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MMIB 2025

LIGHT MICROSCOPY – image formation

magnification/resolution

image spatial frequencies

image of single point

spatial resolution of an image

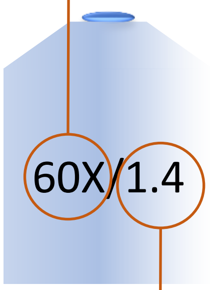
…image sampling

MAGNIFICATION / RESOLUTION

Magnification

"How many times the apparent size of the object is increased"

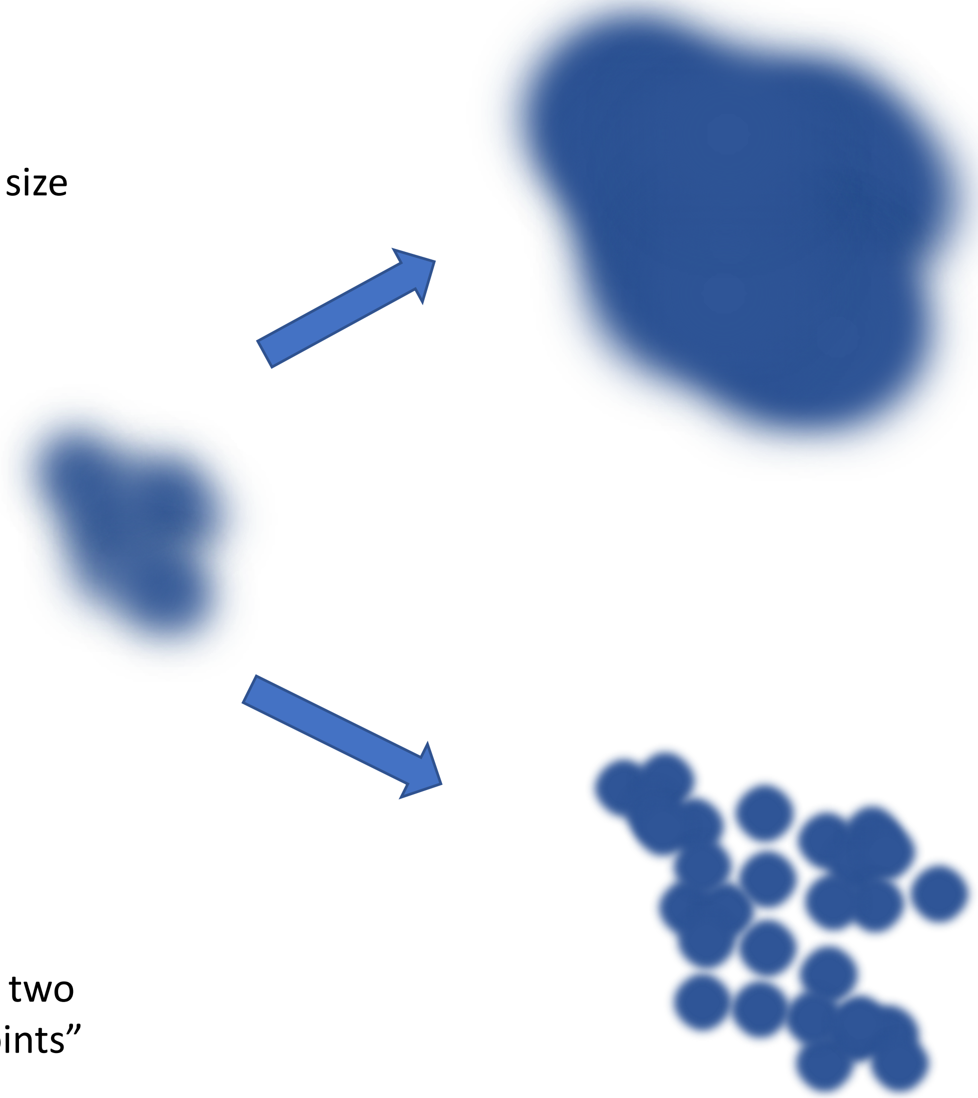
TO SEE THINGS BIGGER



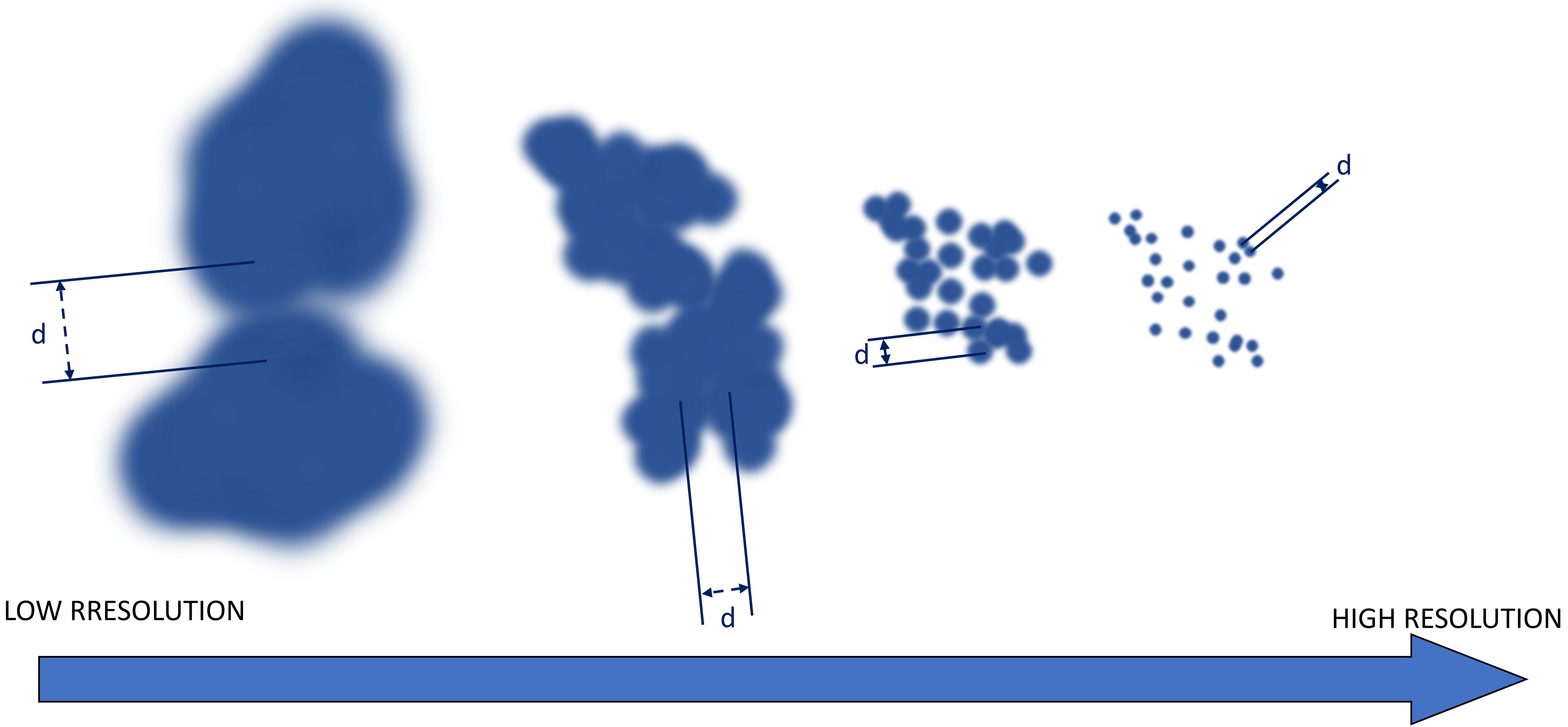
Resolution

"The smallest distance at which two points can be identify as two points"

