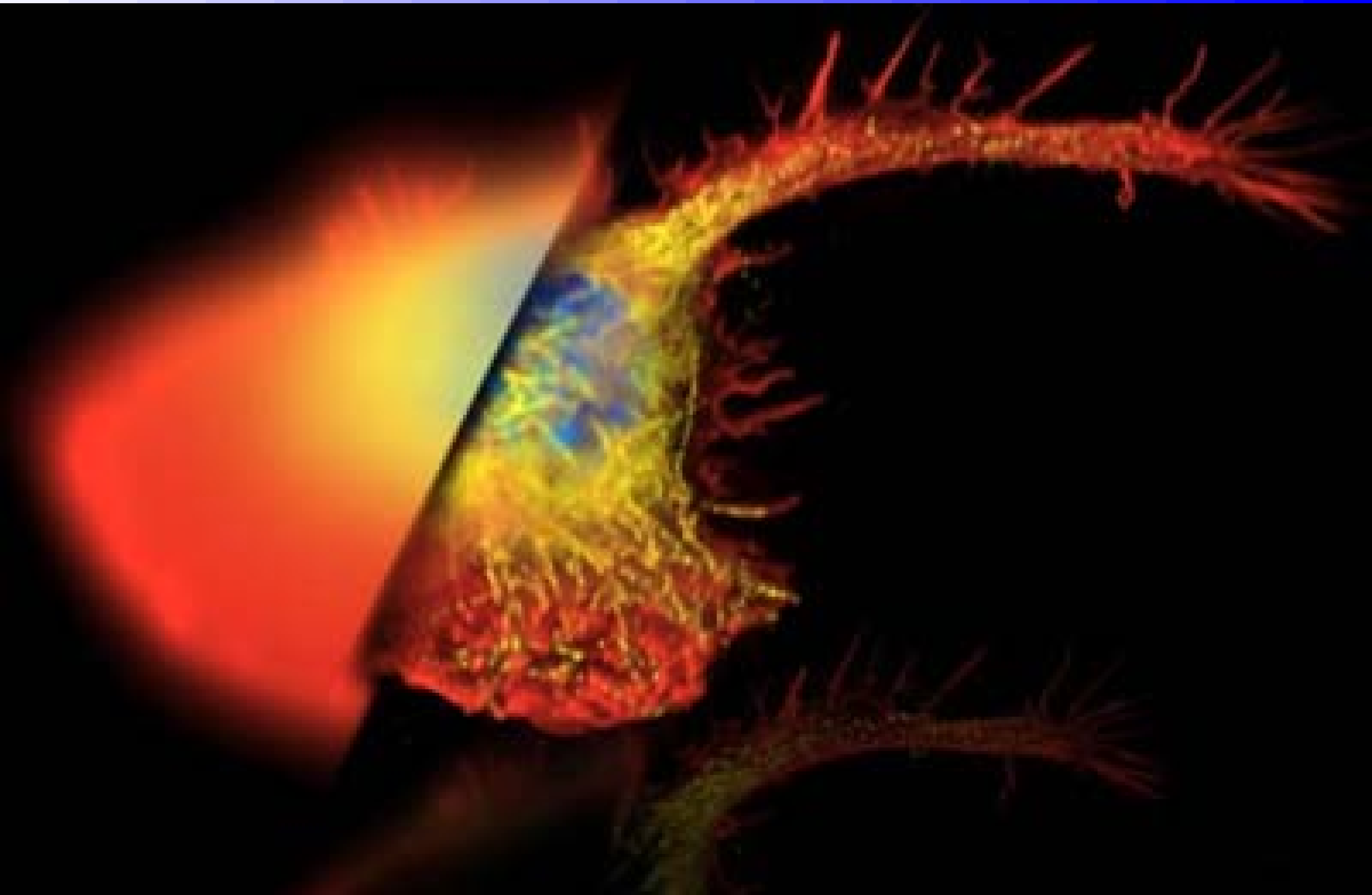
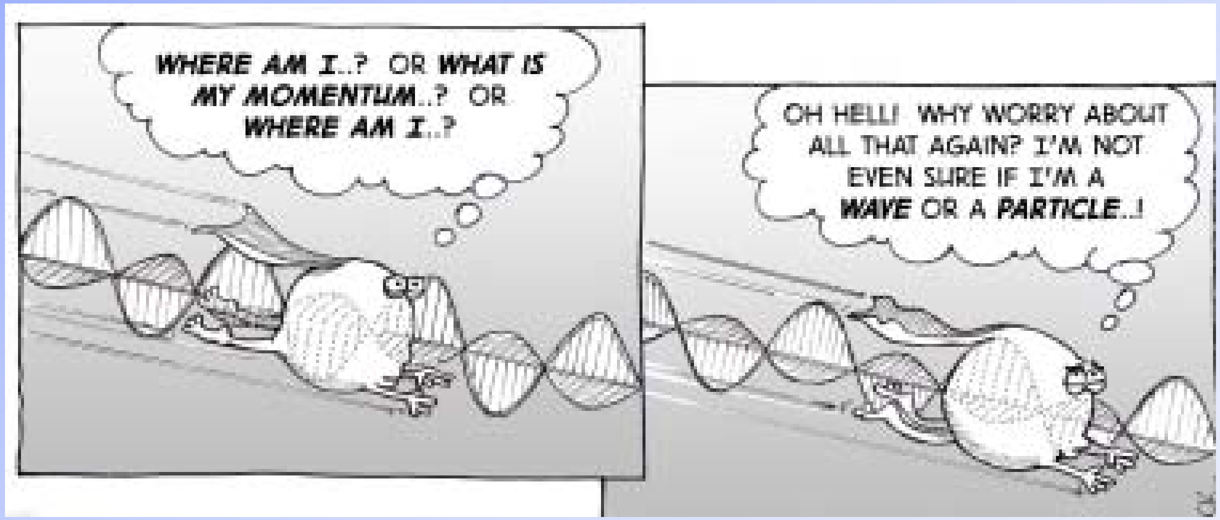
Deconvolution



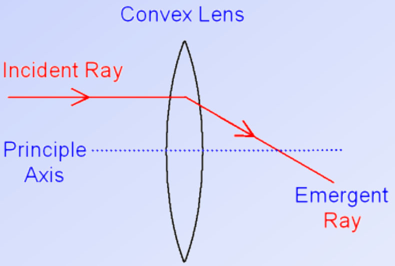
The beginning: Let there be light



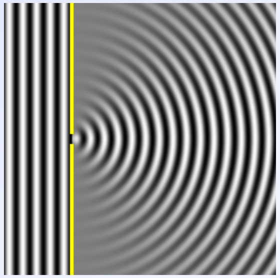
<u>A photon is a wave… and a particle</u>

In microscopy, we exploit mainly wave characteristics:

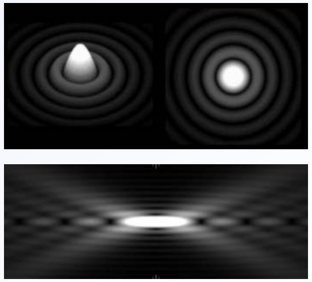
Refraction



Diffraction

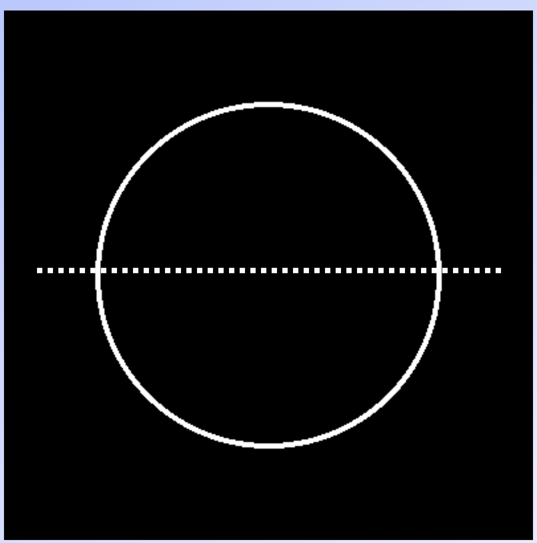
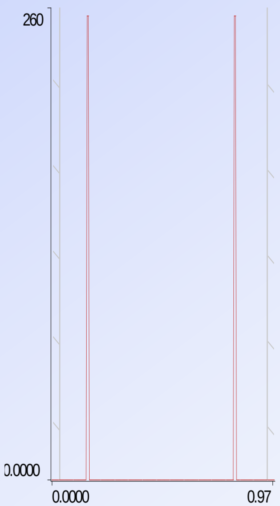


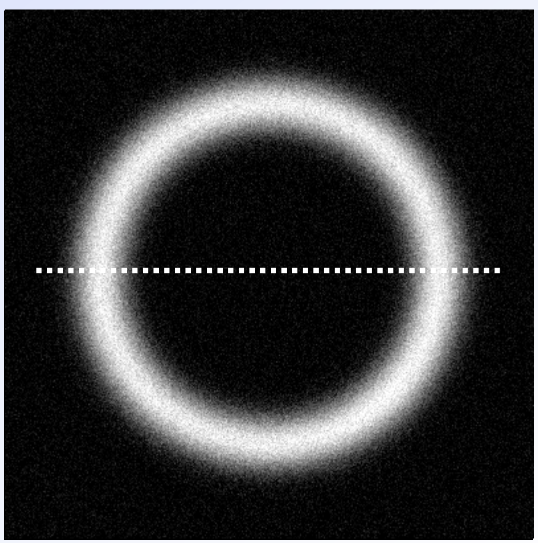
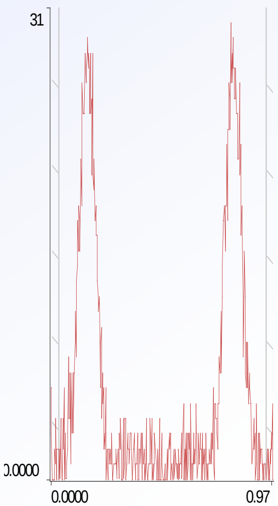
Interference



