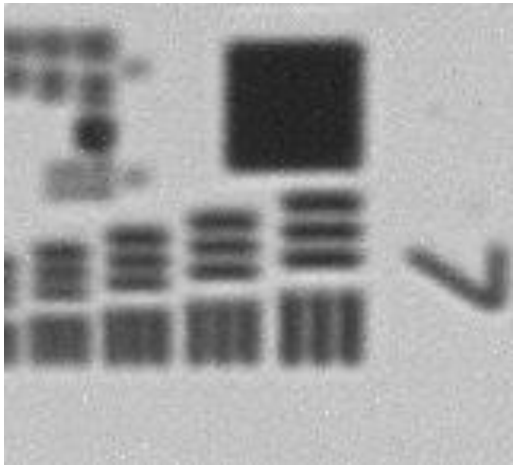
32X

Empty Magnification

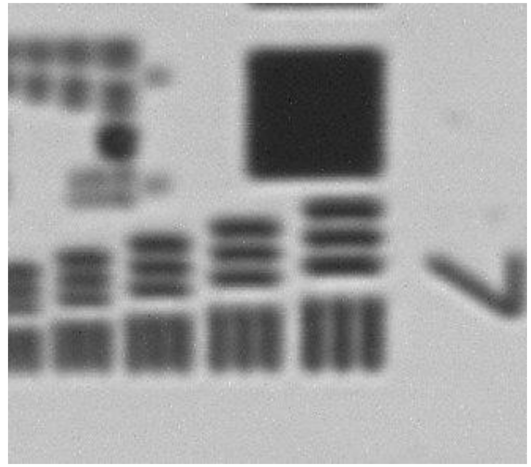


1X 2X 4X 8X

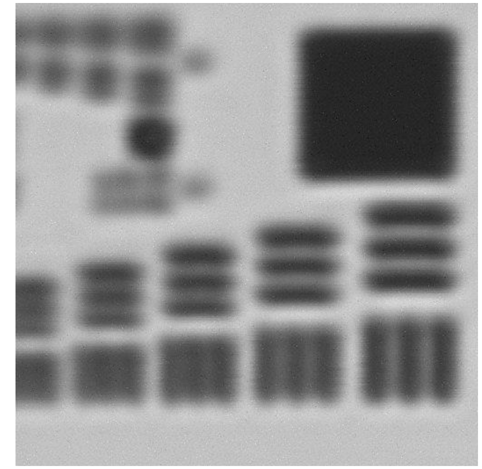
Useful Magnification



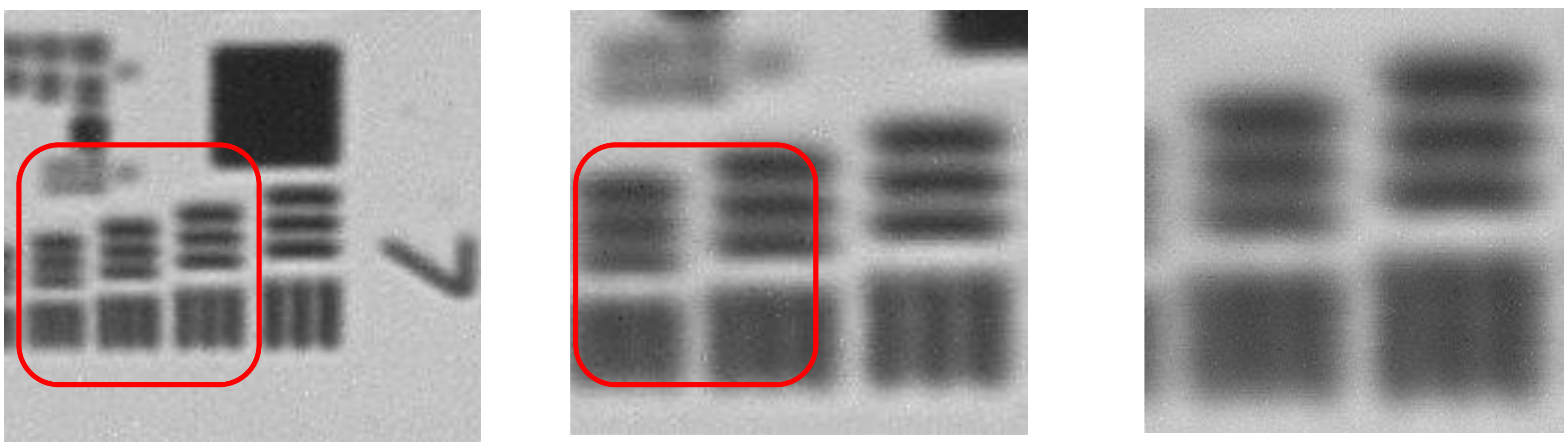
8X



16X



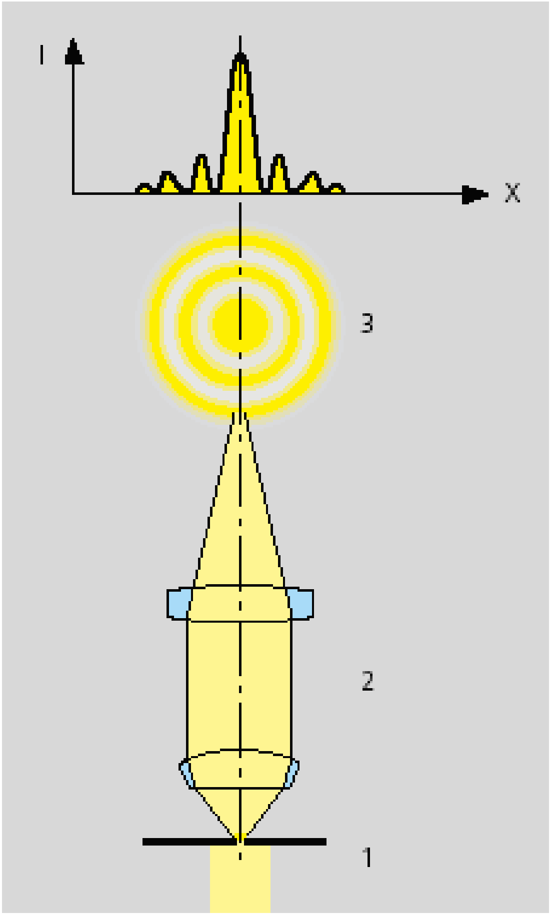
32X



Empty Magnification

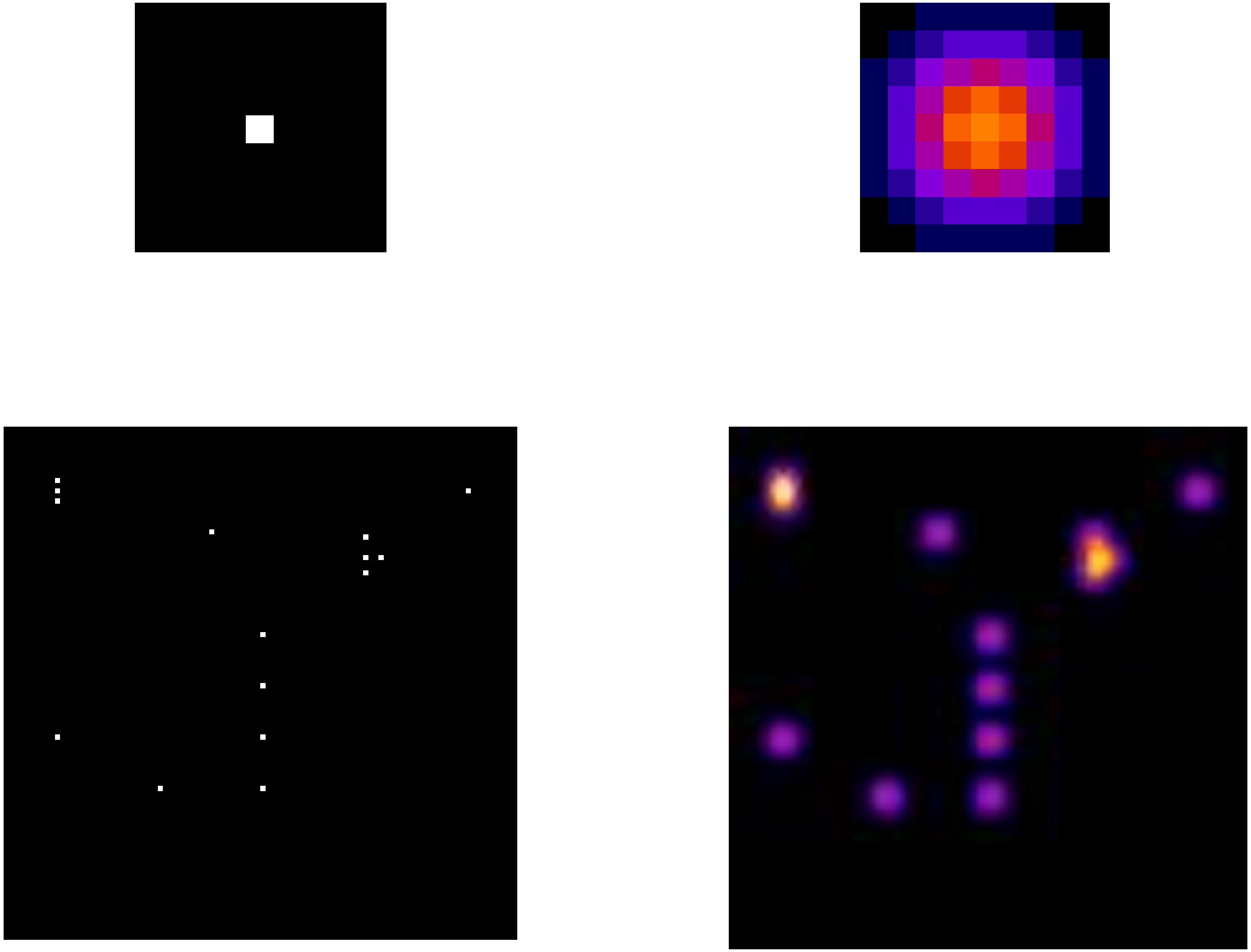
Why there is Empty Magnification ?