TO SEE MORE DETAILS



RESOLUTION



SPATIAL FREQUENCIES AND IMAGE FORMATION

https://www.youtube.com/watch?v=k8FXF1KjzY0

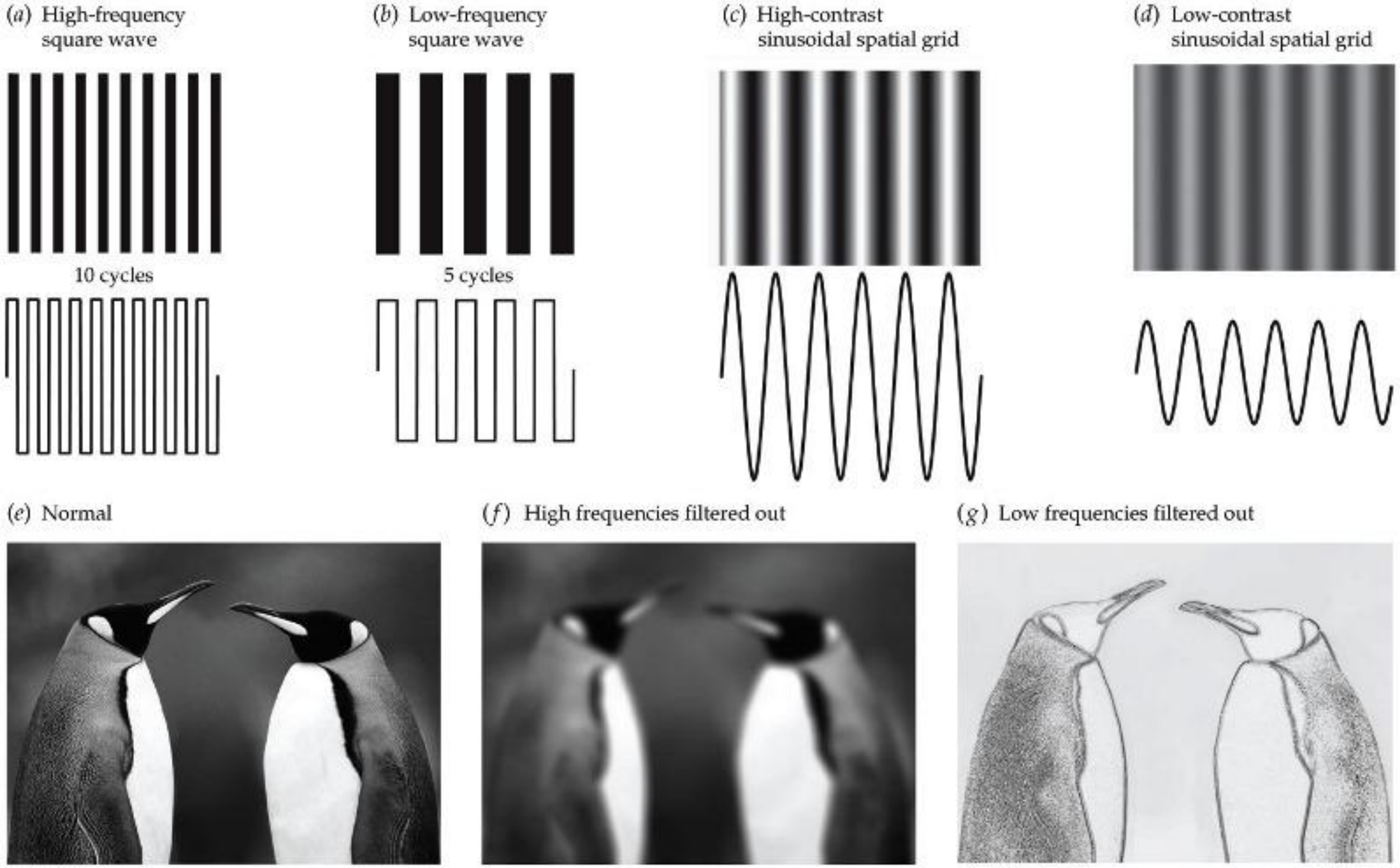
https://www.youtube.com/watch?v=WSkczQe6YxA

https://www.youtube.com/watch?v=cUD1gMAl6W4

https://www.youtube.com/watch?v=eliXYhMtXr8

https://www.youtube.com/watch?v=xhO8iz2qCOE&t=135s

SPATIAL FREQUENCIES AND IMAGE FORMATION



https://2e.mindsmachine.com

Spatial Frequencies

(*a*, *b*) The spacing between dark and light stripes shows that the grating in part a has double the spatial frequency of the grating in part *b*. (*c*, *d*) These visual grids show sinusoidal modulation of intensity: (*c*) high contrast; (*d*) low contrast. (*e*–*g*) A photograph of two penguins subjected to spatial filtering: (*e*) normal photograph; (*f*) high spatial frequencies filtered out; (*g*) low spatial frequencies filtered out.

FOURIER TRANSFORM AND FILTERING OF THE IMAGE

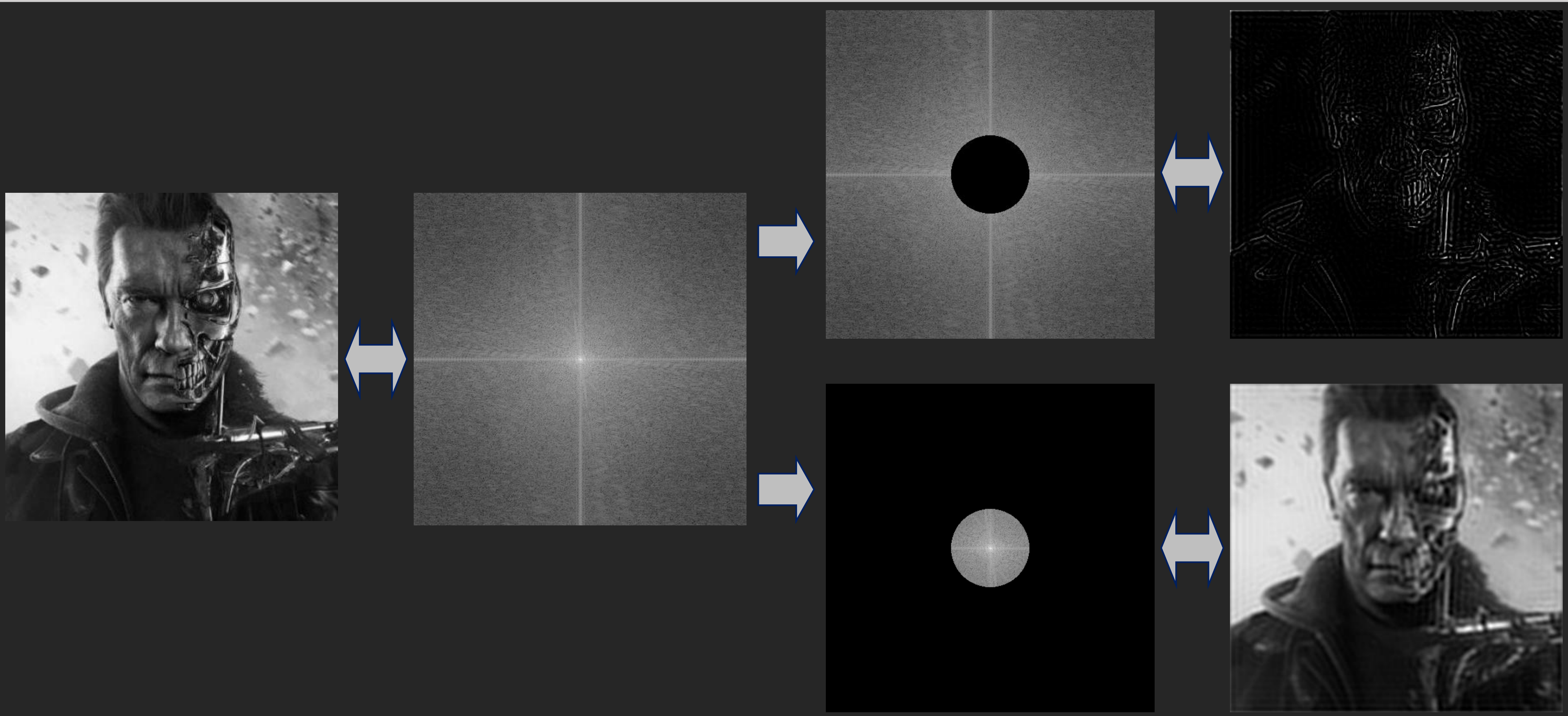
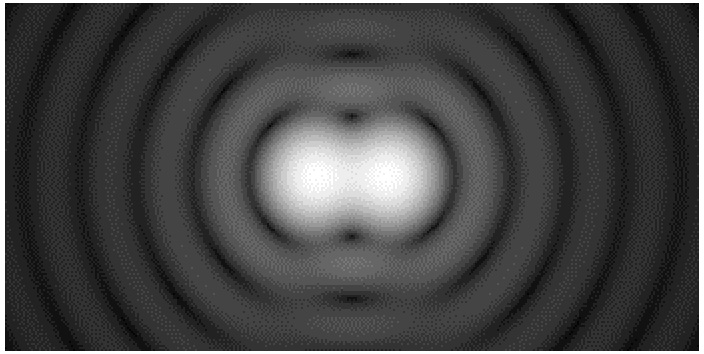


IMAGE OF A POINT, DIFRACTION LIMIT, RESOLUTION

The diffraction limit is **the point where two Airy patterns are no longer distinguishable from each other.**