I **Image Formation**: From an object to an image

- Microscopes read out object information and display it visually.
- This transfer is always partial. No method works without loss of information
- Image quality is limited (mostly) by the microscope‘s resolving power and image noise.
- The resolving power can be limited by aberration or diffraction

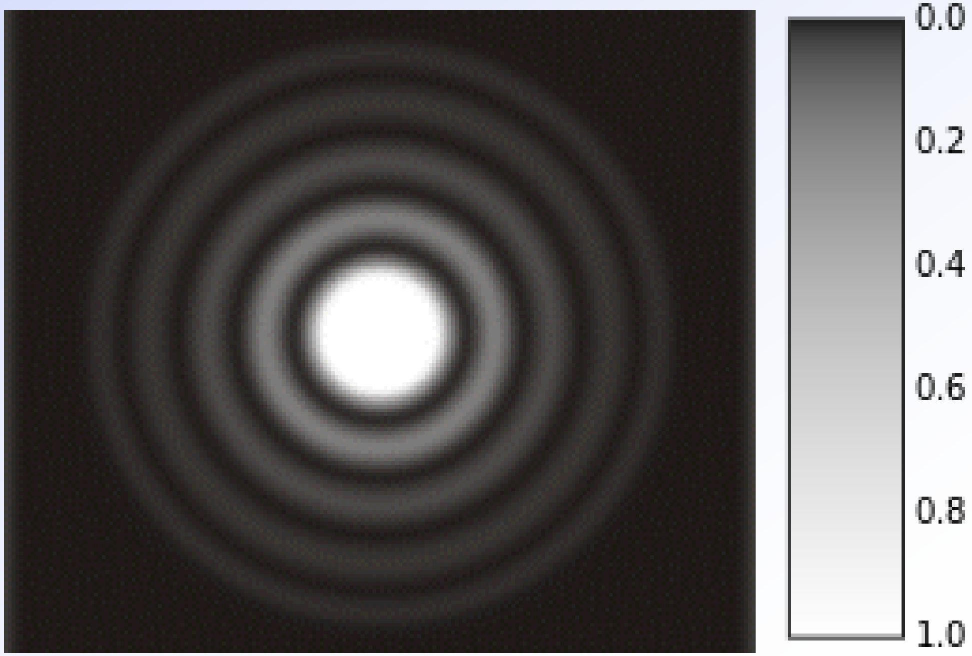
The Airy pattern

„...the star is then seen (in favourable circumstances of tranquil atmosphere, uniform temperature, &c.) as a perfectly round, well-defined planetary disc, surrounded by two, three, or more alternately dark and bright rings, which, if examined attentively, are seen to be slightly coloured at their borders…"

George Biddell Airy

1801 - 1892

Light from a point source passing a circular apperture (e.g. a lens) produces a disc surrounded by concentric rings.