On the image side : a “point” is not a point



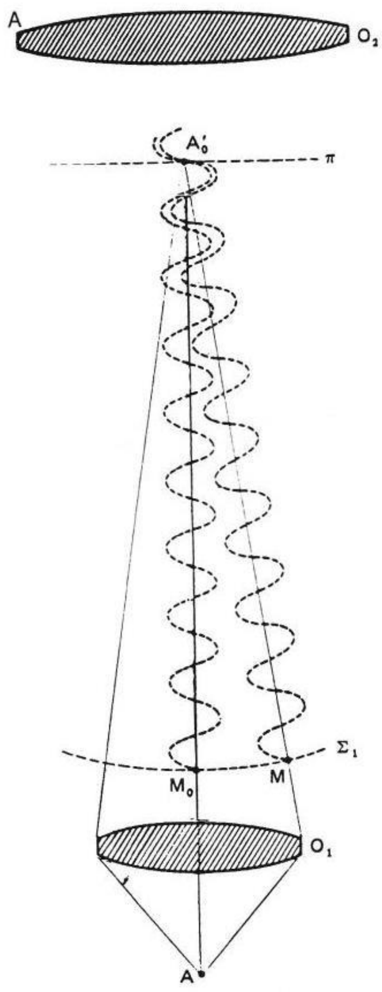
Airy disk

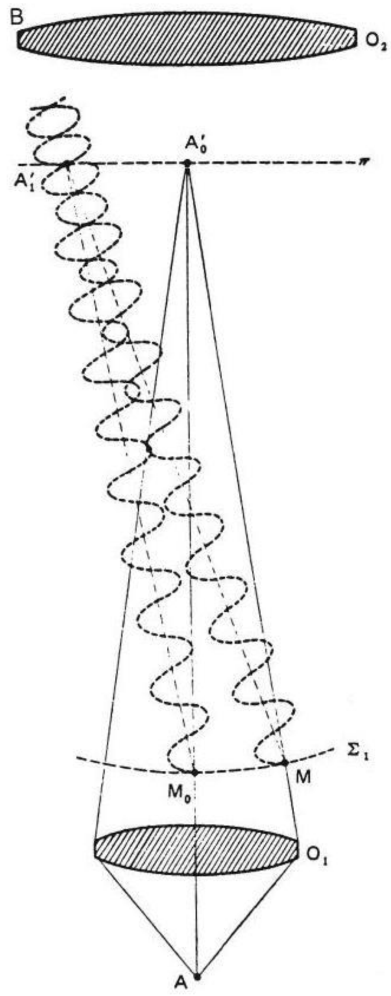
Sir George Biddell Airy
1801-1892

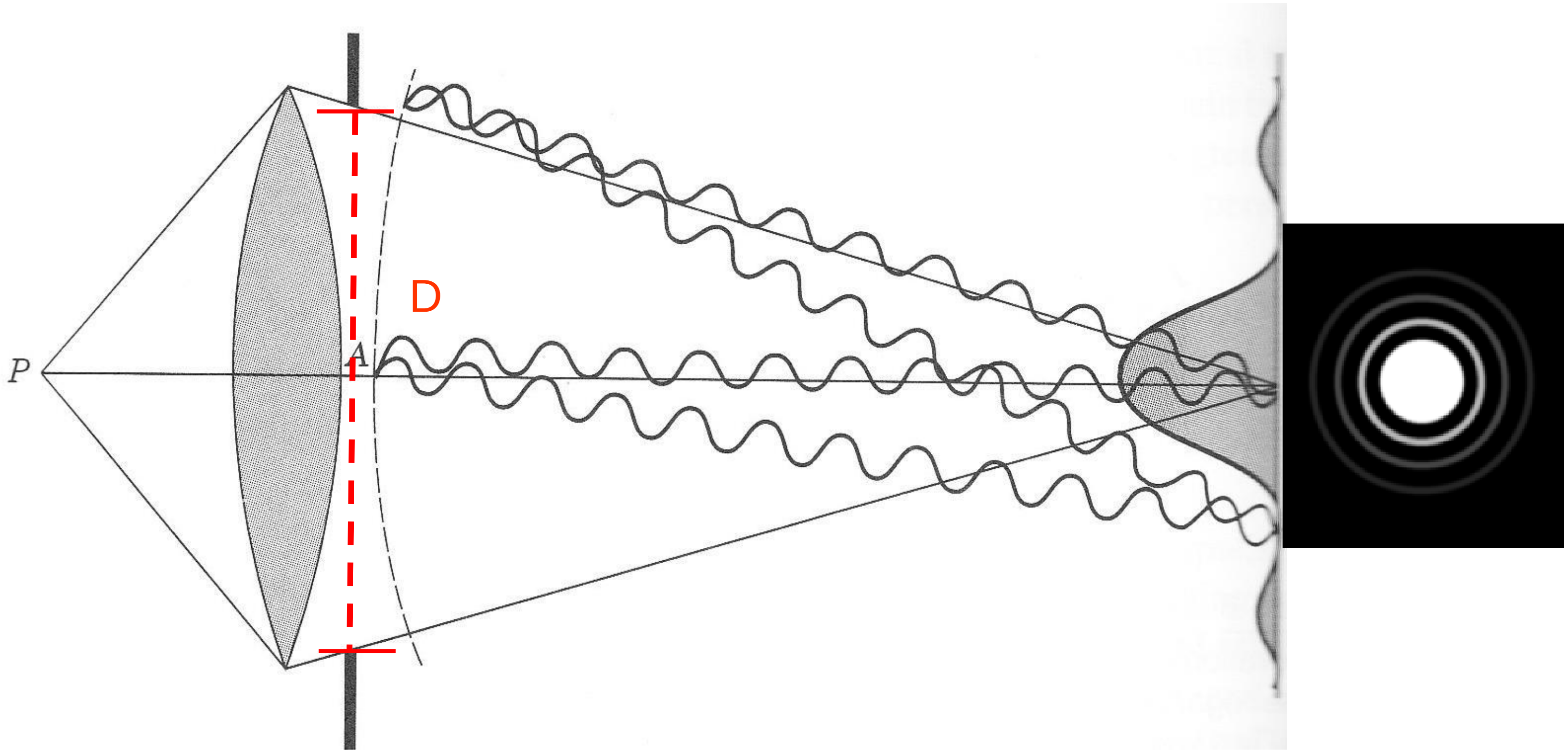


Why does the point become a disk?

Diffraction is key to the Point Spread Function (PSF)

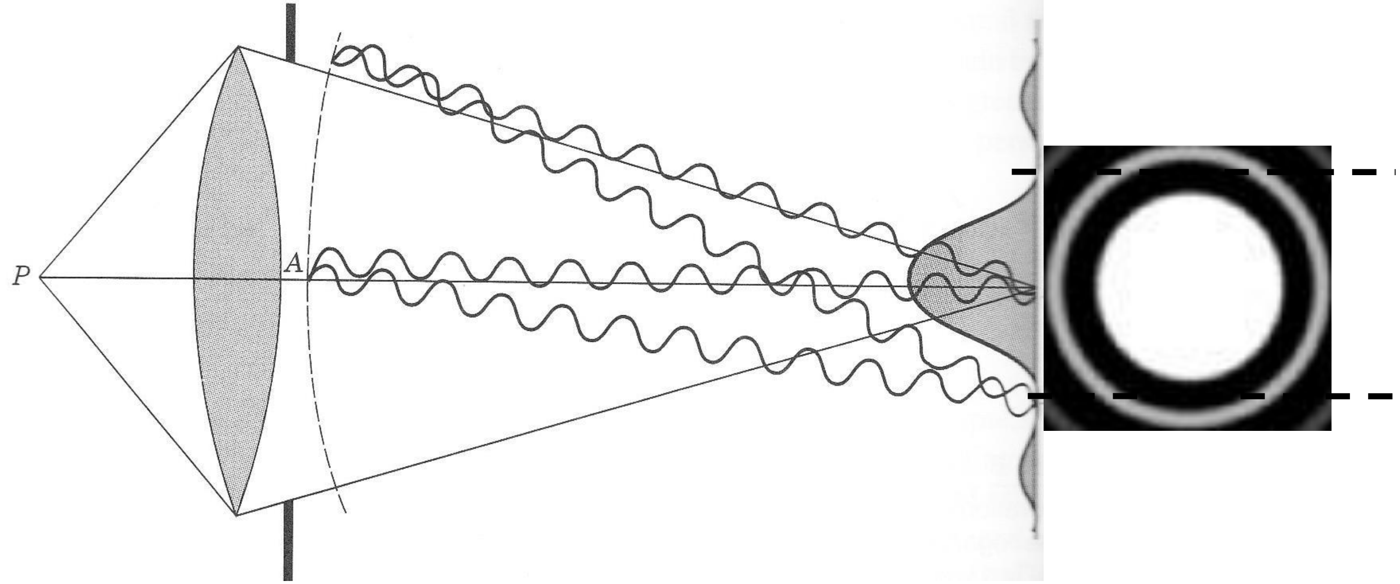


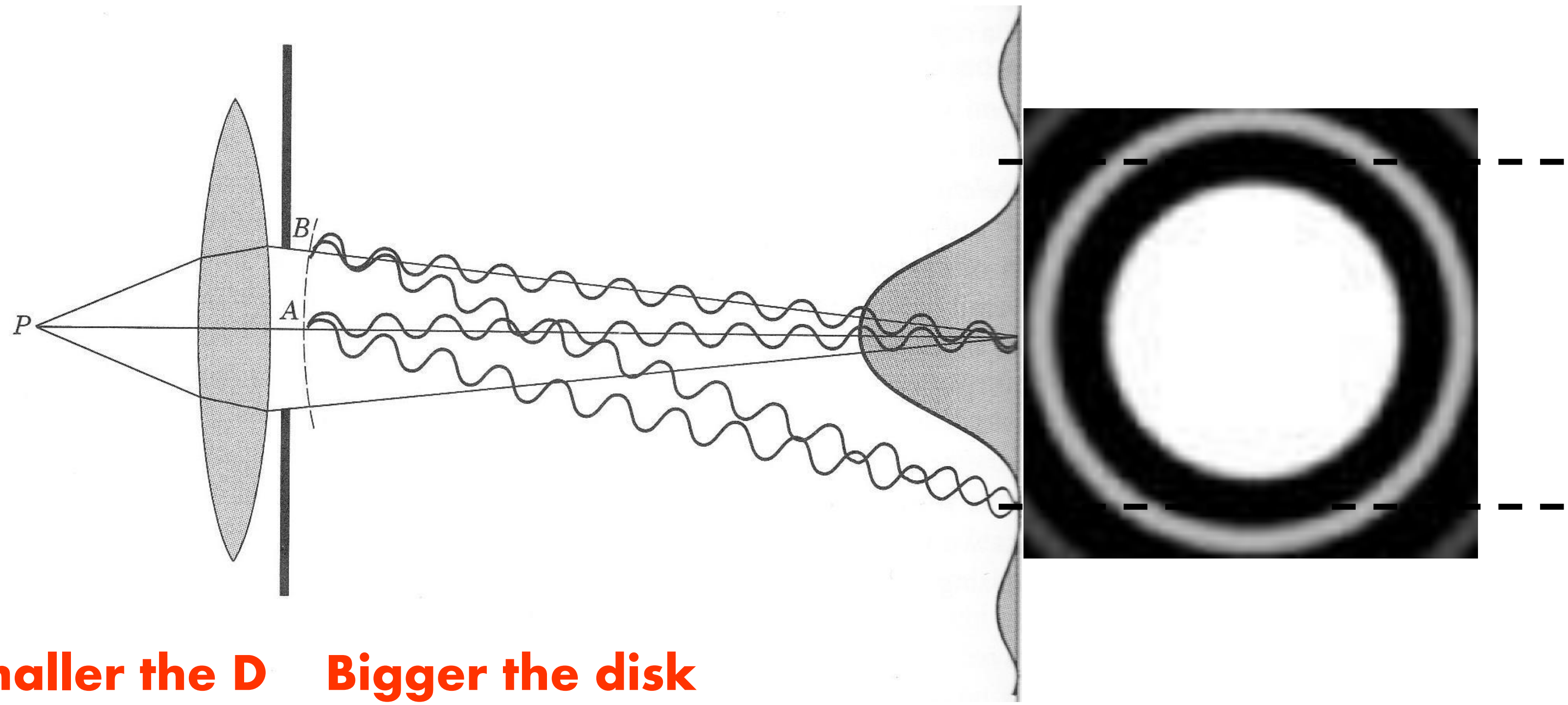




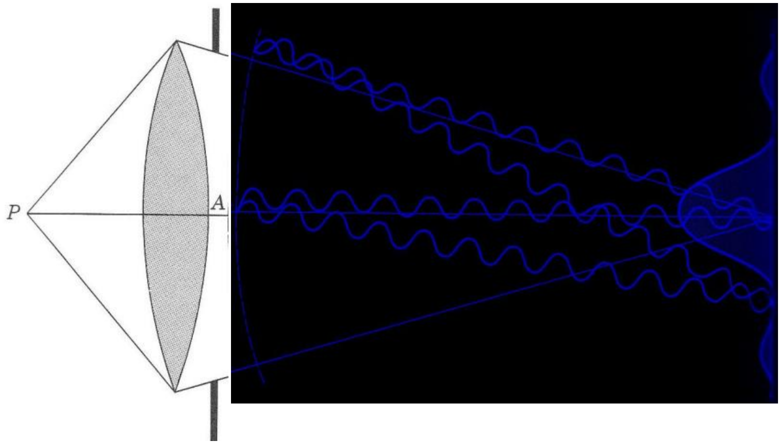
Size of the (airy) disk is a function of