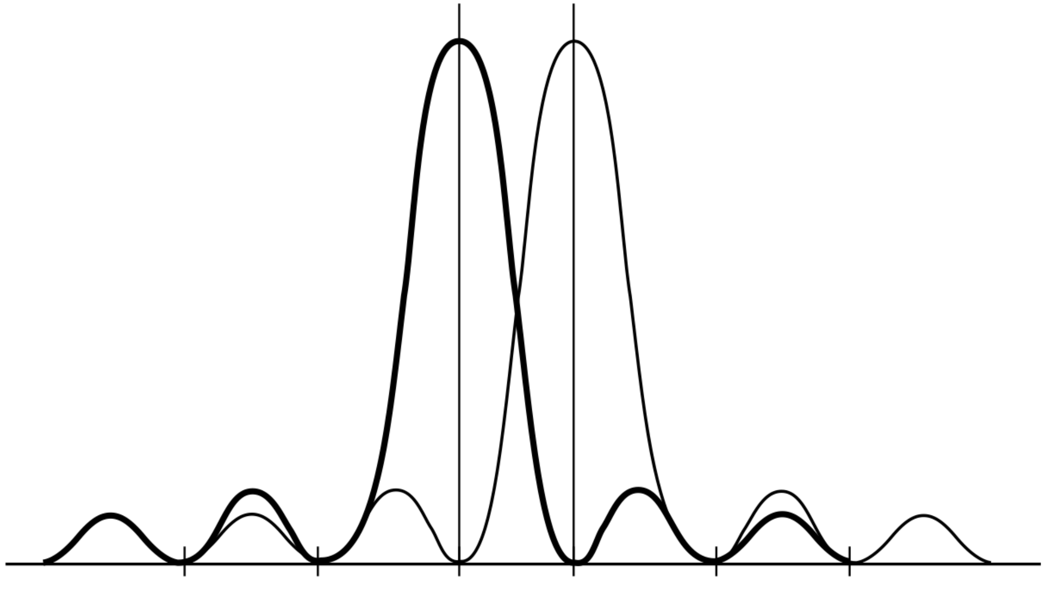
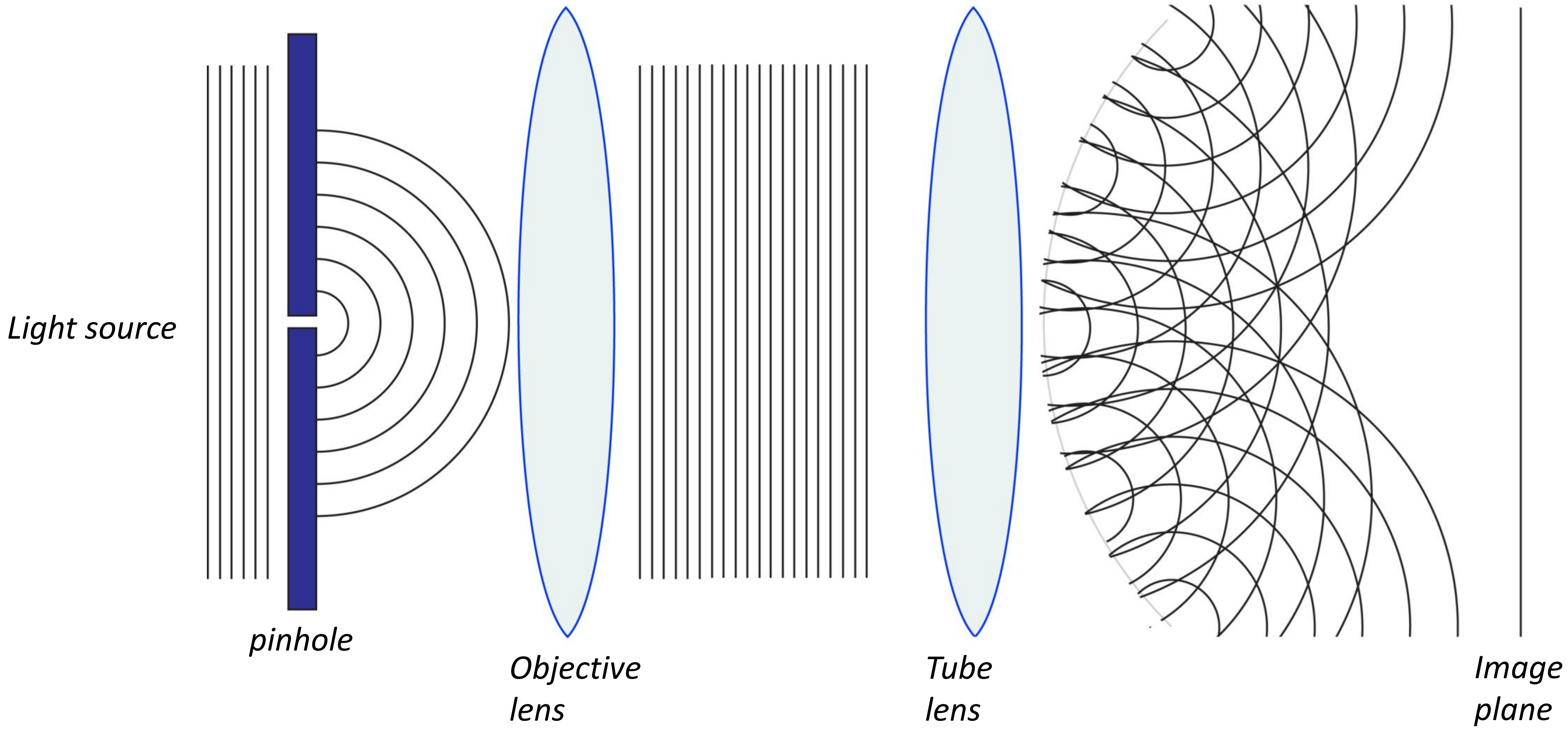