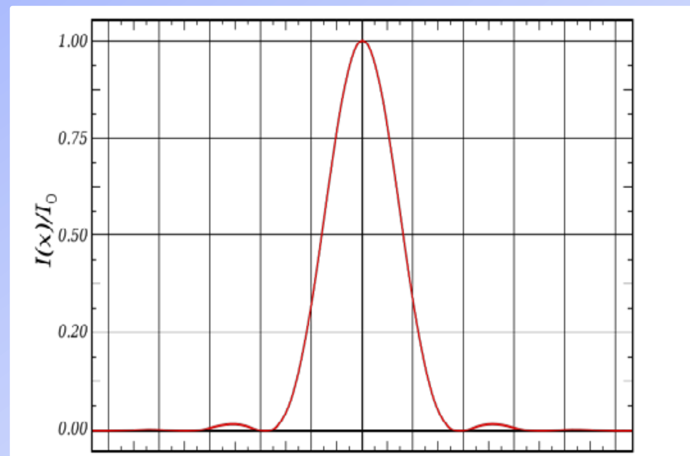
Huygens‘ principle

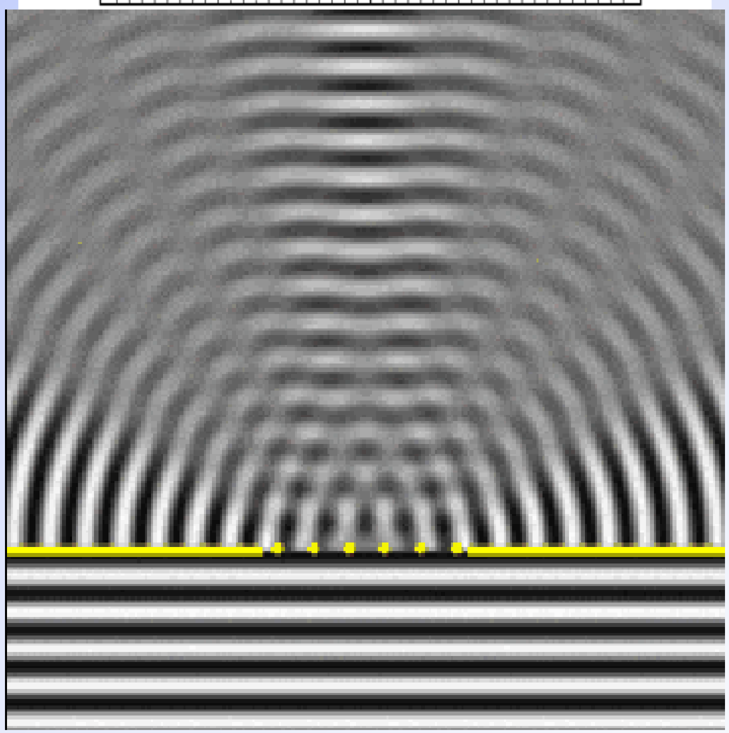
Christiaan Huygens

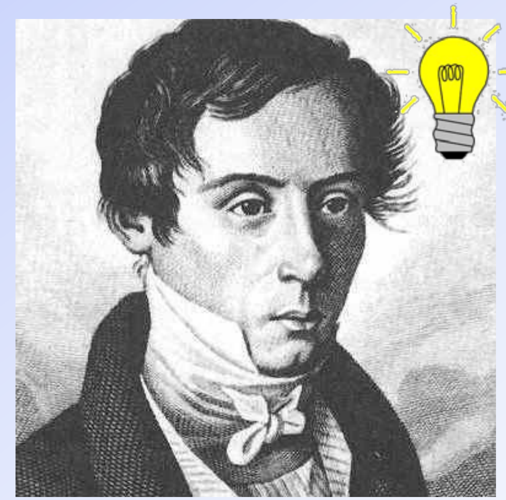
1629 - 1695

Siméon Poisson

1781 - 1840

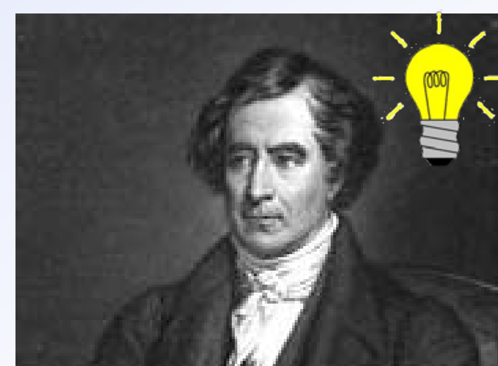






Augustin-Jean Fresnel

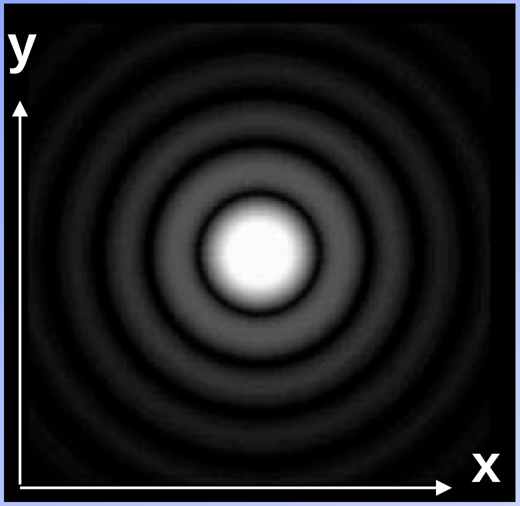
1788 - 1827



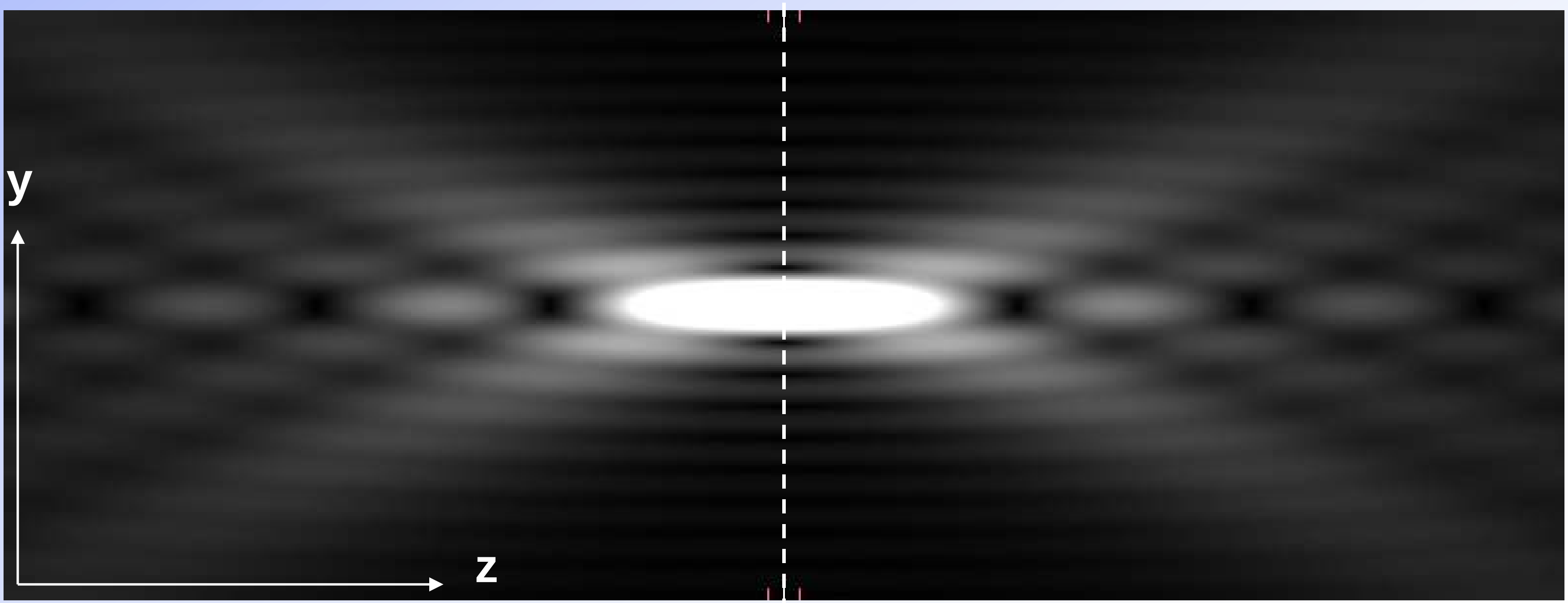
Francois Arago

1786 - 1853

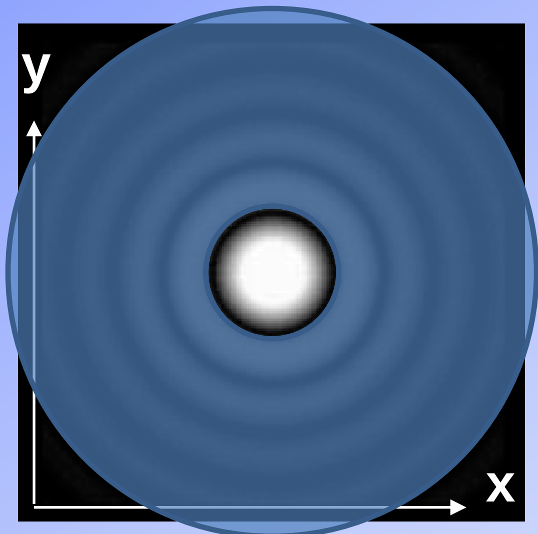
The point spread function (PSF)



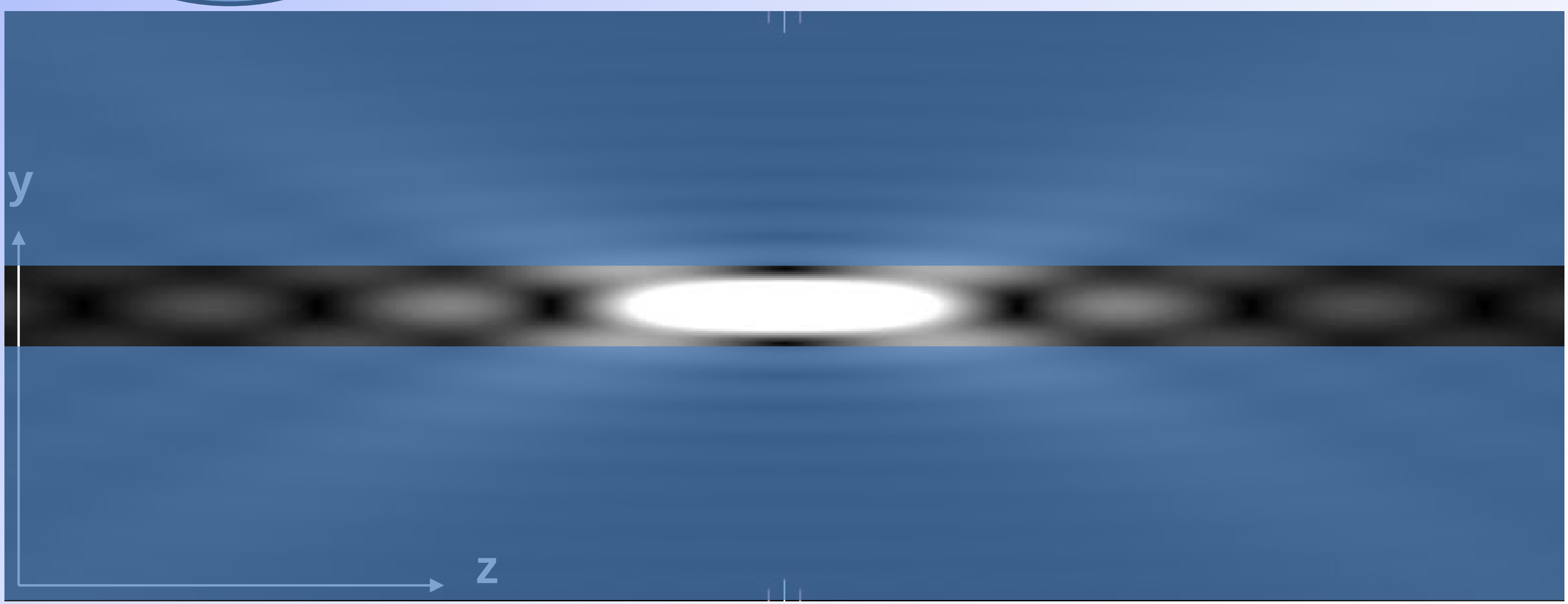
- The PSF is the image of a point source
- It is defined by aberration and diffraction
- The PSF is elongated along the z axis
- Its size limits the microscope‘s resolving power in x/y and in z



The confocal pinhole



- The pinhole cuts off most out-of-focus light
- Its size is variable. It is usually set to one Airy unit (1AU)
- 1AU corresponds to the size of the Airy disc.
- This size depends on a number of factors



Ernst Abbe
1840 - 1905

Equation of Abbe

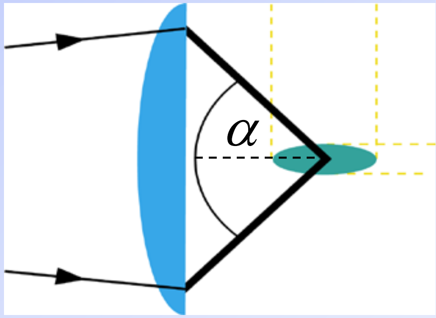
lateral

$$\Delta x \approx \frac{0{,}61\lambda}{n \sin\alpha} = \frac{0{,}61\lambda}{NA}$$