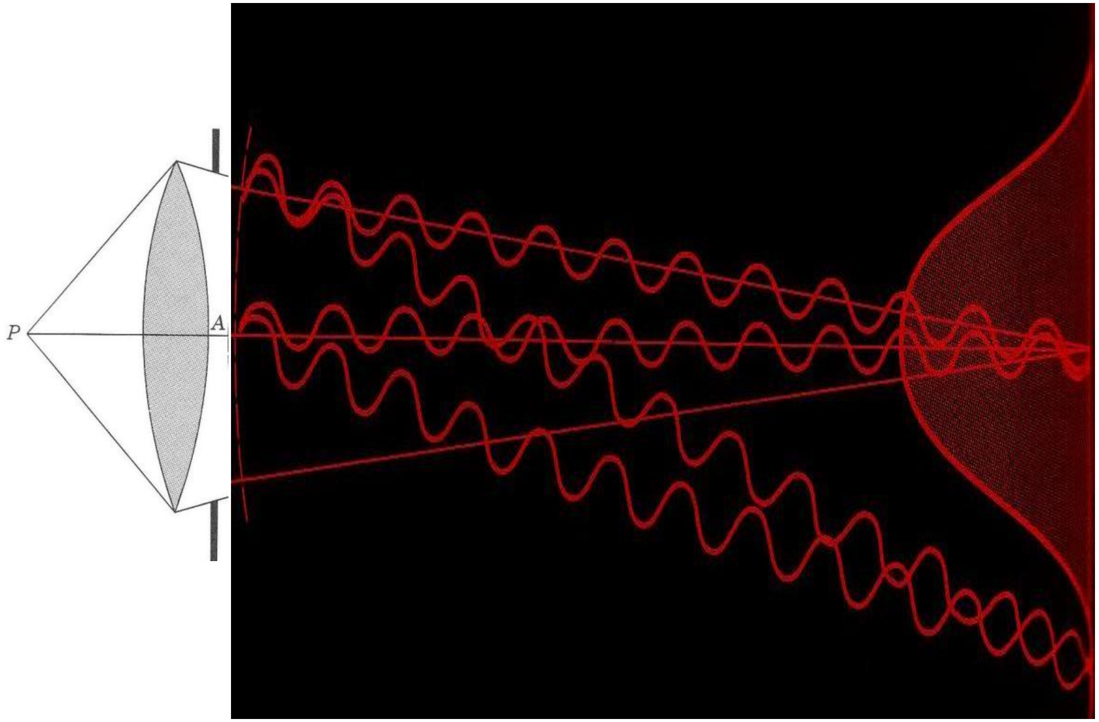
D and λ



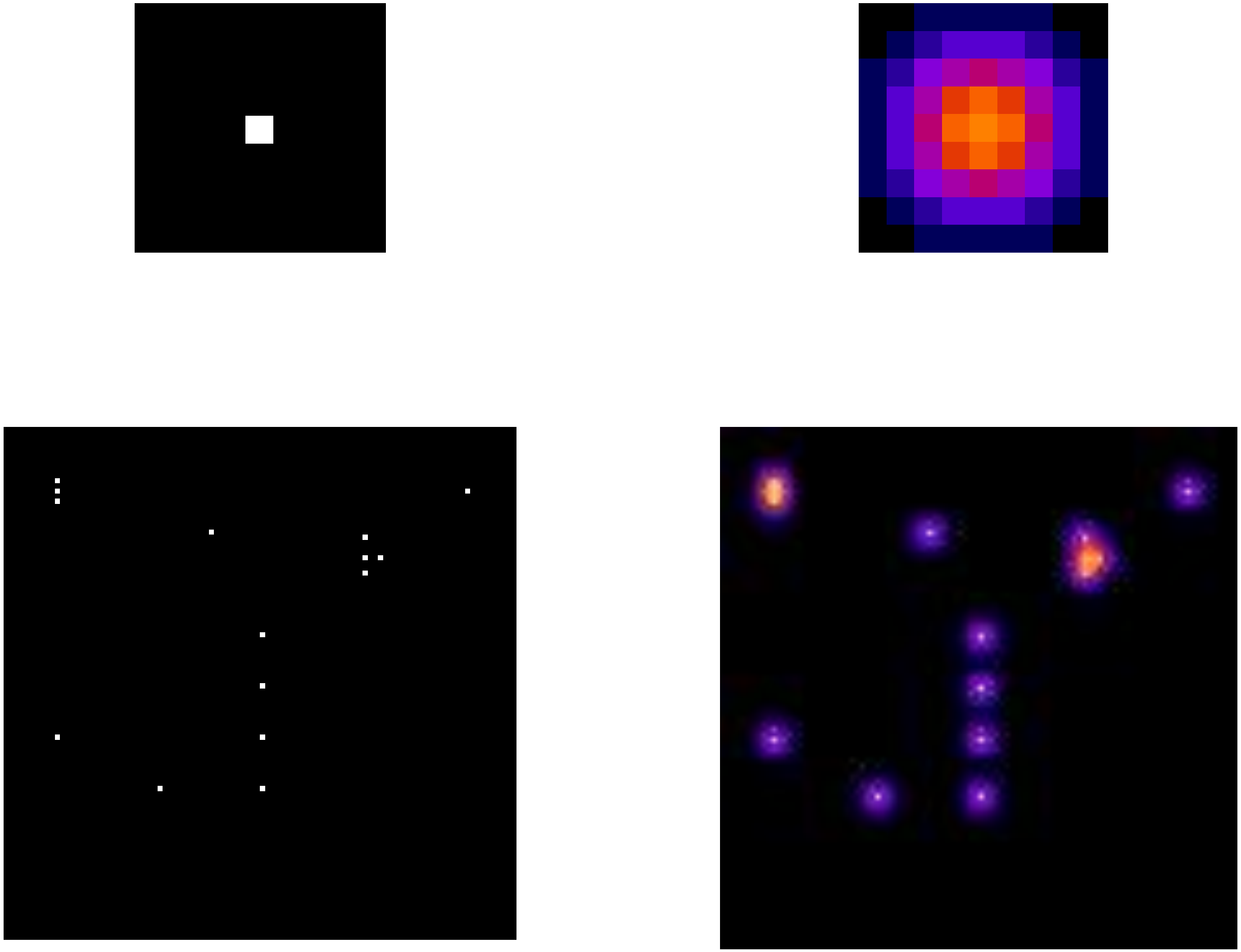


Smaller the D   Bigger the disk





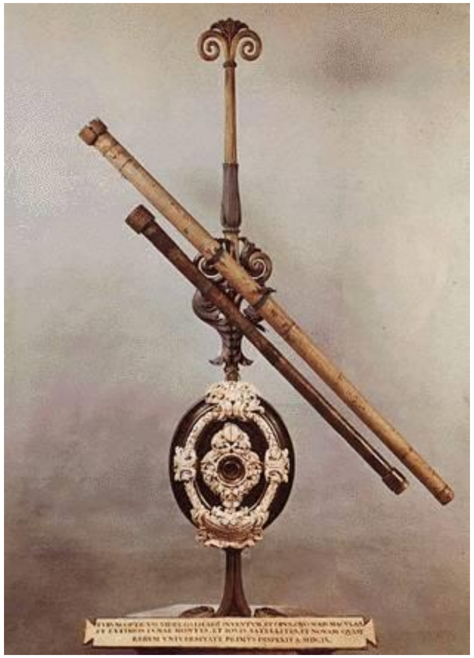
Longer the λ   bigger the disk



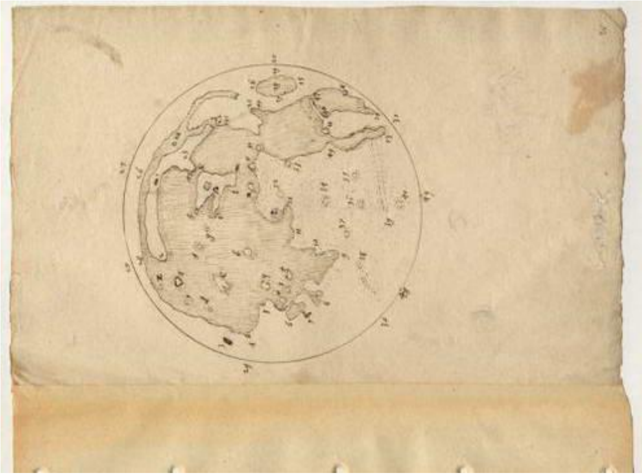
PSF size determine the resolution limit

Trends of Telescope

Size MATTERS!!!!



Galileo ~1600
D ~ 5cm



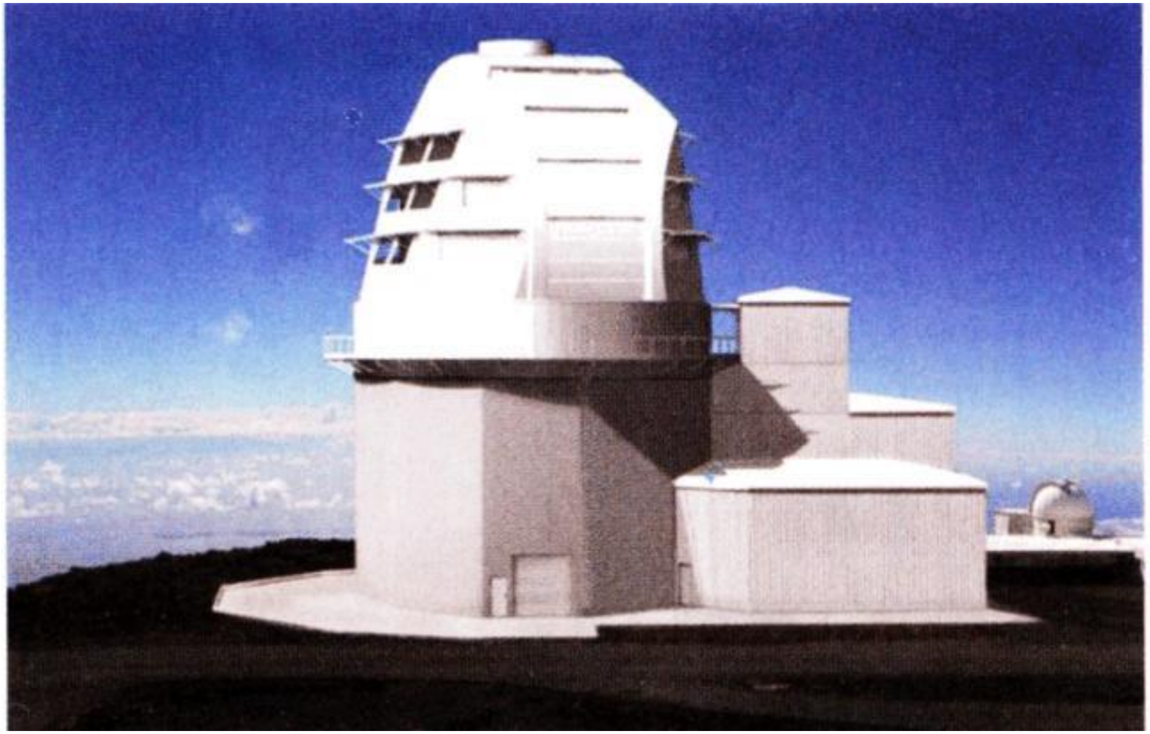
Moon

Hubble 1990
D ~ 2.4m

Courtesy of the European Space Agency

Herschel Space Telescope

Location: L2 Lagrange point
Launch: 2009
First Observations: 2010
Principal Investigator: European Space Agency
Primary Mirror Diameter: 3.5 m
Observed Wavelength Range: 60 to 670 μm



Courtesy of Tom Kekona, K.C. Environmental Inc.