IMAGE OF A POINT, DIFRACTION LIMIT, RESOLUTION



SPATIAL FREQUENCY DEFINES THE FINAL RESOLUTION

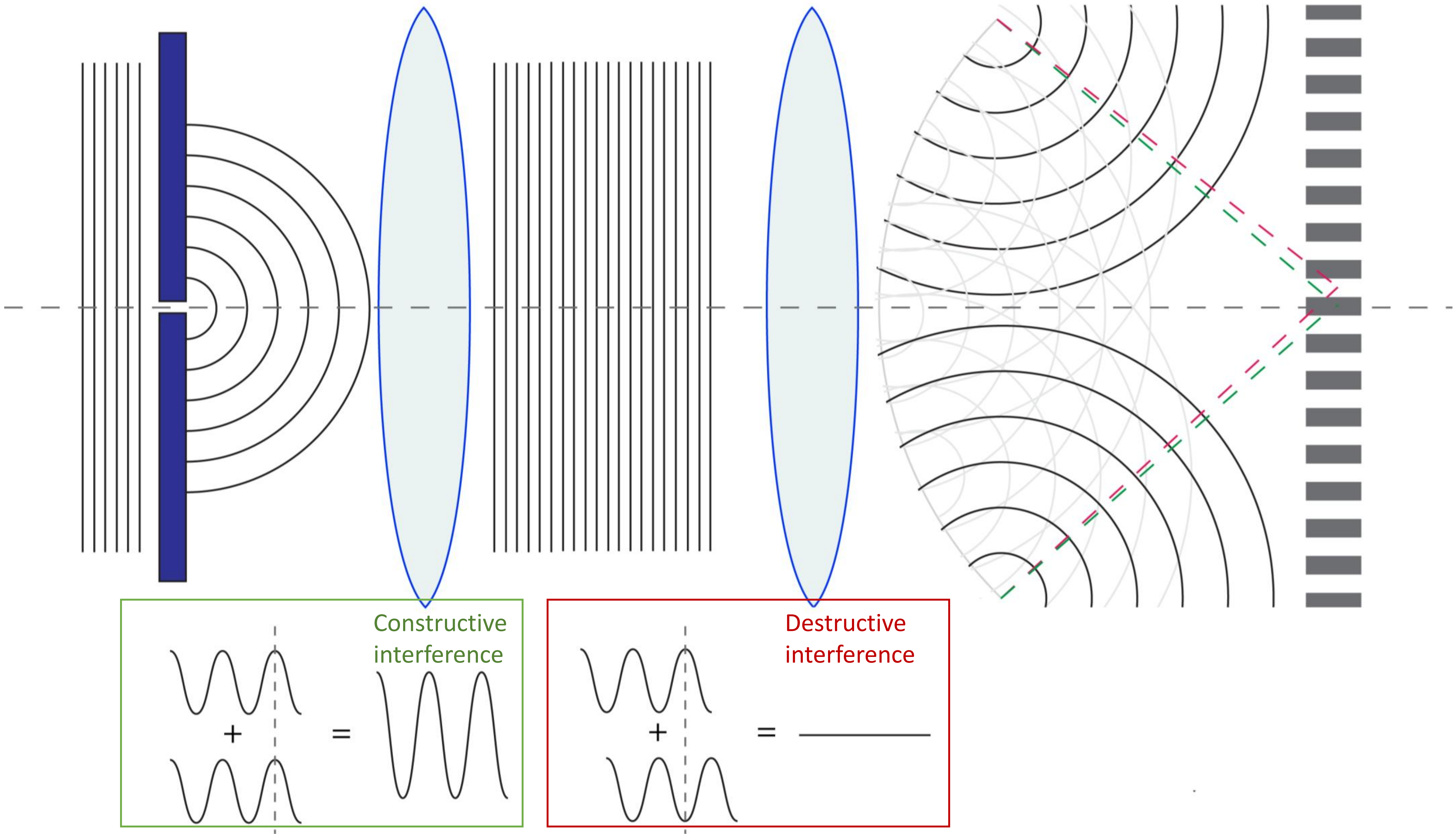
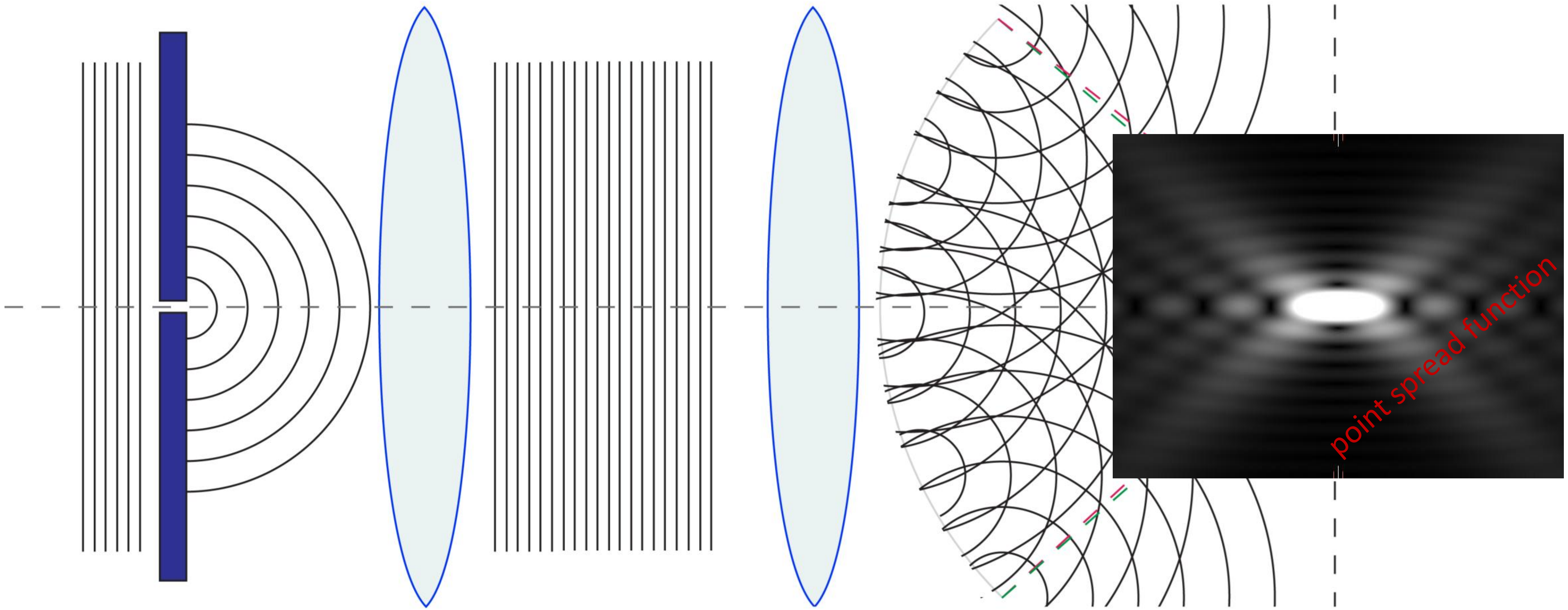
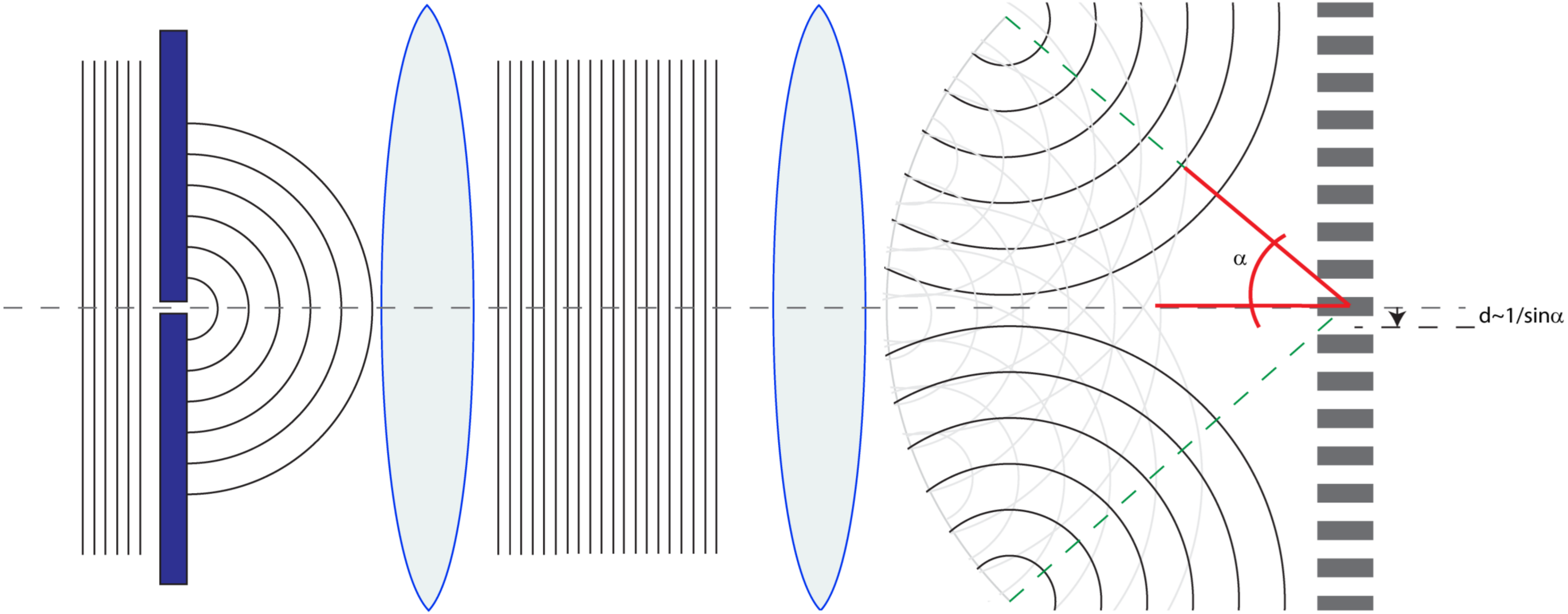