axial

$$\Delta z \approx \frac{\lambda}{2n\sin\alpha} = \frac{2\lambda n}{(NA)^2}$$

confocal **axial** **> 500 nm**



lateral
> 200 nm

Δx = resolution

λ = wavelength

n = refractive index of medium

α = opening angle of the objective

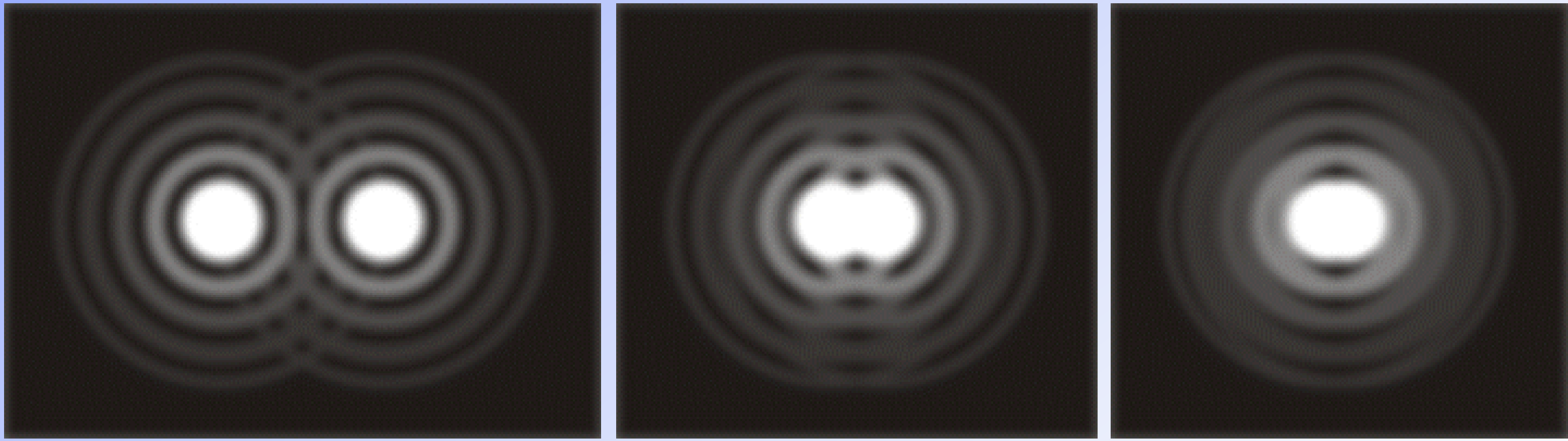
NA = Numerical Aperture

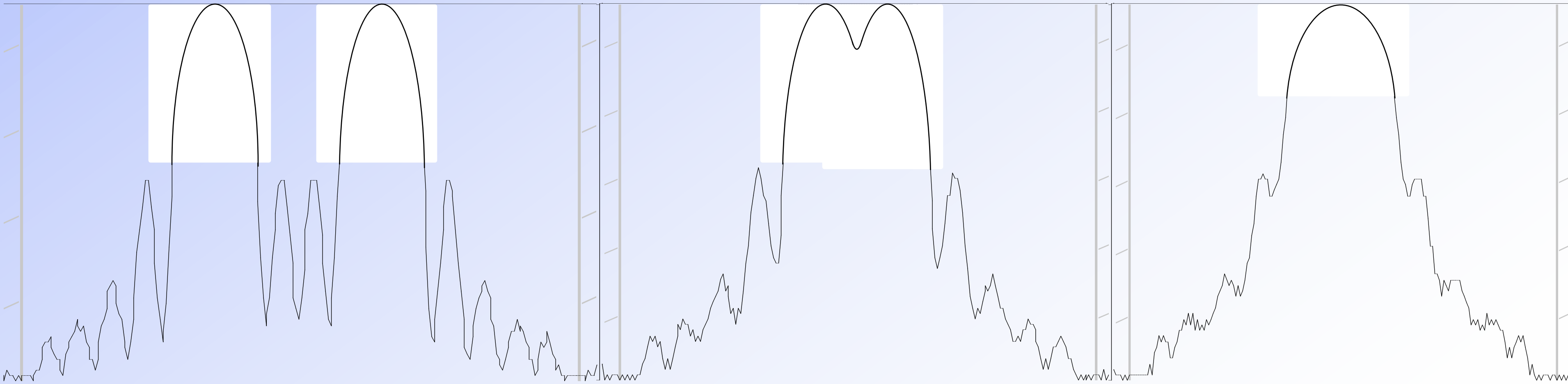
→ **improving resolution:**

- Immersion medium with high refractive index (oil)
- Objectives with a high refractive index
- Lowering of the wavelength

Resolution: the Rayleigh criterion

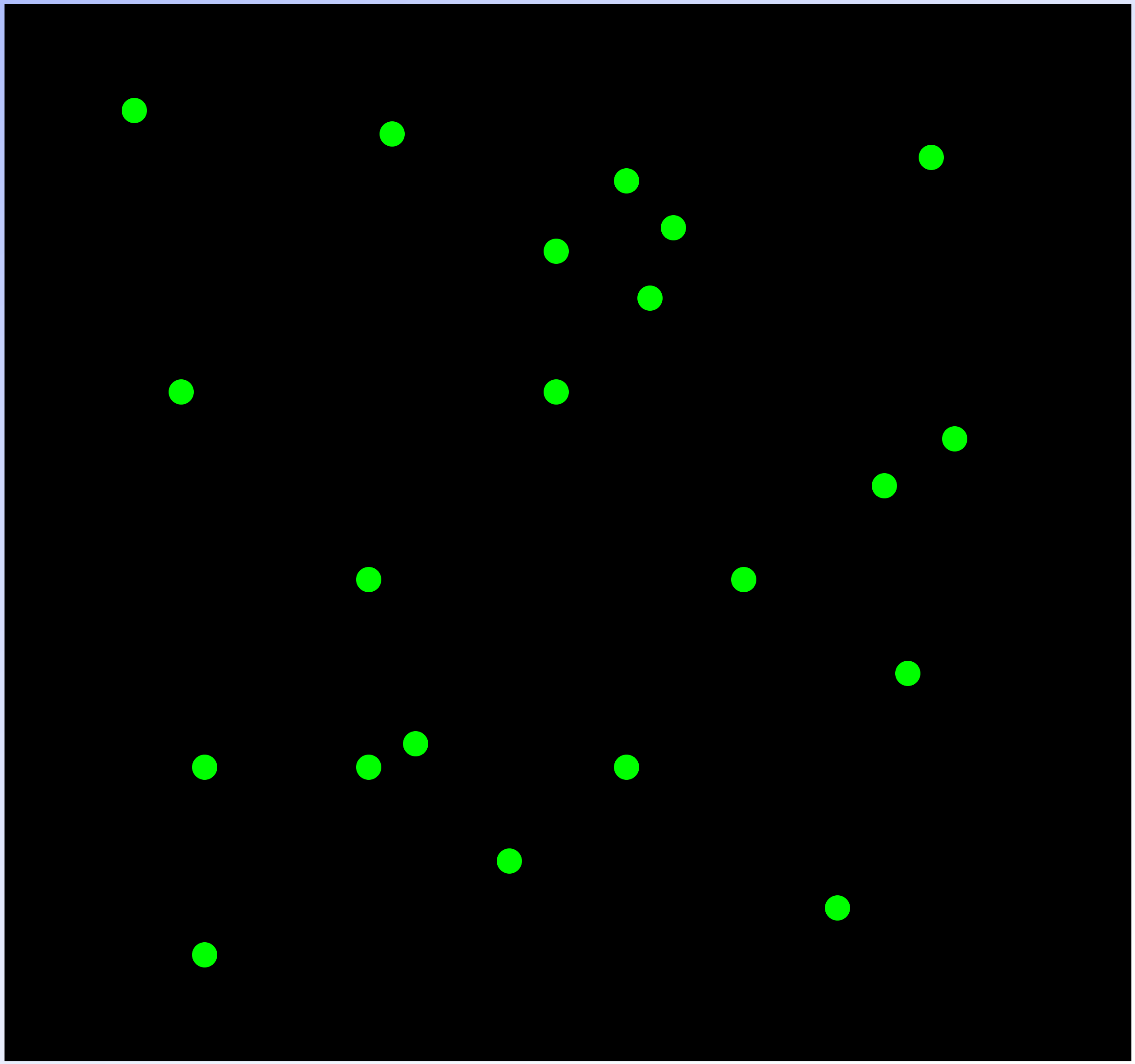
Two points can be resolved at a minimum distance corresponding to the distance between the center of the Airy pattern and the first minimum





In the final image, every point source is replaced by the microscope‘s point spread function (PSF)

Image formation is a convolution of object data with the microscope‘s PSF.