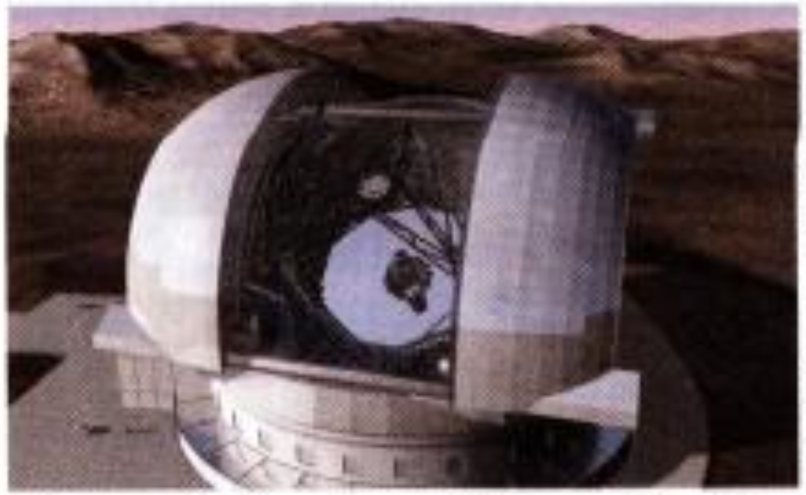
Advanced Technology Solar Telescope (ATST)

Location: Haleakala, Hawaii
Construction: 2009
First Observations: 2016
Principal Investigator: National Solar Observatory
Primary Mirror Diameter: 4.24 m
Field of View: 5 arcmin
Resolution: 30 km, or 5× the current state of the art
Observed Wavelength Range: 300 to 28,000 nm

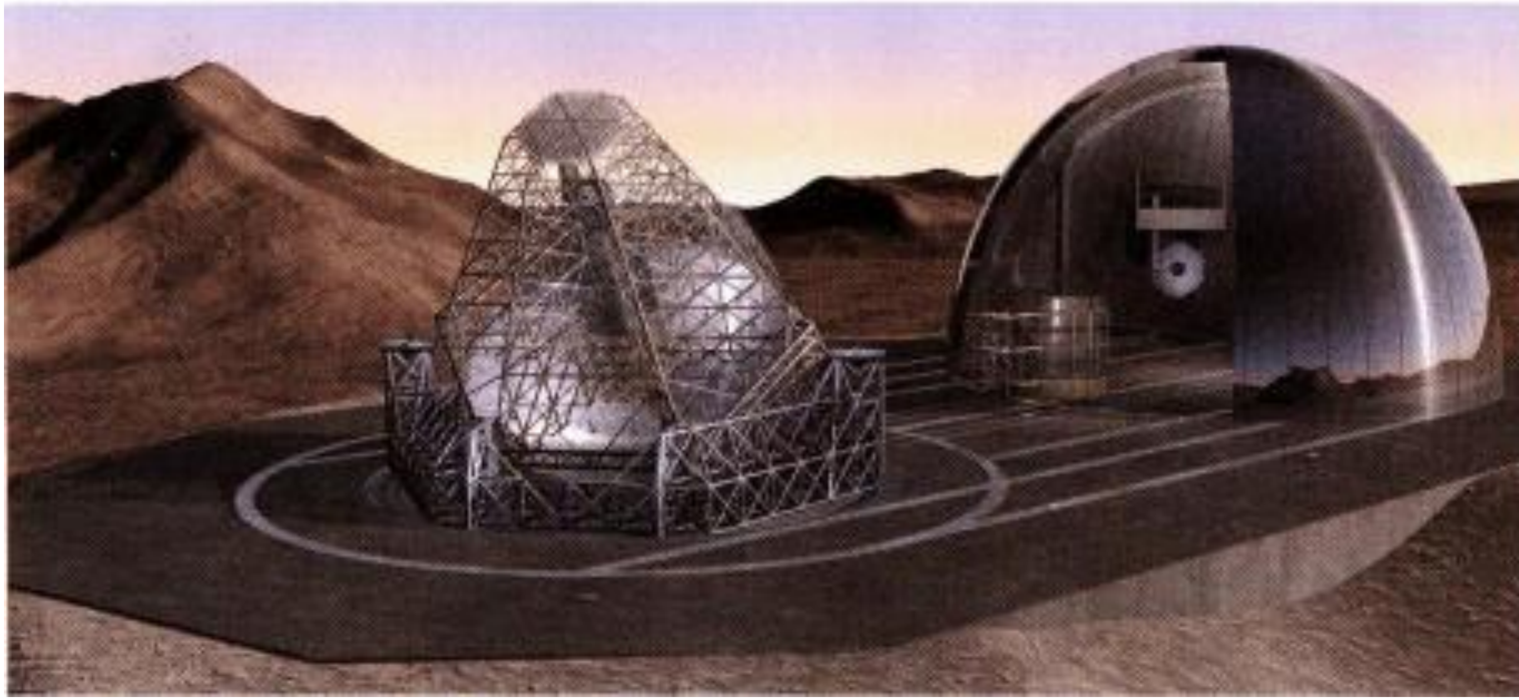
Large Synoptic Survey Telescope (LSST)

Location: Cerro Pachón, Chile
Construction: *Undergoing preliminary design review, though mirror blanks are under way*
First Observations: 2015
Principal Investigator: LSST Corp.
Primary Mirror Diameter: 8.4 m
Field of View: 9.6 degrees square
Observed Wavelength Range: 320 to 1050 nm

European Extremely Large Telescope (E-ELT)

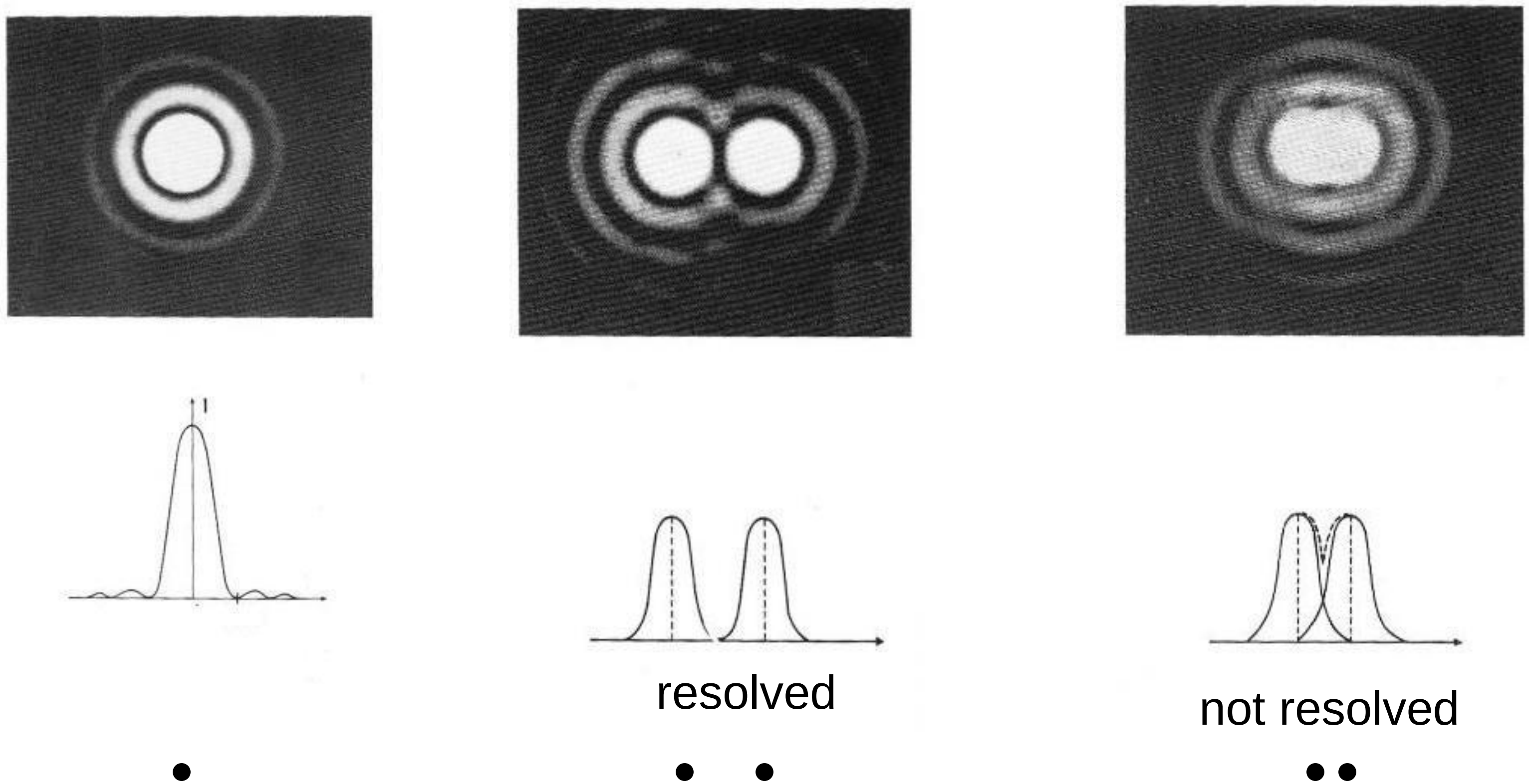


Location: To be determined, likely Chile or Canary Islands
Construction: *Scheduled to begin in 2010*
First Observations: 2017
Principal Investigator: European Southern Observatory
Primary Mirror Diameter: 42 m
Field of View: 10 arcmin diameter
Resolution: 0.001 to 0.6 arcsec
Observed Wavelength Range: Visible to near-IR