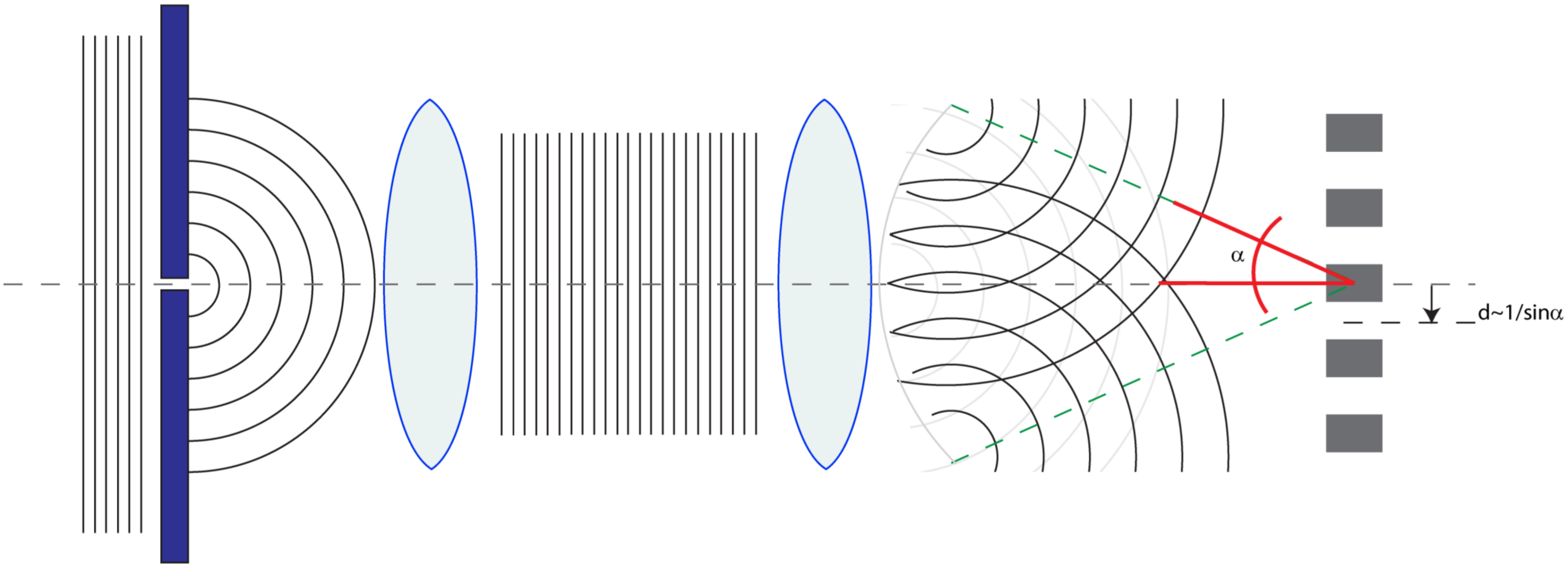
IMAGE OF A POINT CORRESPONDS TO PSF



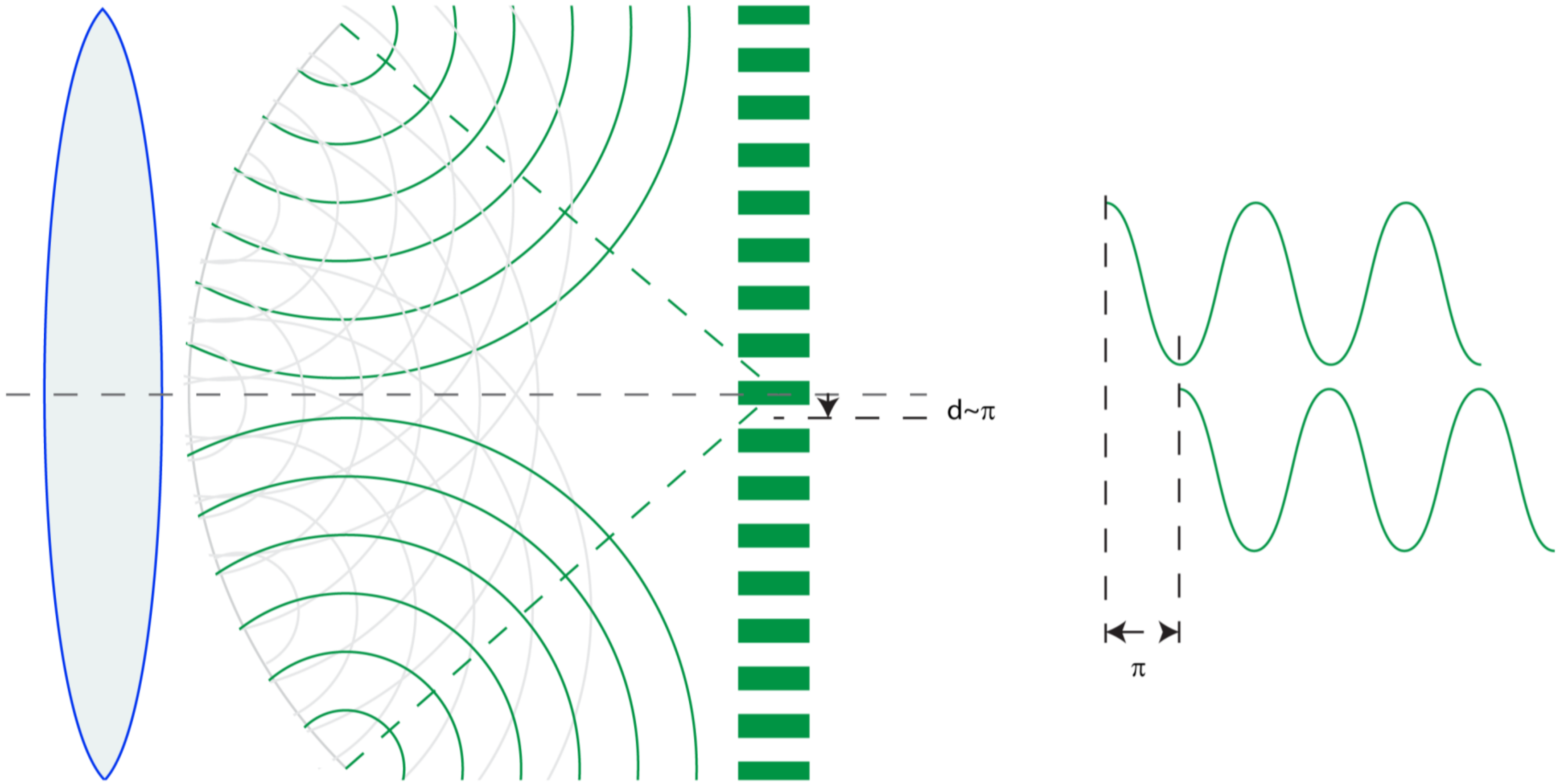
ACCEPTANCE ANGLE DEFINES THE FINAL RESOLUTION



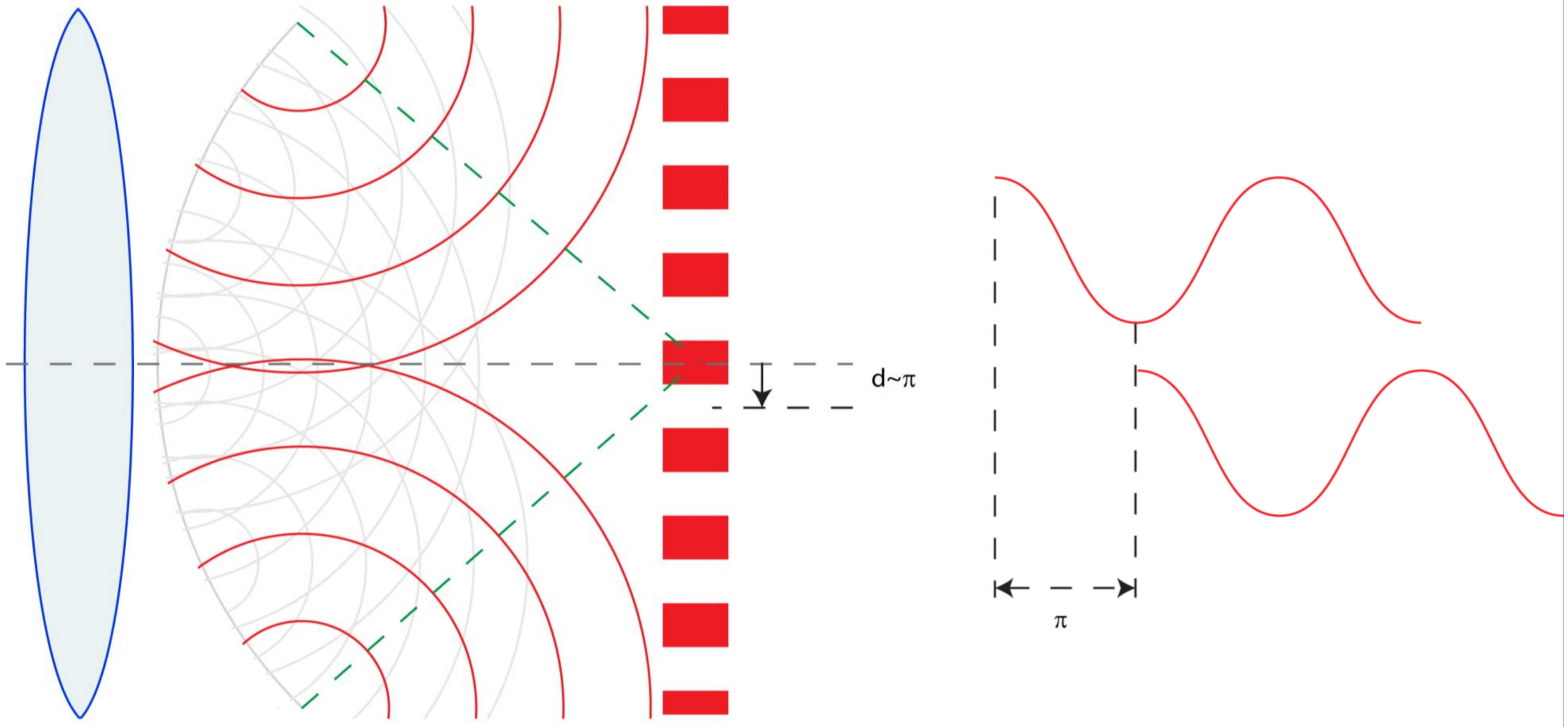
ACCEPTANCE ANGLE DEFINES THE FINAL RESOLUTION



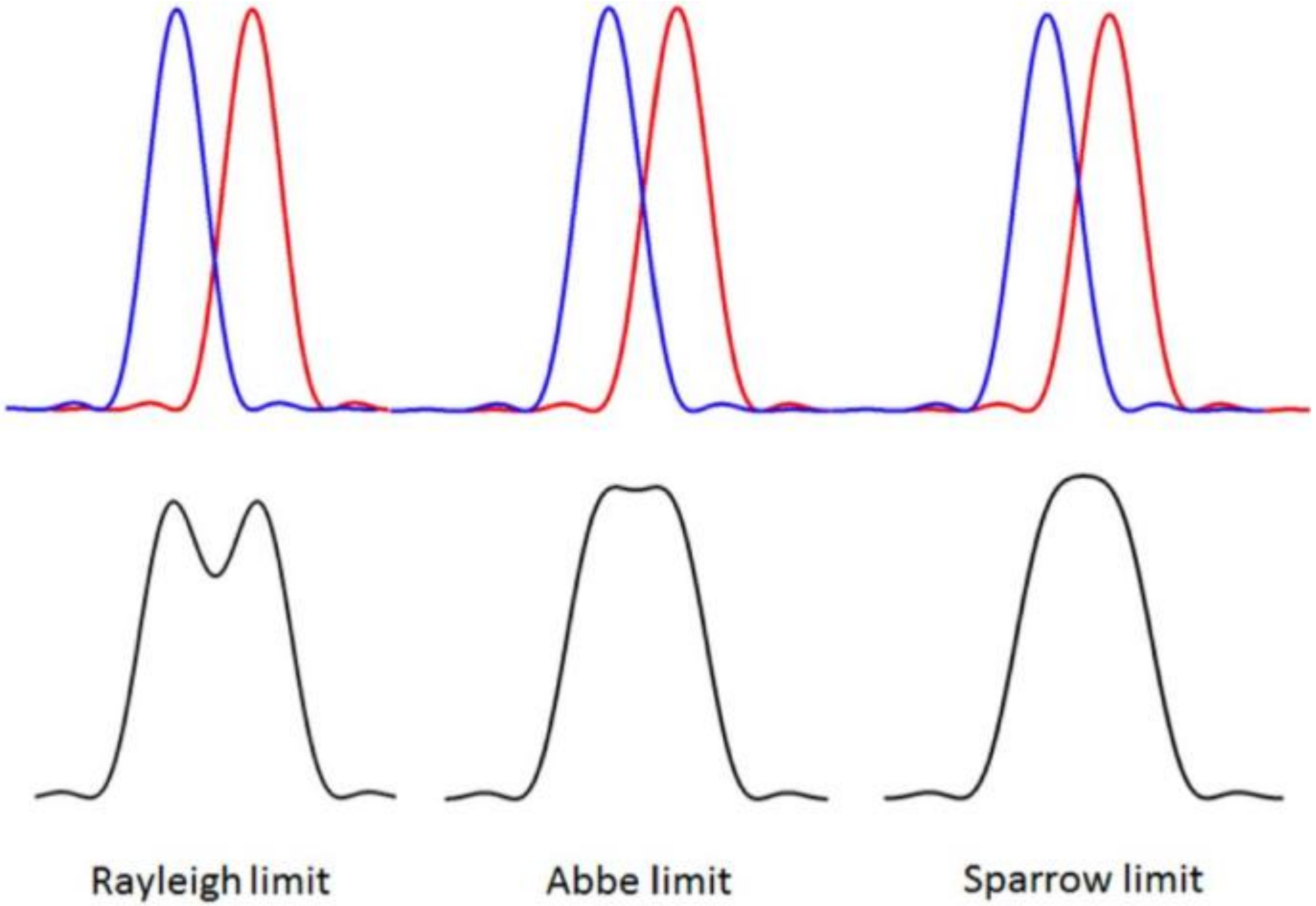
VAWELENGTH DEFINES THE FINAL RESOLUTION



VAWELENGTH DEFINES THE FINAL RESOLUTION



RESOLVED?