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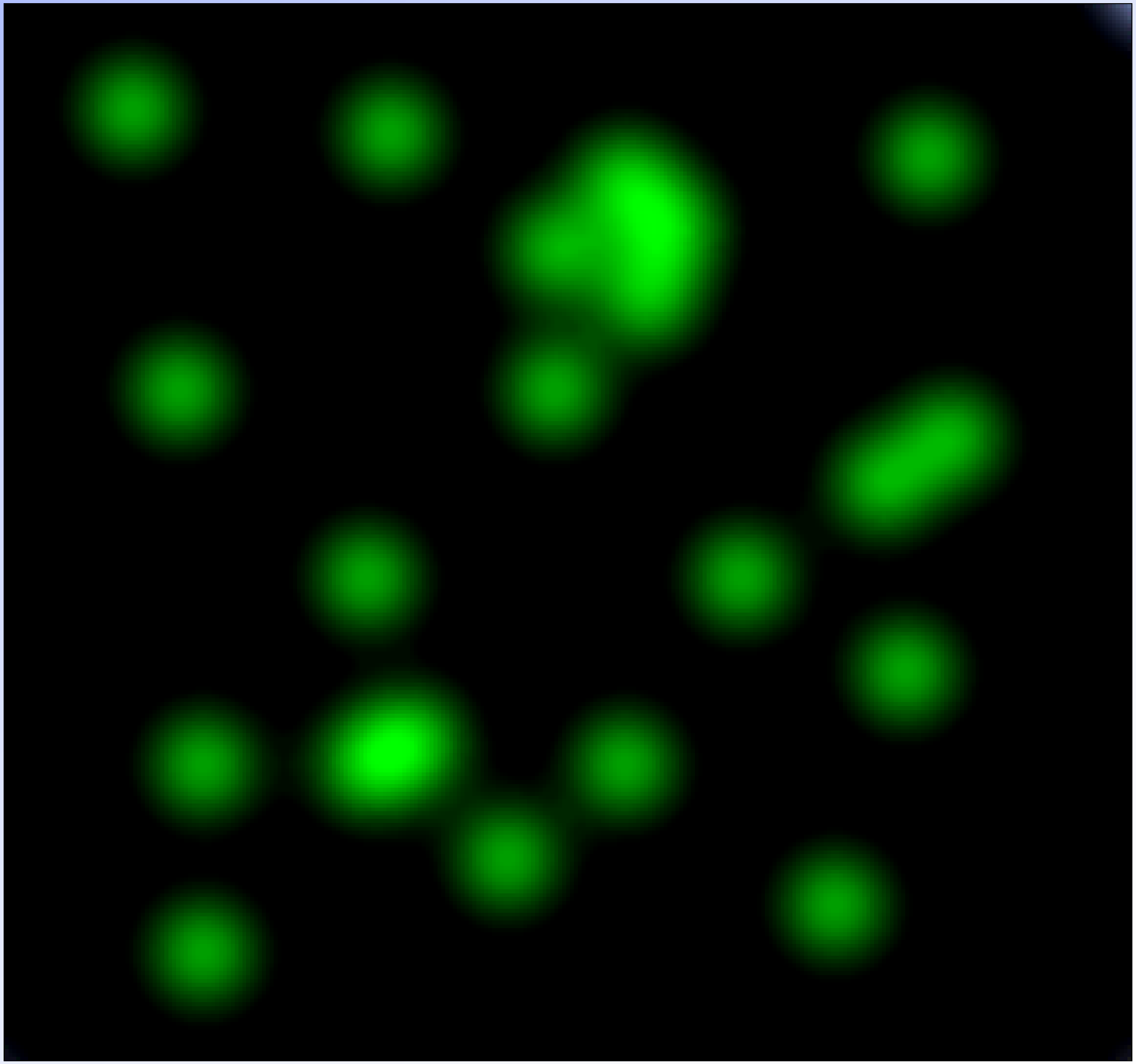
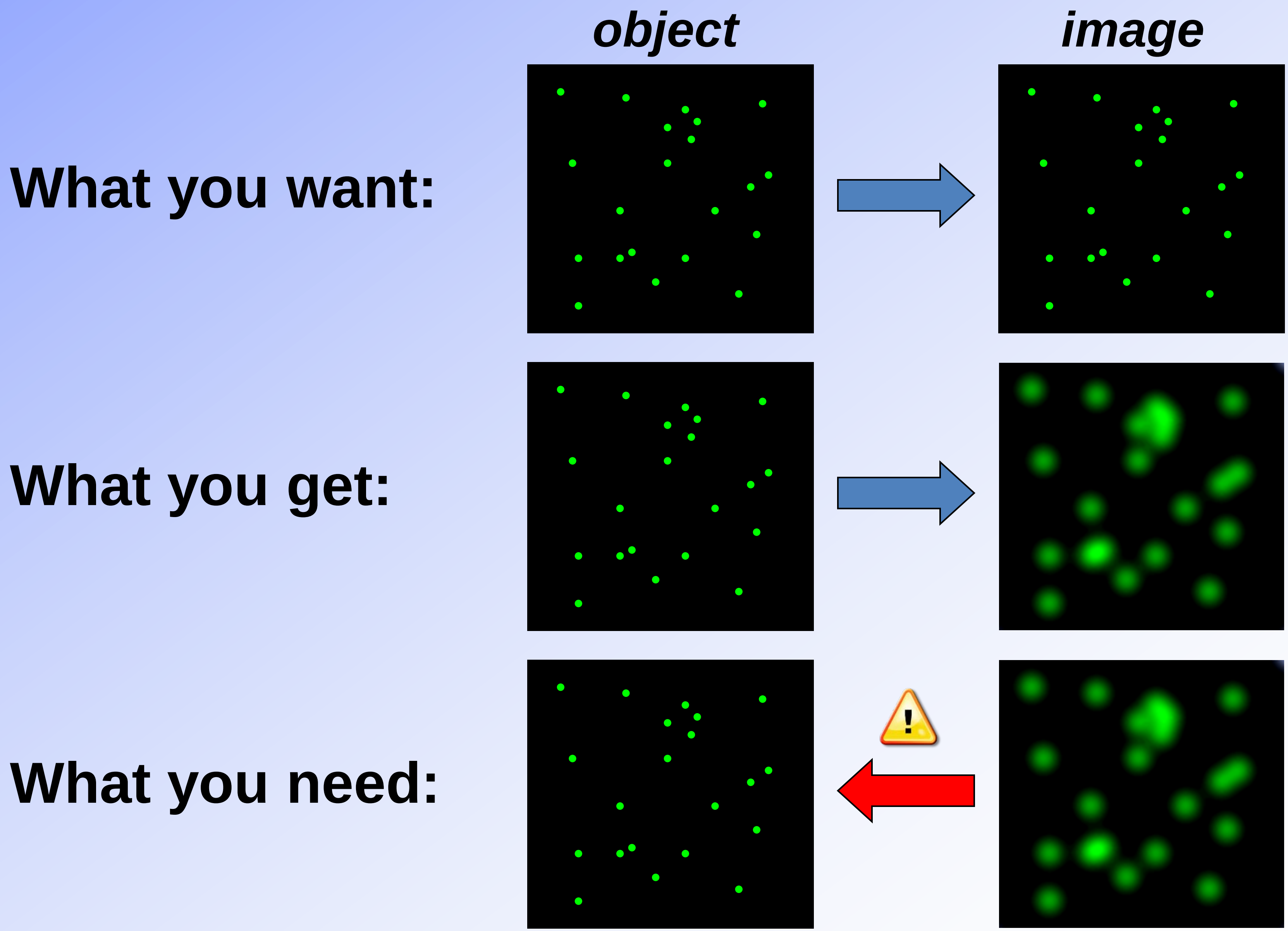


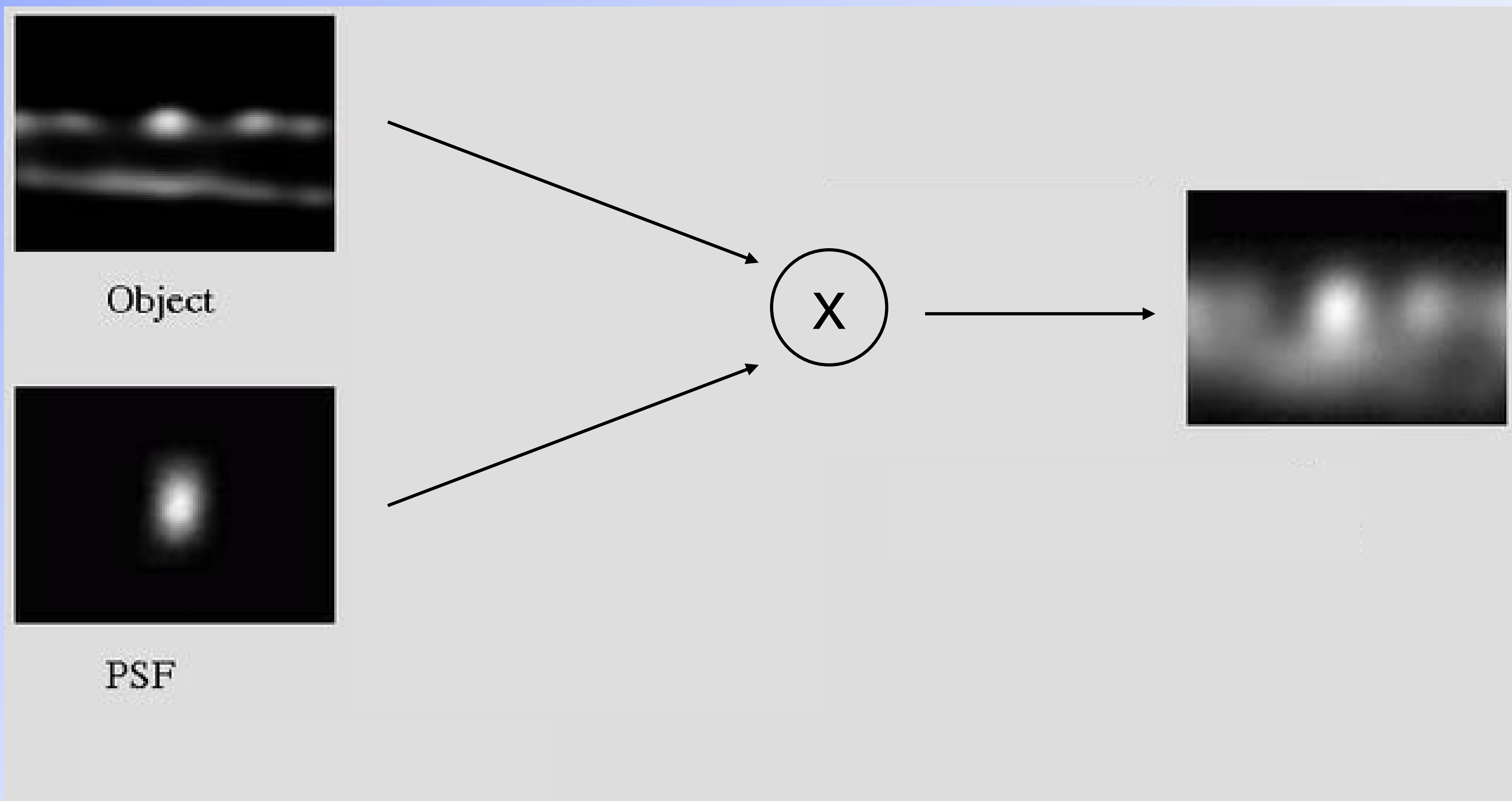
II **Image restoration**: de-convolution