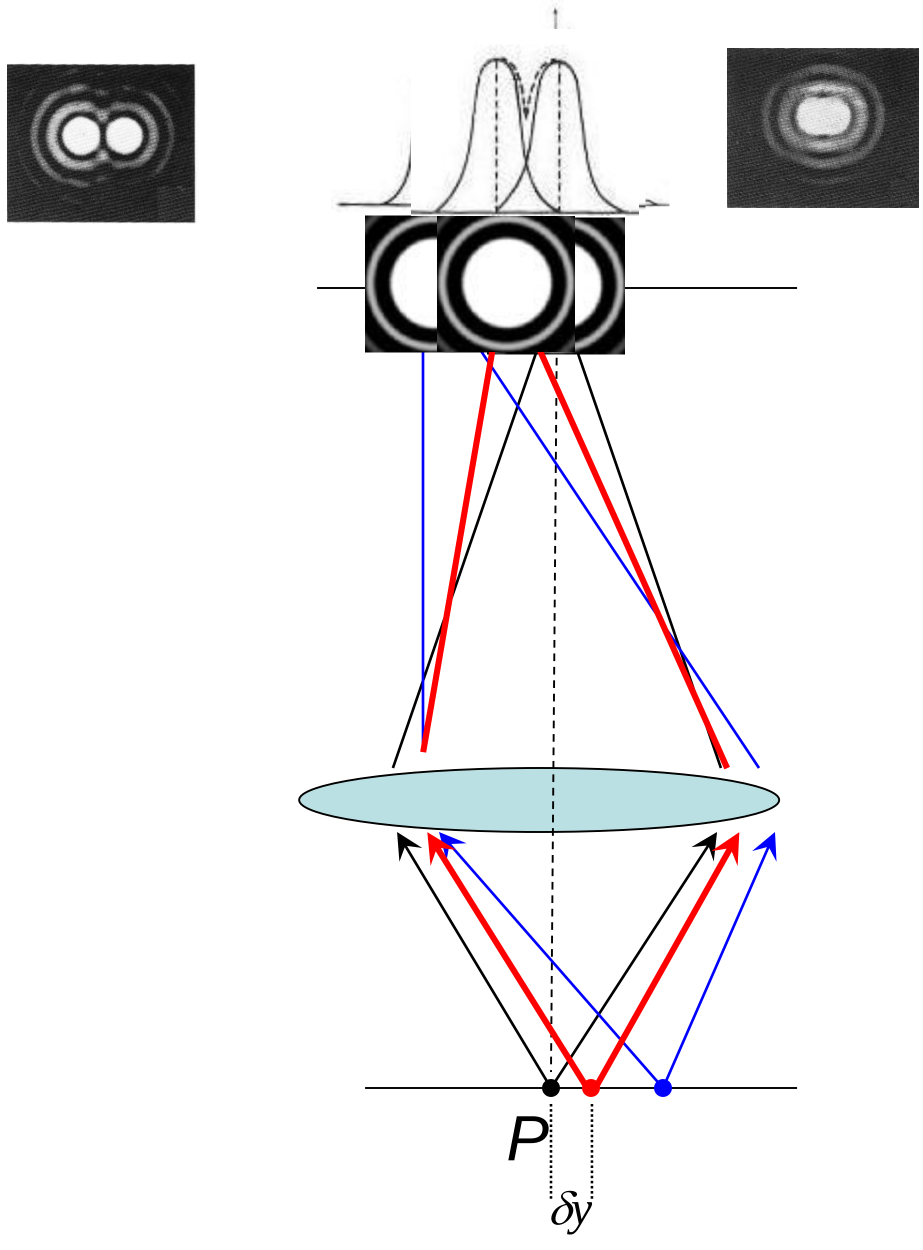
Overwhelmingly Large Telescope (OWL)

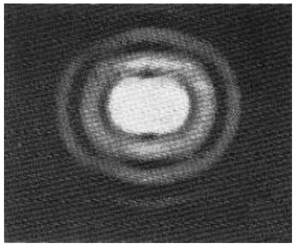
Location: To be determined, likely Chile or Canary Islands
Construction: Currently in design stage
First Observations: 2020
Principal Investigator: European Southern Observatory
Primary Mirror Diameter: 60 or 100 m, spherical
Field of View: 30 arcsec to 10 arcmin
Resolution: 1 milliarcsec
Observed Wavelength Range: 320 to 12,000 nm

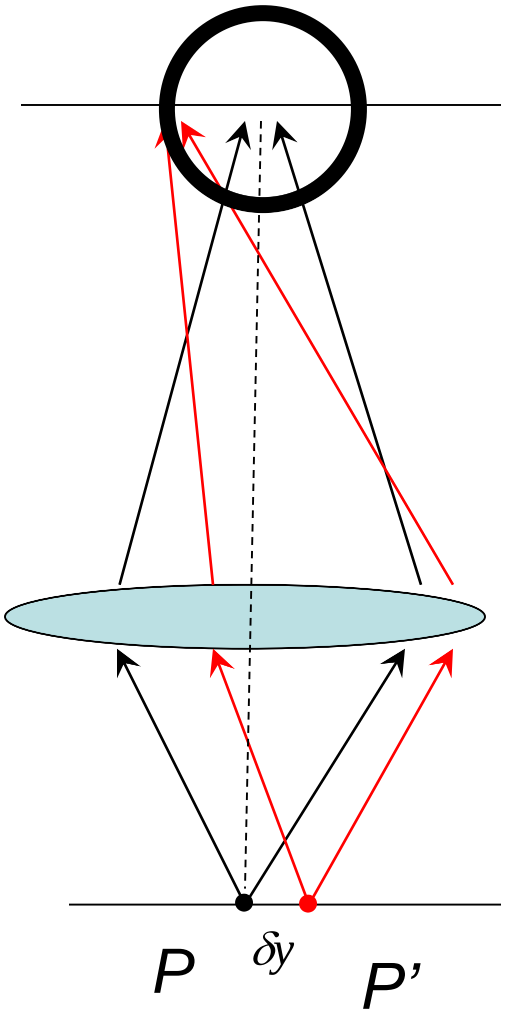


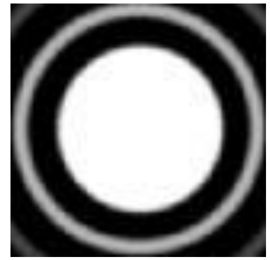
Resolution limit

The distance between two points (on object side), which we can barely resolve them as two distinguished disks on image side


P
δy

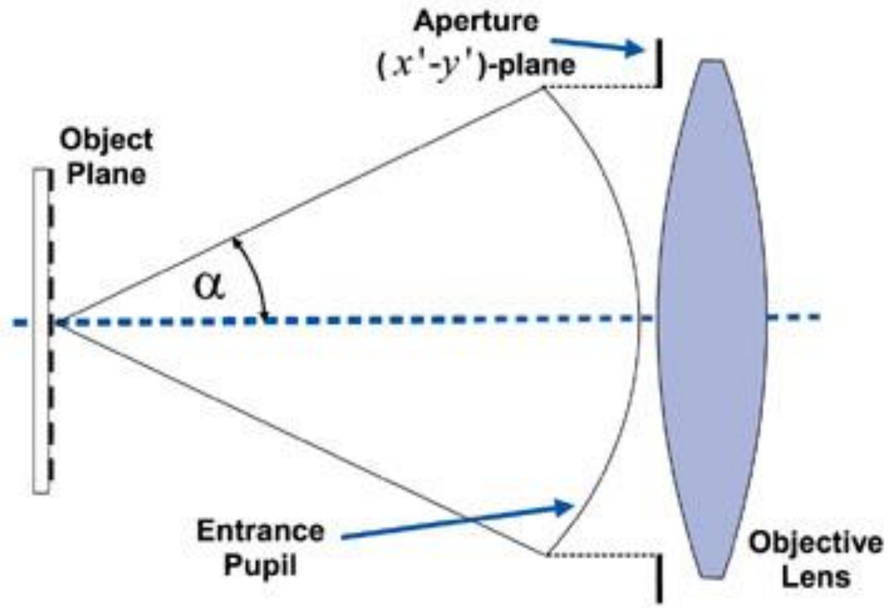






$$\delta y = 0.61\ \lambda\ /\ N.A.$$

Definition of N.A.



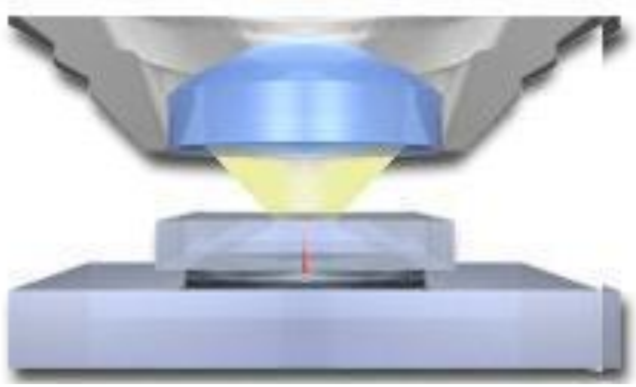
N.A. = n * sin(α)

Resolution (r) = 0.61 λ /N.A. *Lateral resolution*

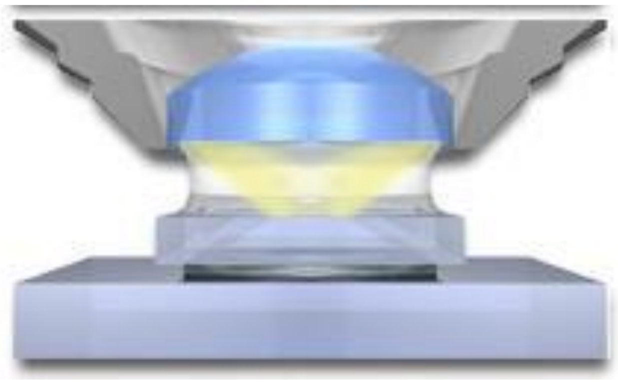
$$Resolution(r) = 0.61\,\lambda / N.A.$$

Resolution(z) = 2 λ • n / N.A.² *Axial Resolution*

$$Resolution(z) = 2\,\lambda \cdot n / N.A.^2$$



air objective n = 1.0



oil objective n = 1.518

	Resolution	
Objective/ N.A.	*x-y*	*z*
10x/0.3	*1000nm*	*11.4um*
25x/0.8	*400nm*	*2.4um*
40x/1.3	*234nm*	*1um*
63x/1.4	*218nm*	*0.8um*
100x/1.4	*218nm*	*0.8um*

$R_{x\text{-}y} = 0.61\ \lambda / N.A$

$R_z = 2\ \lambda \bullet n / N.A.^2$

λ = 500nm

Ernst Abbe
(1840-1905)

The person who laid the physics foundation of modern optics manufacture.

Cofounder of Zeiss

"Based on a precise study of the materials used, the designs concerned are specified by computation to the last detail - every curvature, every thickness, every aperture of a lens - so that any trial and error approach is excluded.“

Ernst Abbe

VIII.—*The Relation* (*f Aperture and Power in the Microscope.**

By Pr(fessor ABBE, Hon. F.R.M.S.

(*Read 10th May*, 1882.)

I.—*General Conside ations as to Wide and Narrow Apertures.*

The smallest dimensions which are within the reach of a given aperture are indicated with sufficient accuracy by taking the limit of the resolving or separating power of that aperture for periodic or *regular* structures, i.e. the minimum distance apart at which given elements can be delineated *separately* with the aperture in question. The numerical expression of that minimum distance is

$$\delta = \tfrac{1}{2}\frac{\lambda}{a},$$

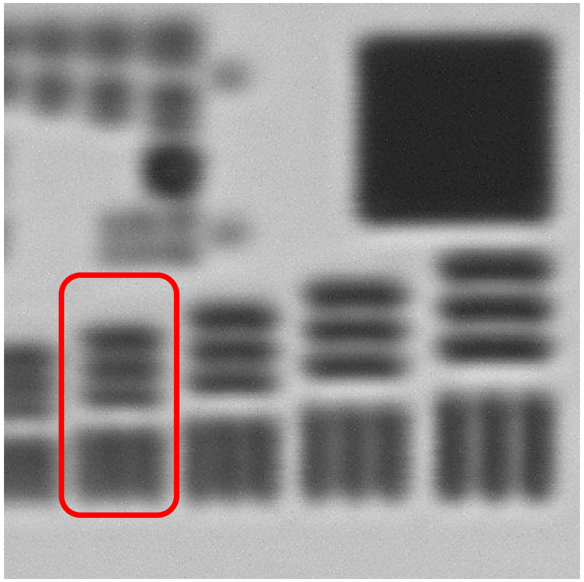
where a denotes the numerical aperture and λ the wave-length of light; a fair average is obtained for the latter element (with observations with the eye and white light), by taking $\lambda = 0 \cdot 55\ \mu$ $= 0 \cdot 00055$ mm.; i. e. the wave-length of green rays between the lines D and E, very near to the point of maximum visual intensity in the diffraction spectrum.