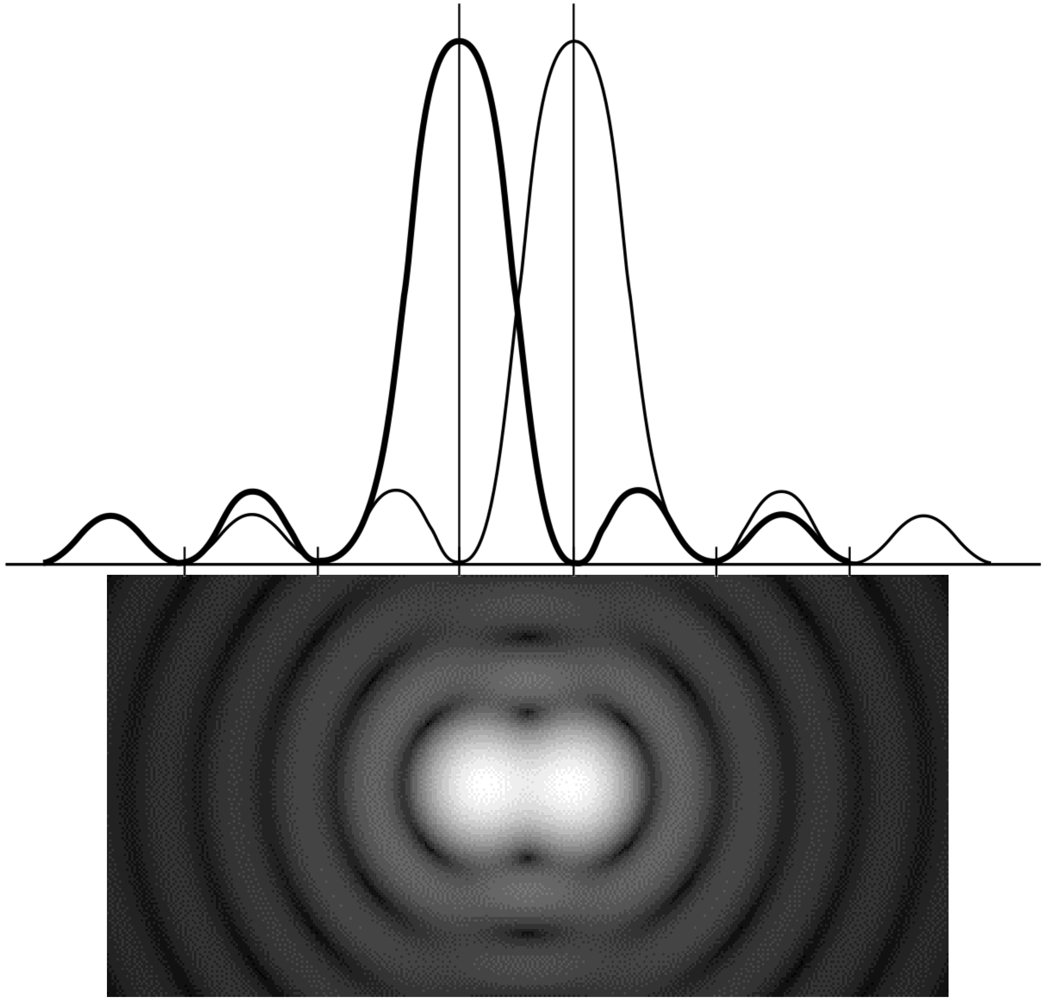


$$d = \frac{0.61 \times \lambda}{NA}$$

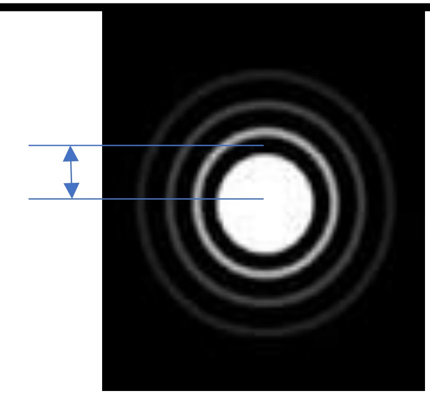
$$d = \frac{0.5 \times \lambda}{NA}$$

$$d = \frac{0.47 \times \lambda}{NA}$$

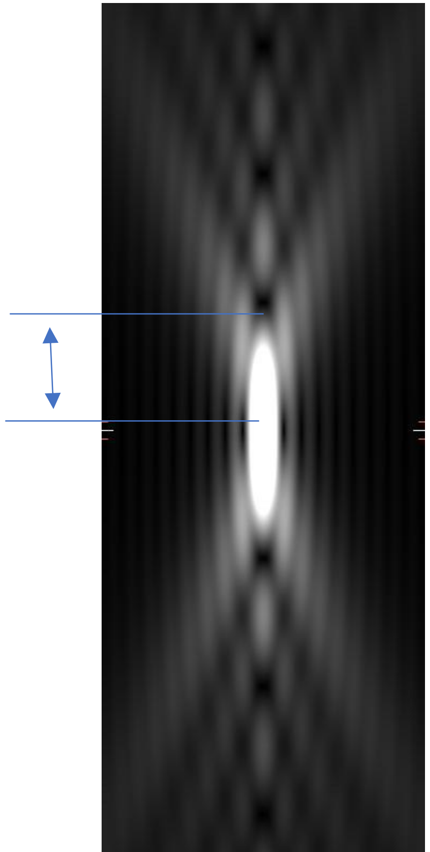
DIFFRACTION LIMIT BY RAYLEIGH



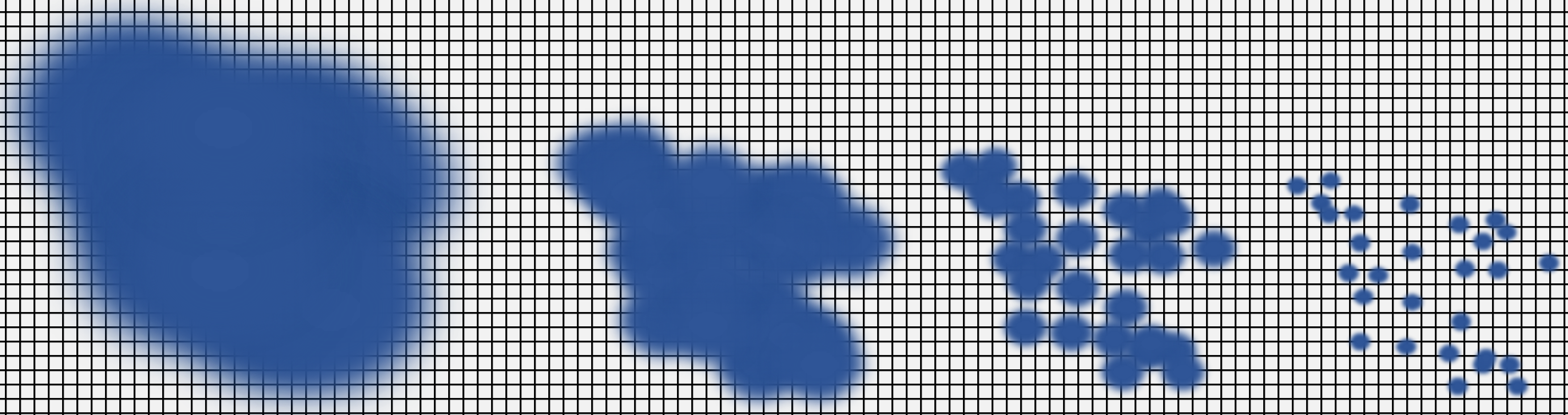
lateral $r = 0{,}61\lambda / NA$



axial $r = 2n\lambda / NA^2$



HOW TO ACQUIRE AND REPRODUCE THE FREQUENCY



HOW MANY PIXELS WE NEED? - - - THE SAMPLING THEORY