Convolution in position space

Per pixel: x*y operations

Per image: $(x*y)^2$ operations



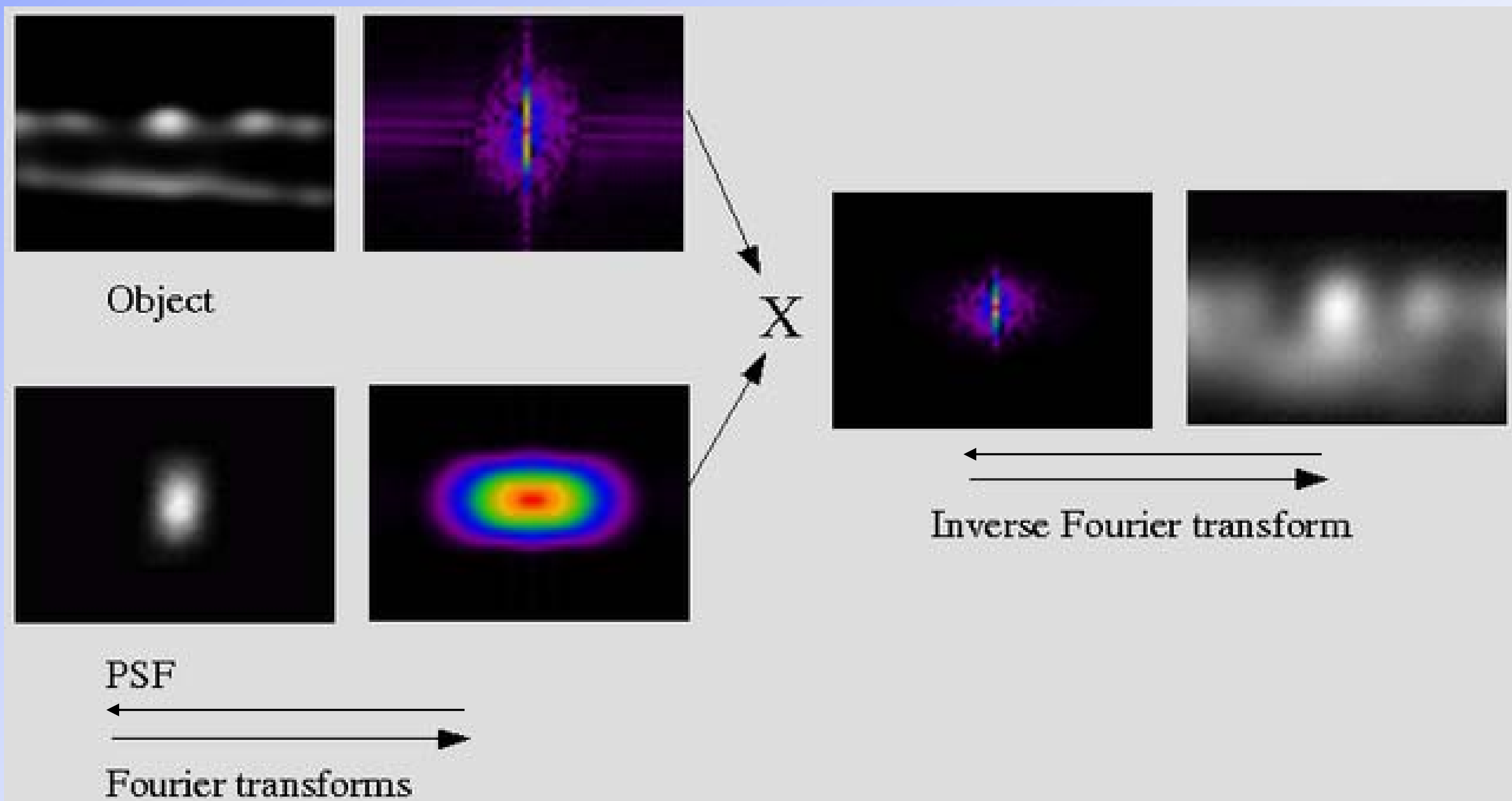
Convolution in frequency space

~~Per pixel: $x*y$ operations~~

~~Per image: $(x*y)^2$ operations~~

Per pixel: 1 operation

Per image: $x*y$ operations



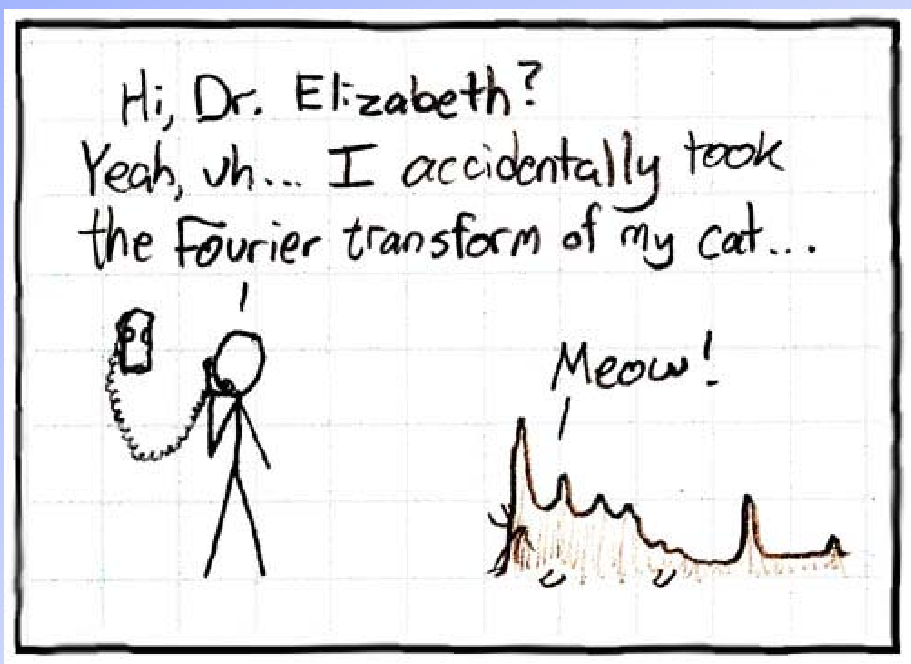
Convolution in position space

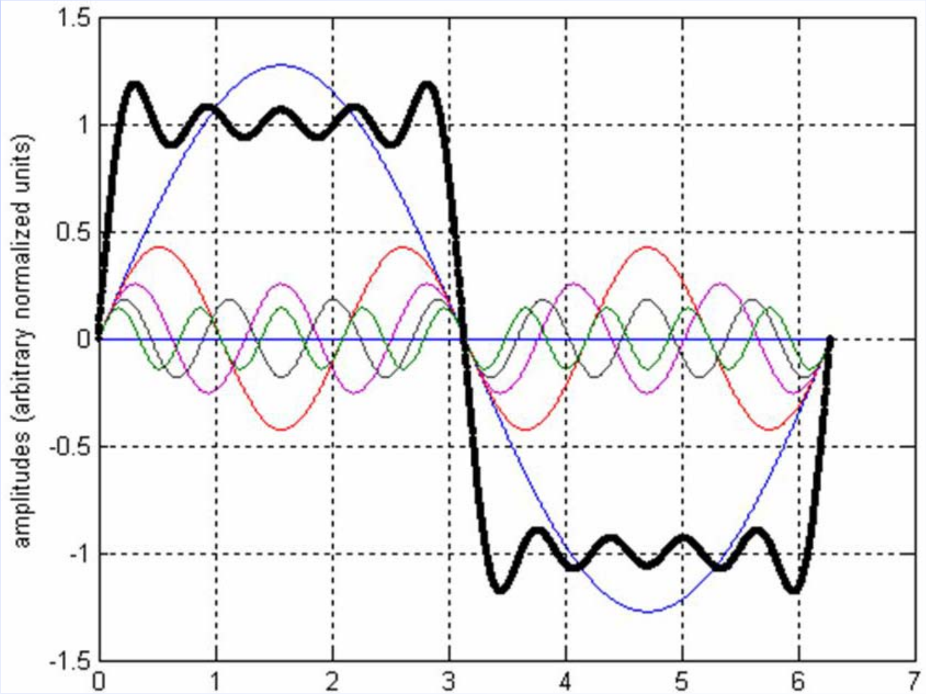