Practical Limit on Magnification

Theoretical limit *~200nm*	limit of human eyes ~ 0.15mm (at 25cm distance)

↓

Practical magnification : 0.15mm/200nm = 750x



2.5X/0.12 Objective

Rxy ~ 2.54um

Group 7, Element 4

Group	Element	Linewidth (micron)
0	1	500.00
	2	446.43
	3	396.83
	4	354.61
	5	314.47
	6	284.09
Group 1… Group 6		
7	1	3.91
	2	3.47
	3	3.11
	4	2.76
	5	2.46
	6	2.19
8	1	1.95
	2	1.74
	3	1.55
	4	1.38
	5	1.23
	6	1.10
9	1	0.98
	2	0.87
	3	0.78

Objective/ N.A.	Resolution	
	x-y	z
10x/0.3	1000nm	11.4um
25x/0.8	400nm	2.4um
40x/1.3	234nm	1um
63x/1.4	218nm	0.8um
100x/1.4	218nm	0.8um

$R_{x\text{-}y} = 0.61\ \lambda / N.A$

$R_z = 2\ \lambda \cdot n\ /\ N.A.^2$

λ = 500nm

The resolution limit of an objective is ONLY dependent on its N.A.

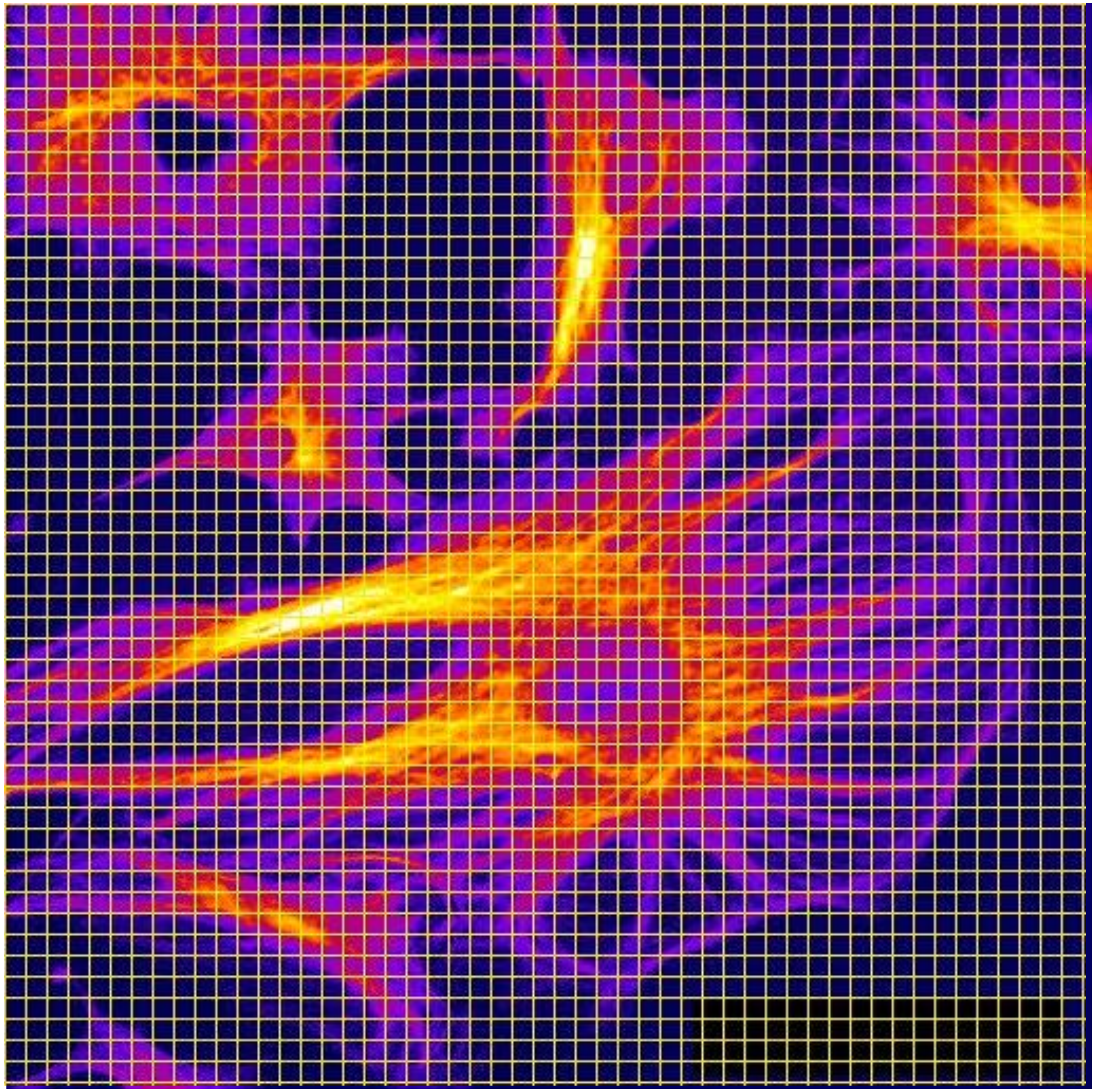
So, 63x/1.4 and 100x/1.4

Which one is better?

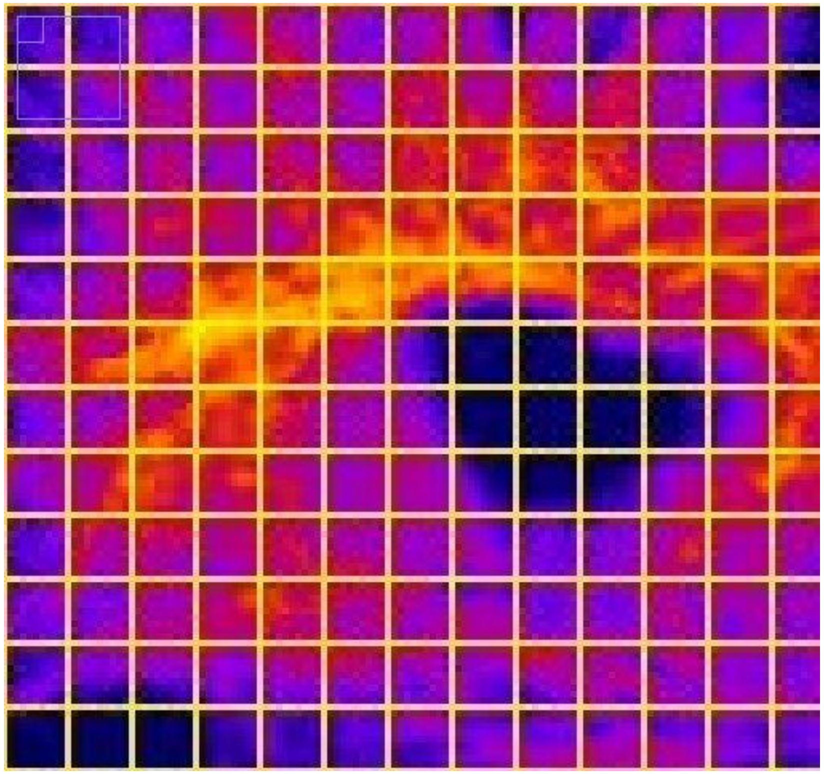
The magnification REALLY doesn’t matter on resolution?