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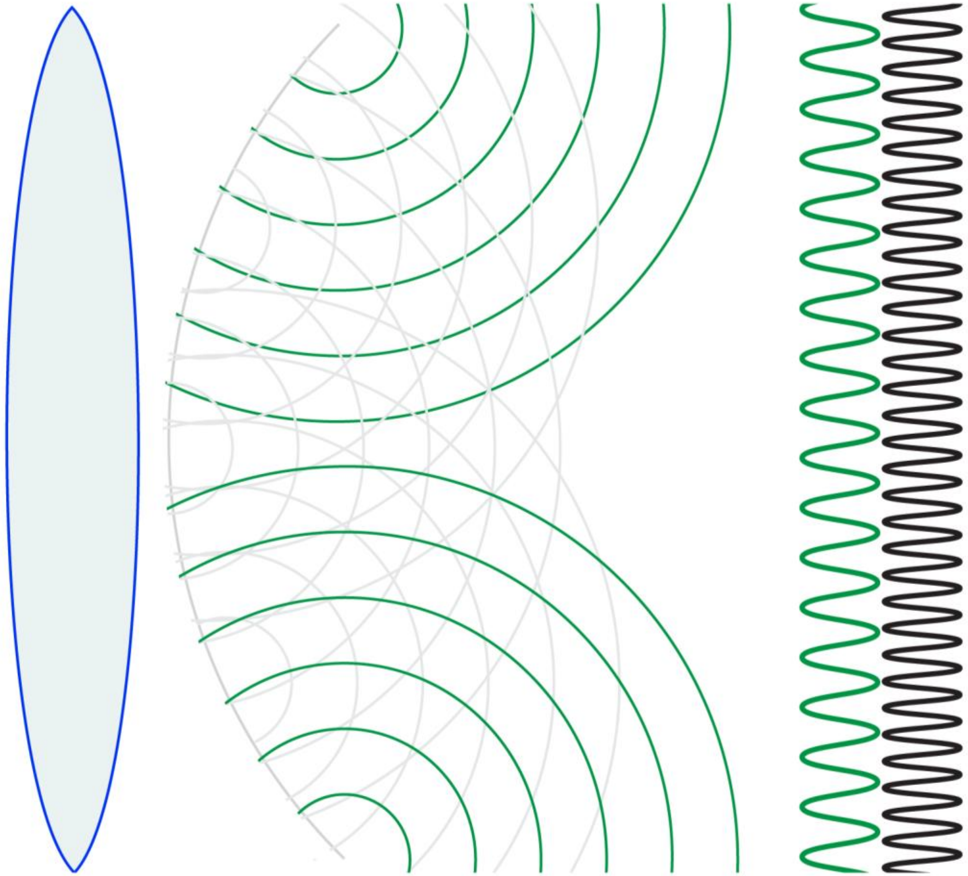


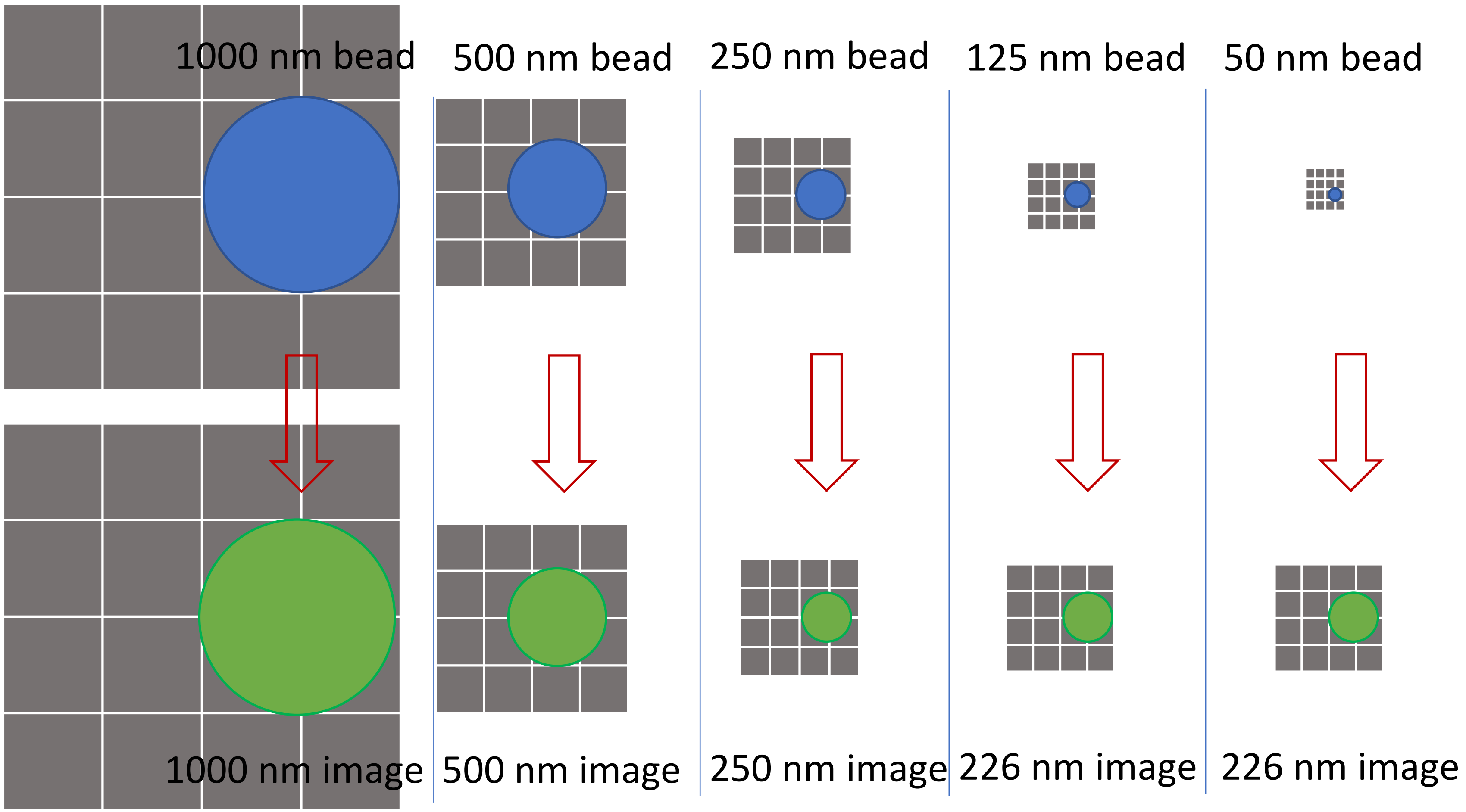
Harry Nyquist (1889–1976)

Nyquist, Harry. "Certain factors affecting telegraph speed". *Bell System Technical Journal*, 3, 324–346, 1924

Nyquist determined that the **number of independent pulses that could be put through a telegraph channel per unit time is limited to twice the bandwidth of the channel**.