~~Per pixel: x*y operations~~

~~Per image: (x*y)² operations~~

Per pixel: 1 operation

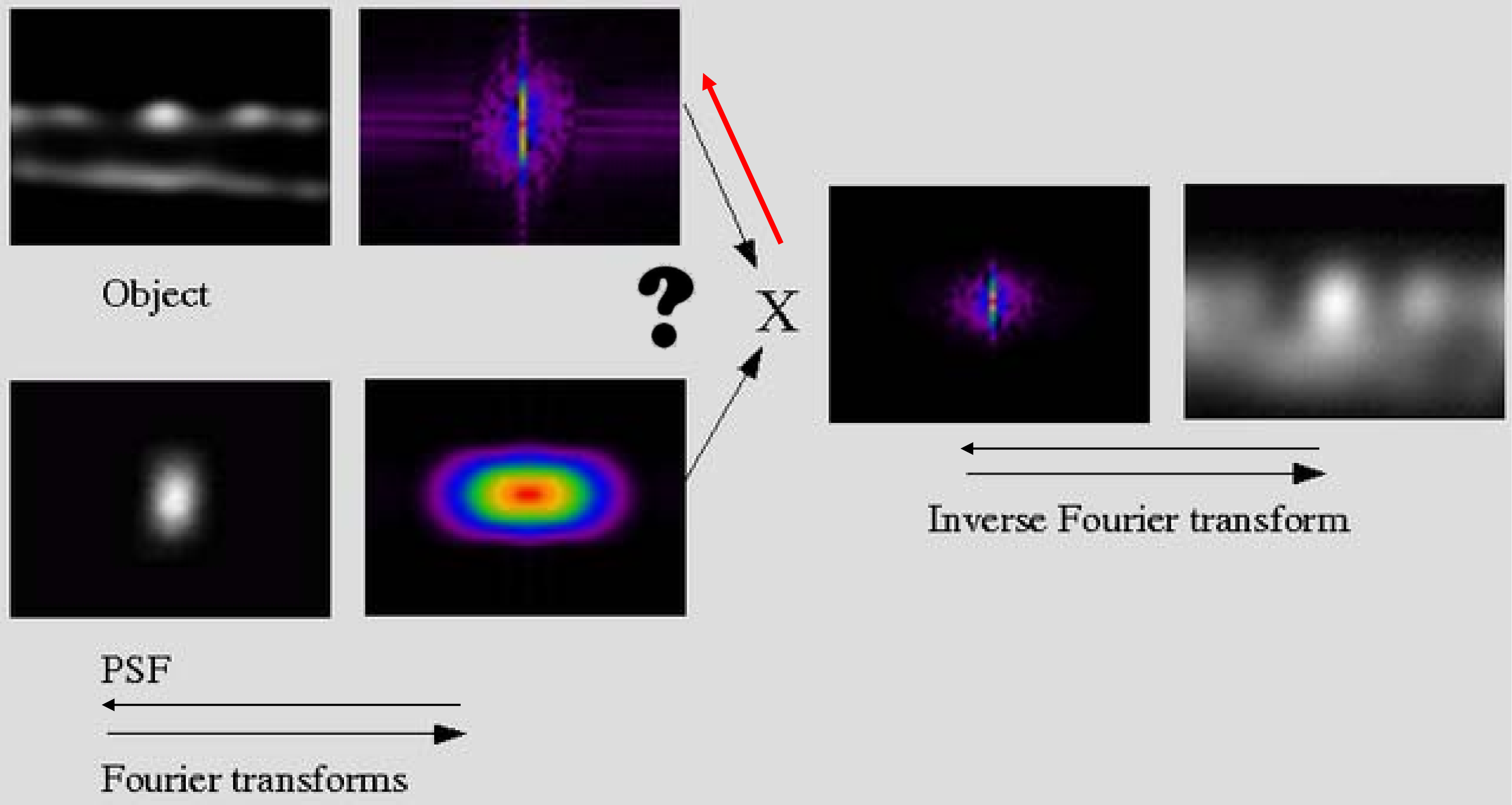
Per image: x*y operations





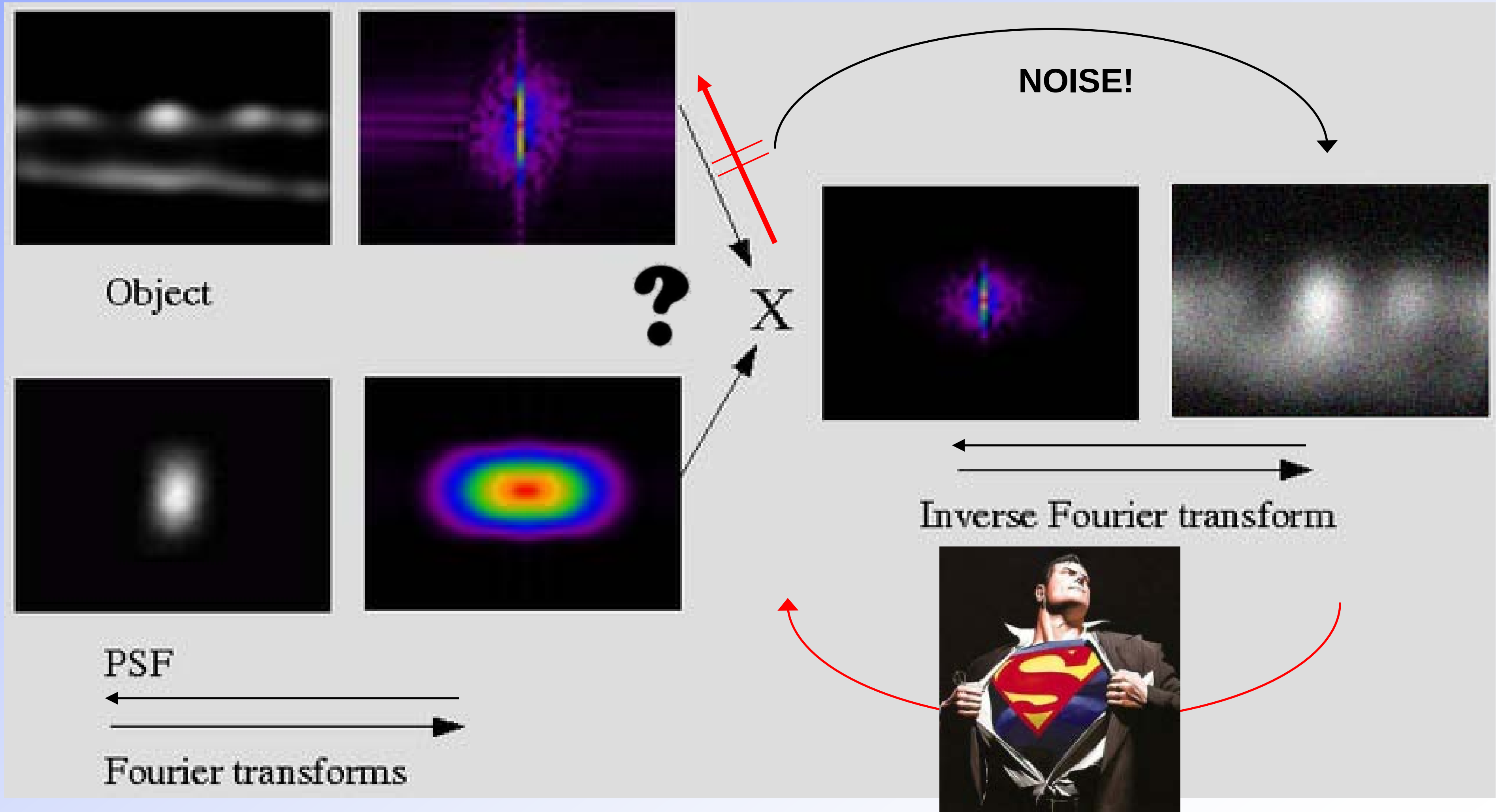
Is de-convolution simply convolution in reverse?

→ **Linear deconvolution** enhances high frequencies.

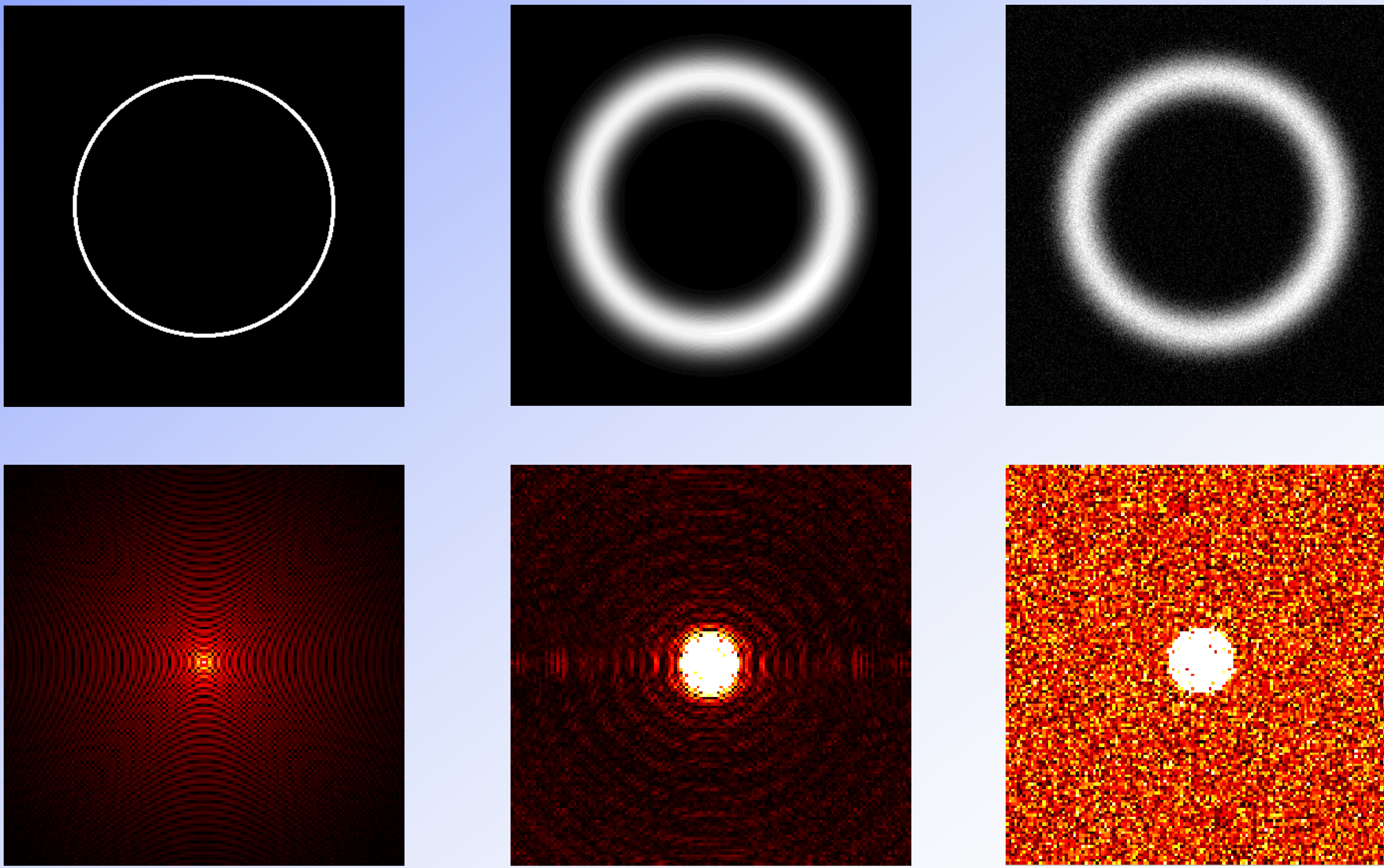


If noise dominates the high frequencies, enhancement creates artifacts.