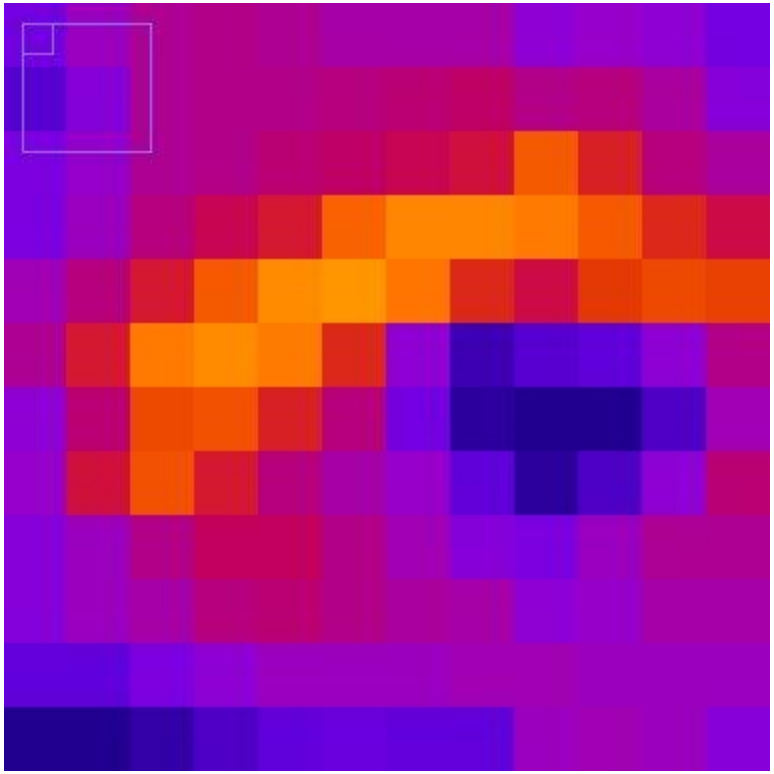
For Objectives, it does not

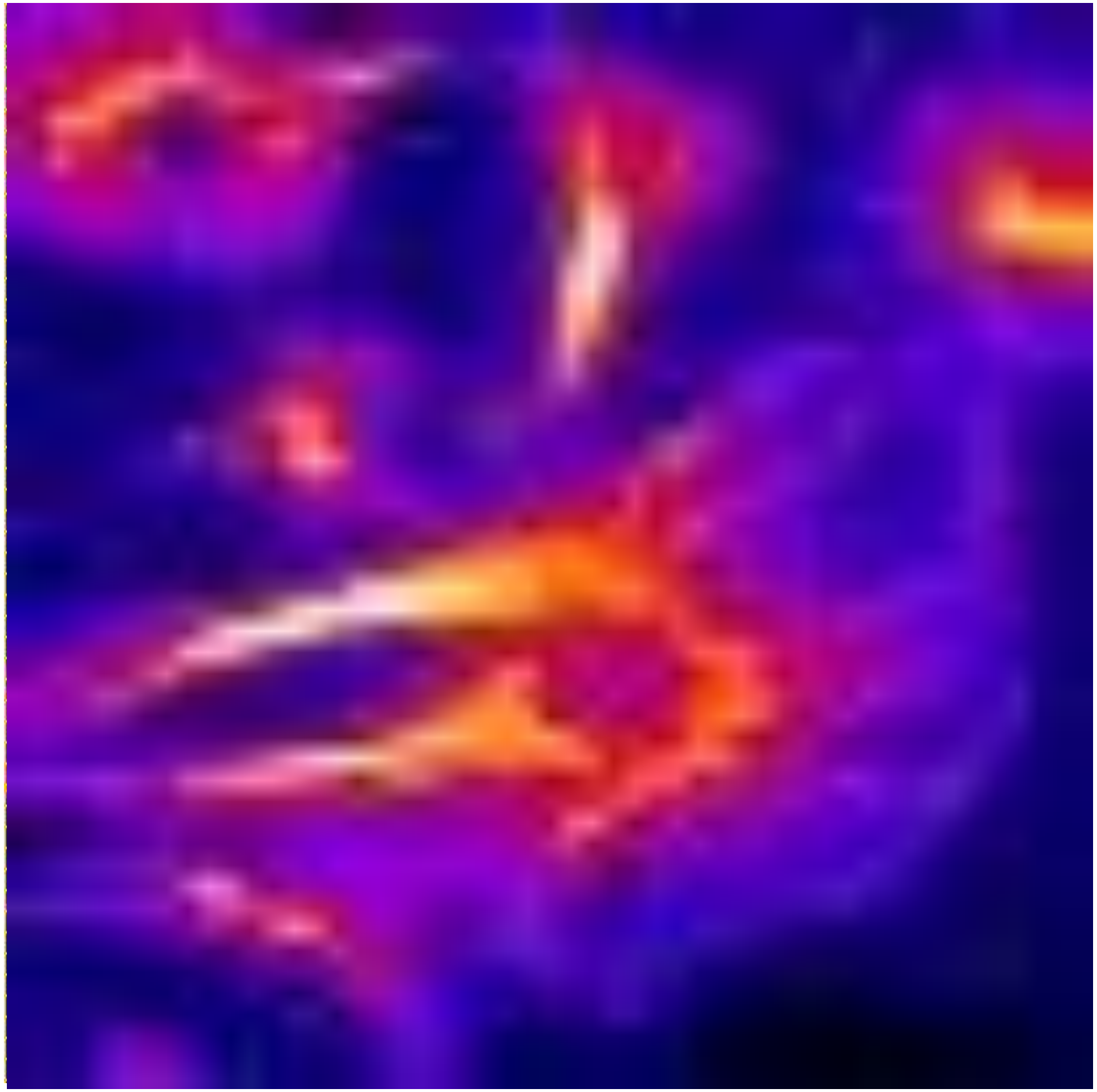
But for your “WHOLE” imaging system, it DOES!



CCD based optical imaging system





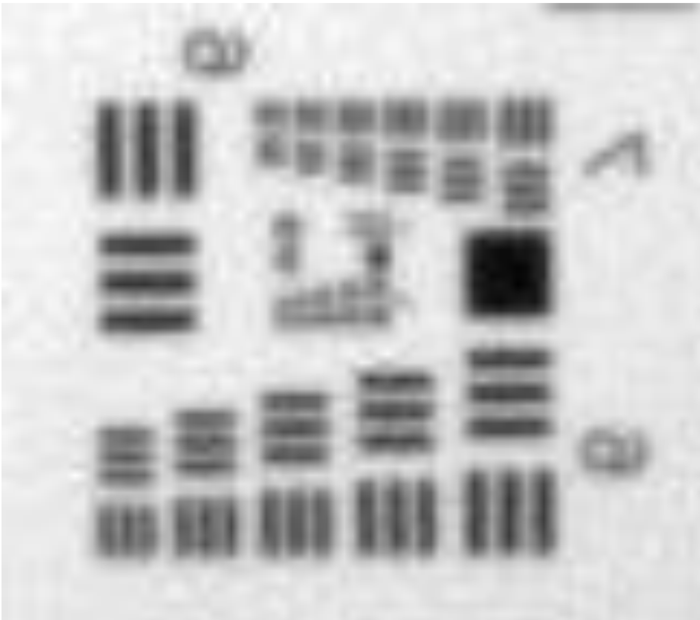


Live demo CCD based system on USAF slide

4X/0.13	2.35um	Group 7 – Element 5
10X/0.3	1.02um	Group 8 – Element 6 or Group 9 – Element 1

Resolution on objective N.A.

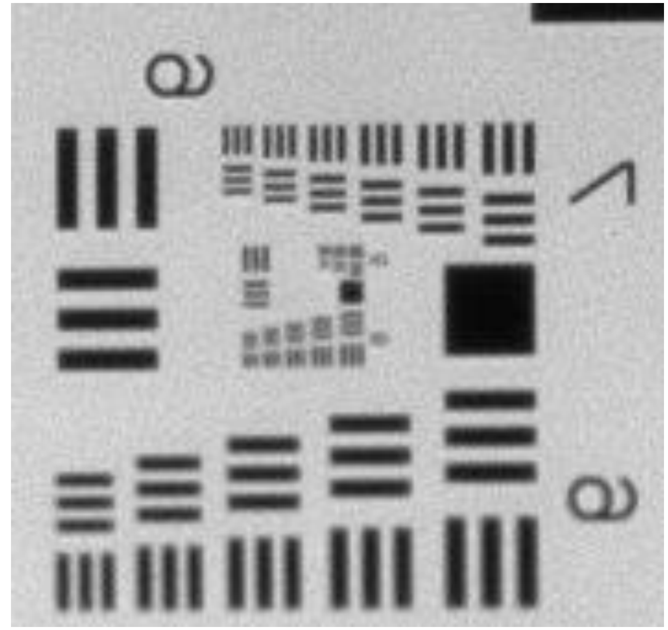
Group	Element	Linewidth (micron)
0	1	500.00
	2	446.43
	3	396.83
	4	354.61
	5	314.47
	6	284.09
Group 1... Group 6		
7	1	3.91
	2	3.47
	3	3.11
	4	2.76
	5	2.46
	6	2.19
8	1	1.95
	2	1.74
	3	1.55
	4	1.38
	5	1.23
	6	1.10
9	1	0.98
	2	0.87
	3	0.78



4X

Best guess group 6 – Element 6

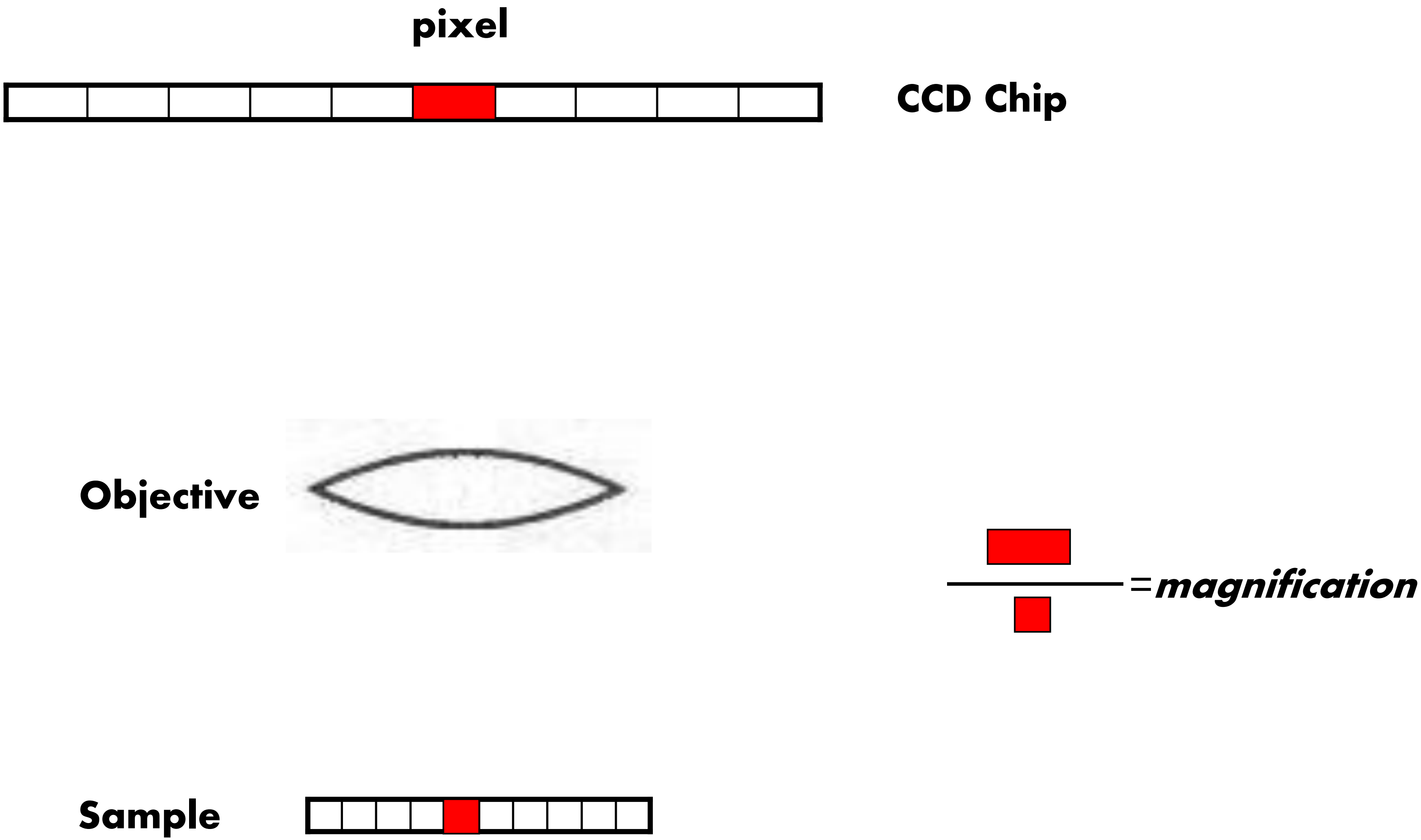
4.40um



10X

Best guess group 8 – element 1

1.95um

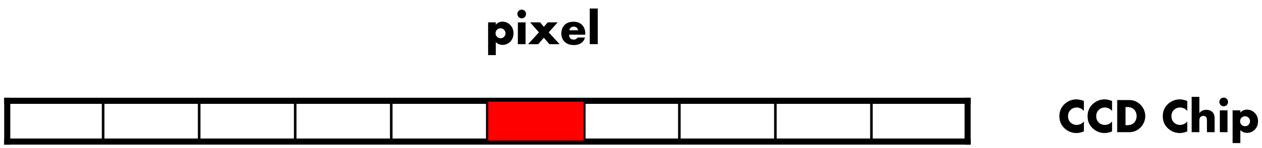

pixel
CCD Chip
Objective
=magnification
Sample

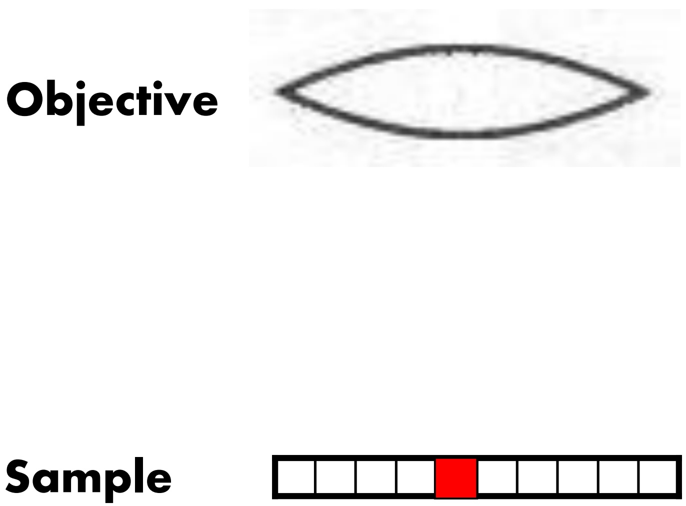
Scientific Camera CCD pixel size

Photometric Evolve 16um x 16um

Hamamatsu C9100-02 8um x 8um

Qimaging Retiga-SRV 6.4um x 6.4um





magnification	pixel size	pixel size on sample
4x	16um	4um
10x	16um	1.6um
100x	16um	160nm

objective magnification	CCD pixel size	pixel size on sample	N.A.	resolution
4x	16um	4um	0.13	2.35um
10x	16um	1.6um	0.3	1.02um
100x	16um	160nm	1.4	160nm