WHY WE NEED TO CALCULATE THE RESOLUTION? TO KNOW HOW MANY PIXELS WE NEED! - - - THE SAMPLING THEORY

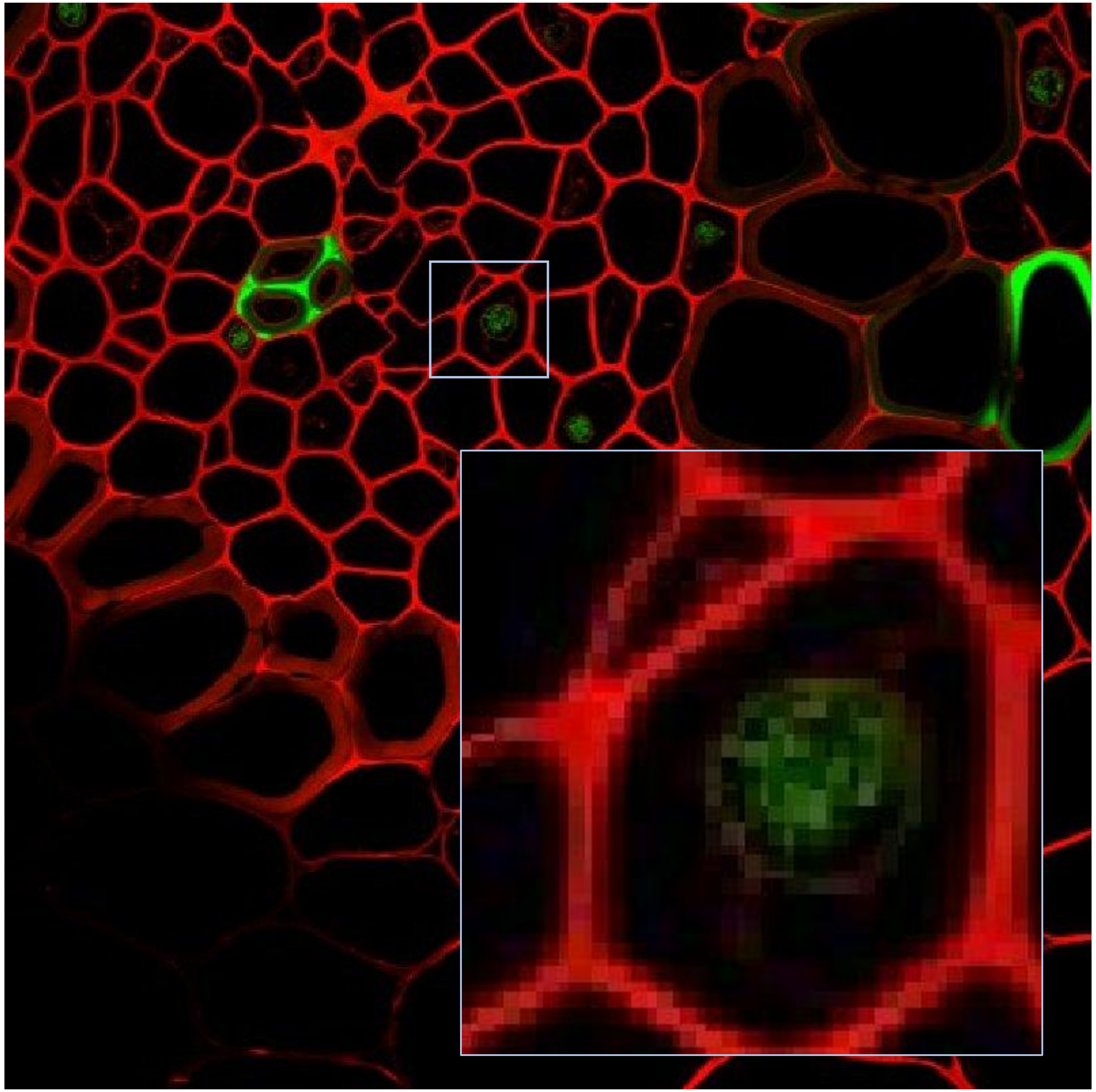
e.g., for λ = 520 and NA = 1.4, the resolution is 226.6 nm:

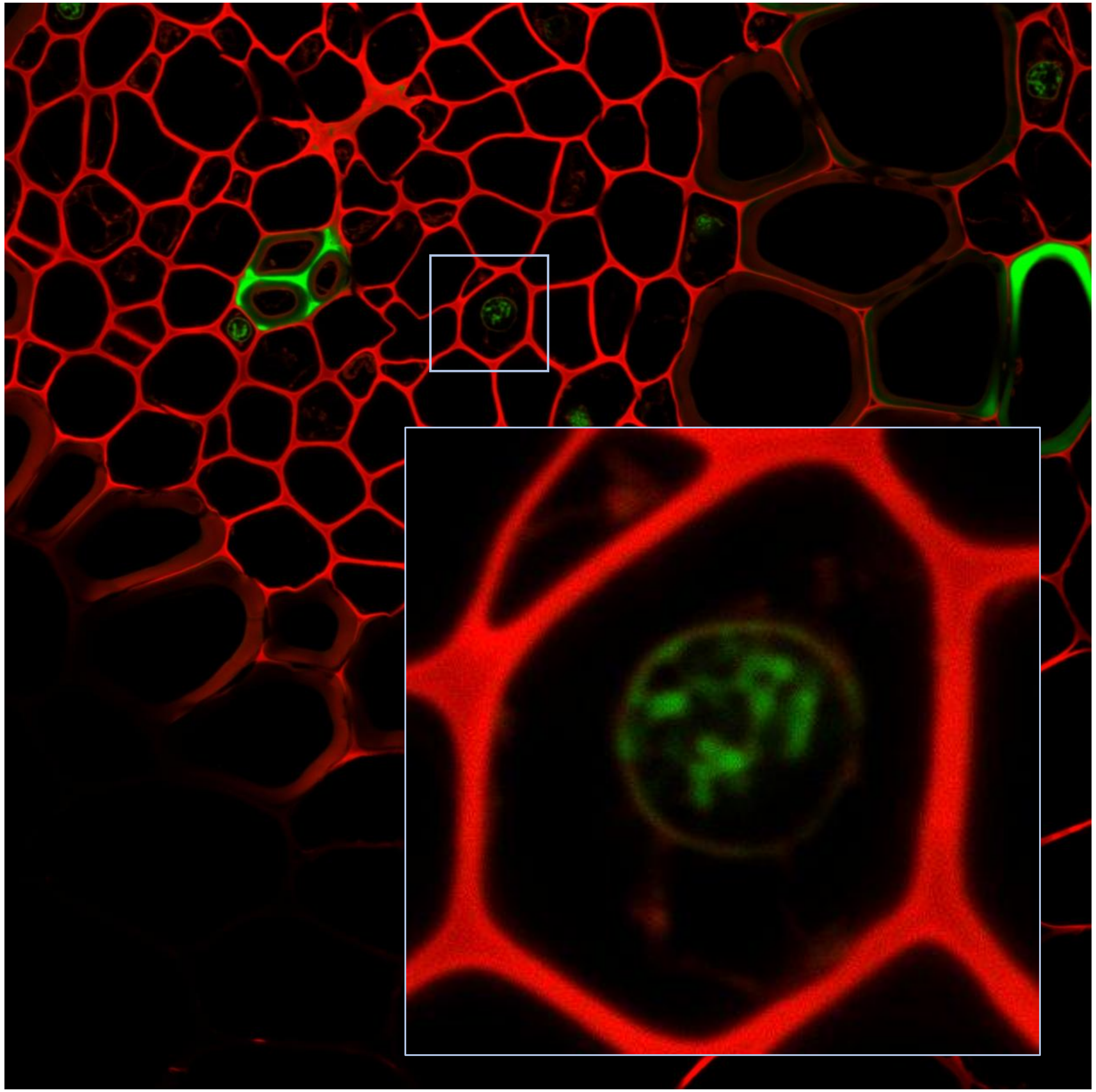


SAMPLING THEORY IN REAL WORLD

SAMPLING THEORY IN REAL WORLD

DEPENDS ON WHAT YOU NEED TO SEE





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LIGHT MICROSCOPY – image formation

THANK YOU FOR YOUR ATTENTION!