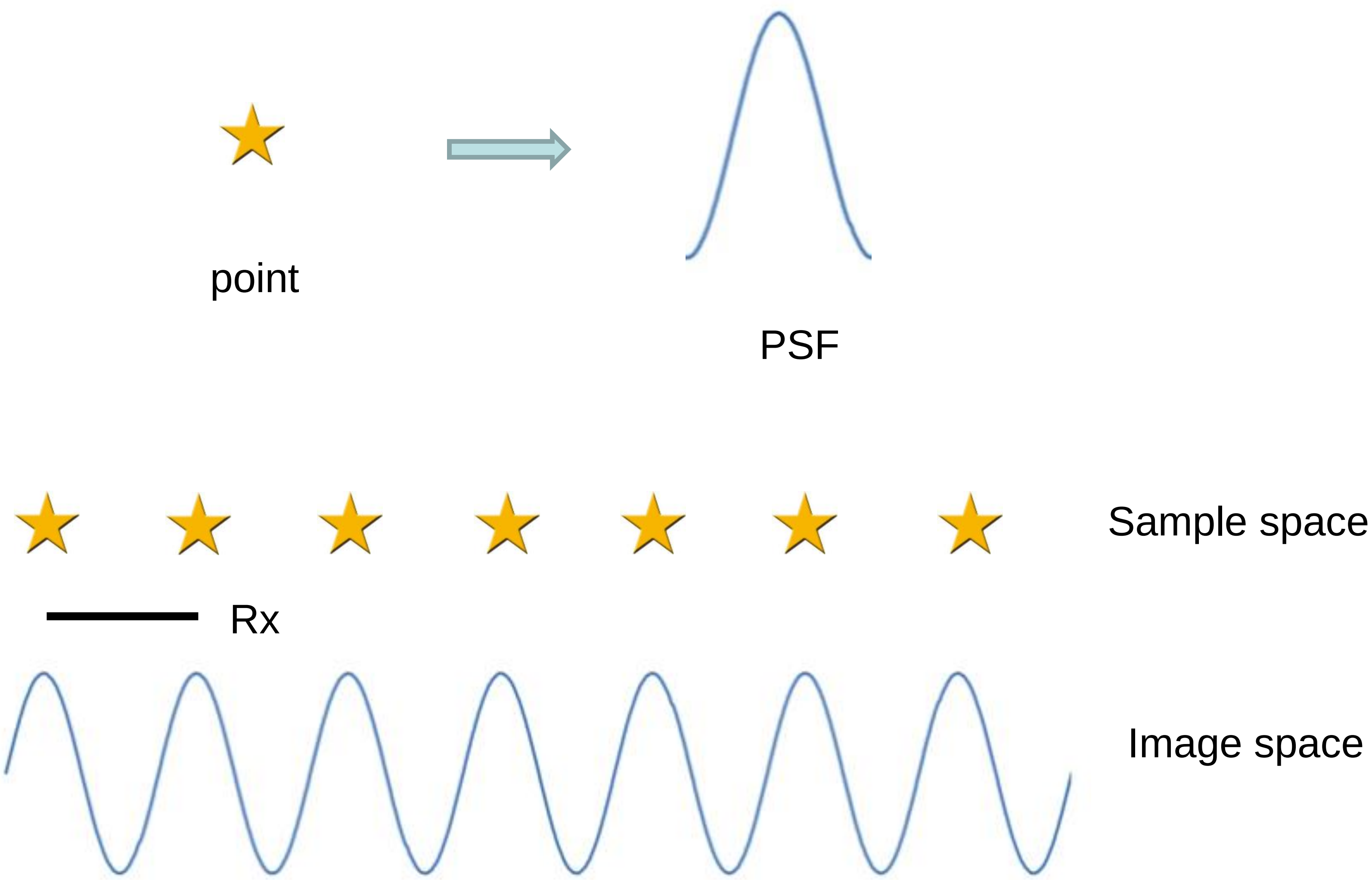
CCD pixel size combined with objective N.A. determines the resolution limit of the "WHOLE" imaging system

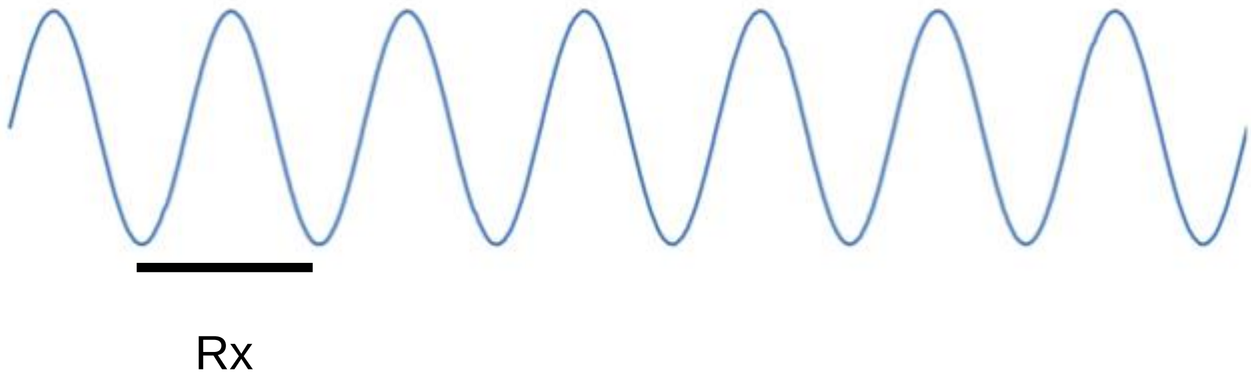
Actual resolution we achieved ~4.38um (4X) and ~1.95um (10X)

Knowing the resolution limit of the objective, what sampling size (pixel scale) we should choose to “realize” such resolution limit?

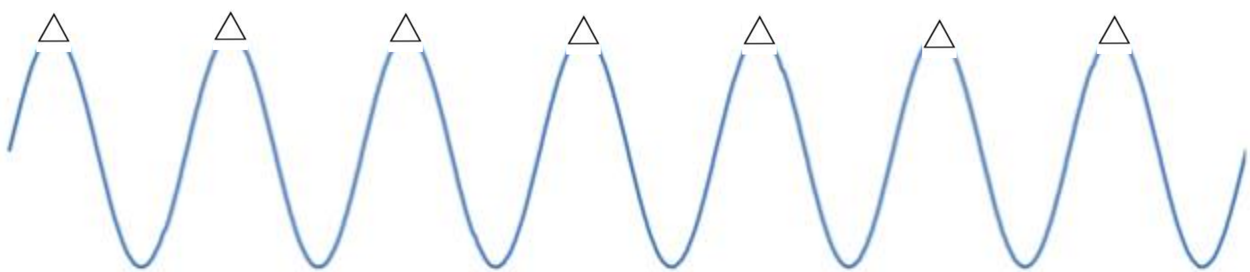
Nyquist–Shannon sampling theorem

Exact reconstruction of a continuous-time baseband signal from its samples is *possible* if the signal is bandlimited and the sampling frequency is greater than *twice* the signal bandwidth.


point
PSF
Sample space
Rx
Image space

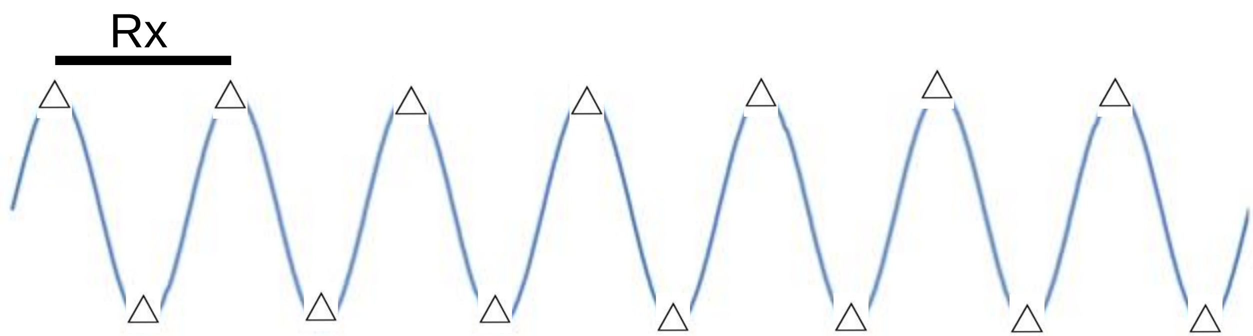


Sampling rate is same as Rx



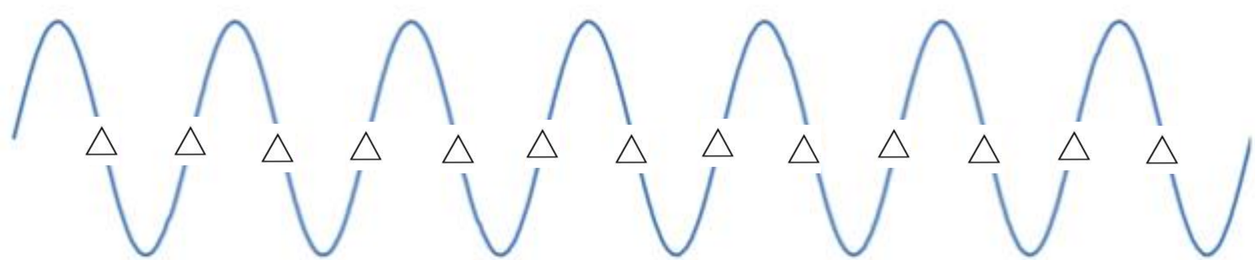
We got a flat signal

Sampling rate is same as Rx / 2

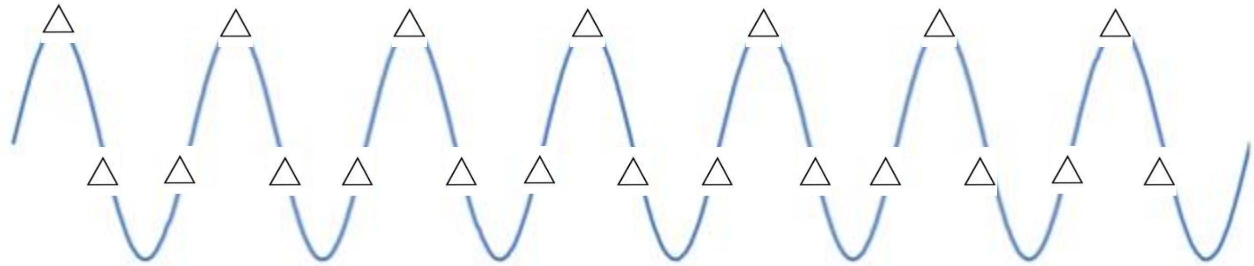


Seems good.

However, if you are unlucky (and normally, you ARE)



Sampling rate is the same as Rx / 3



Or