Wiener filtering can reduce noise amplification (noise info required).



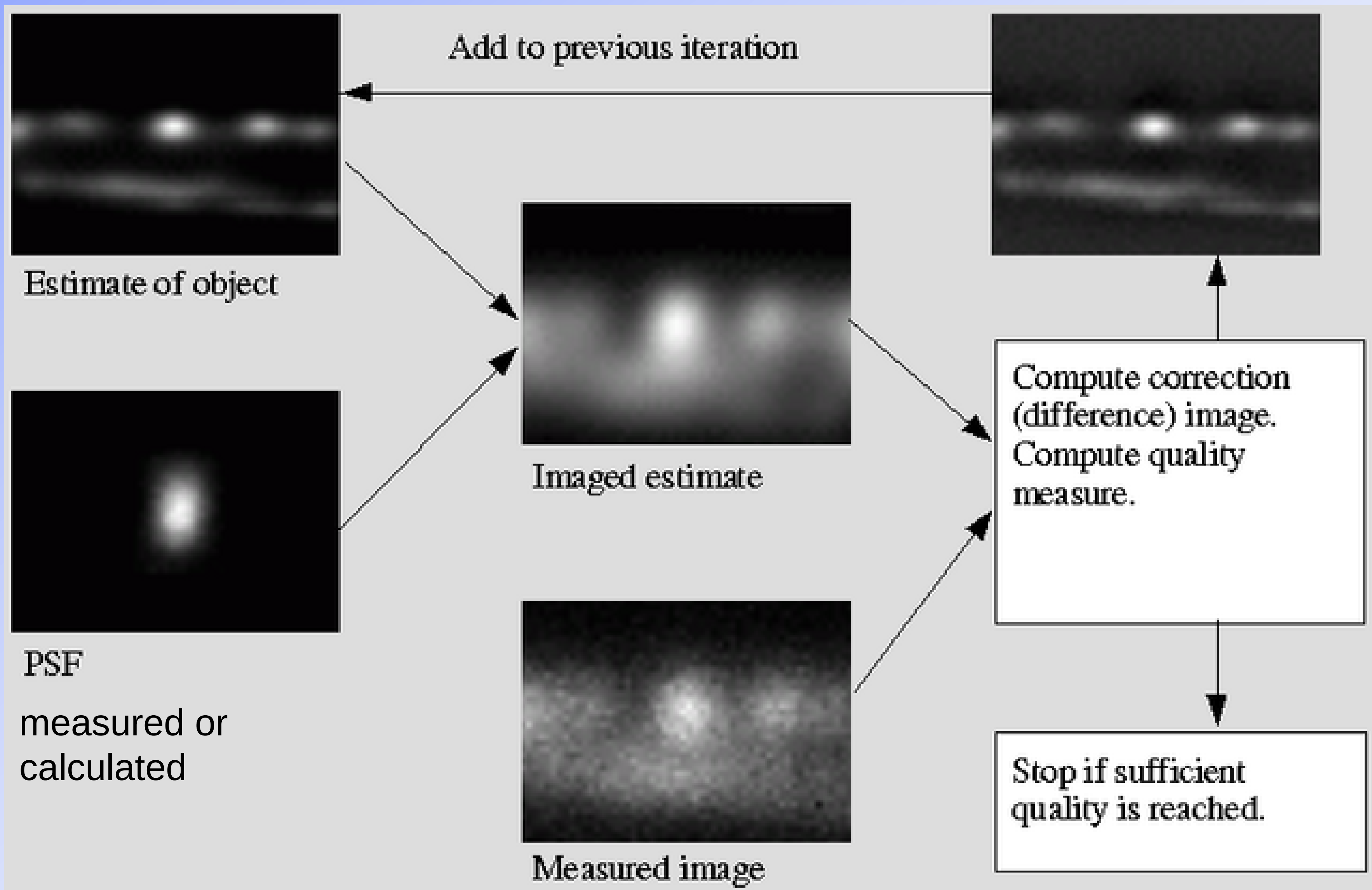
Object, image and noisy image in position space and frequency space



The signal-to-noise ratio (SNR) limits deconvolution quality

- High frequencies (→ fine details) are attenuated at image acquisition.
- Noise is present at all frequencies.
- The inversion problem: With increasing frequency, the SNR decreases. Reversing convolution can boost high frequency noise to the signal level or higher.

Iterative deconvolution: classical maximum likelihood estimation (CMLE)



The solution: Regularisation and a quality measure

- The iterative process does not converge, because the noise is not an effect of convolution and thus cannot be reflected in the estimate.
- When stoped, the result will contain sub-diffraction artifacts.
- A regularisation parameter forces the estimate to be smooth. With low SNR images, regularisation has to be stronger.
- The quality measure stops the process as soon as the difference between convolved estimate and acquired image is satisfactory.
- Constraints such as non-negativity increase the output image resolution

Function to be minimized: [(conv.est – image) – α]

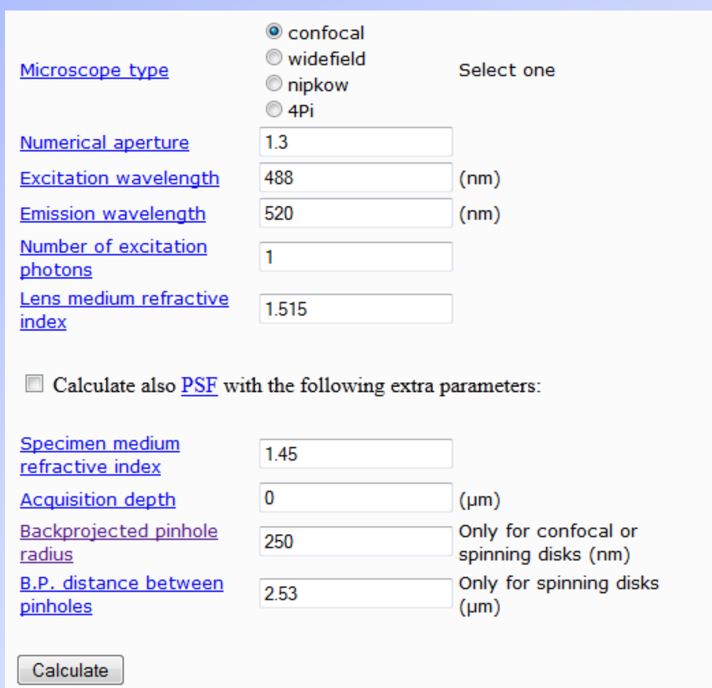
α = regularisation parameter

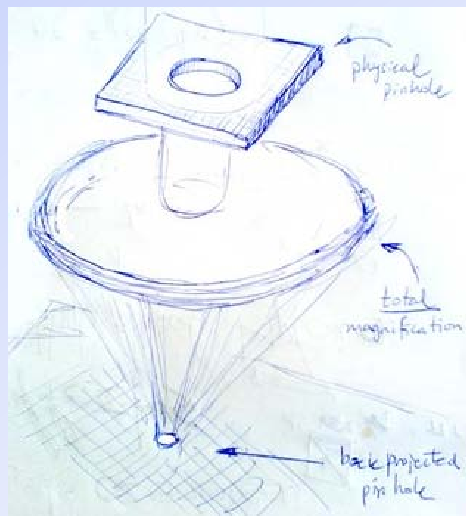
Assumptions and pitfalls

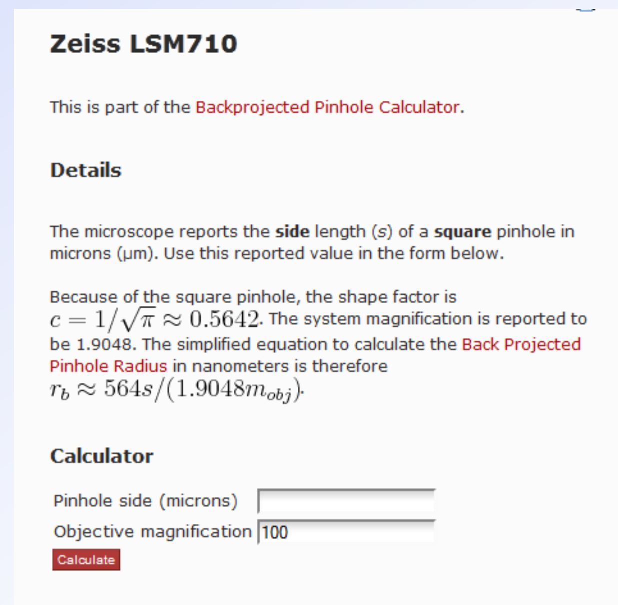
1. Avoid undersampling! Images that have not been recorded according to the Nyquist-criterion benefit less from deconvolution, or not at all. This is especially true for a too large step size.

The Nyquist criterion

- The sampling rate has to be at least twice as fine as the optical resolving power.
- This makes image reconstruction possible, but finer sampling is still better.







Huygens: Nyquist-Calculator (http://www.svi.nl/NyquistCalculator)

Assumptions and pitfalls

1. Avoid undersampling! Images that have not been recorded according to the Nyquist-criterion benefit less from deconvolution, or not at all. This is especially true for a too large step size.
2. Image acquisition has to include all relevant data! Clipping of images, especially along the z-axis, results in loss of information. Detector saturation has to be avoided.
3. The PSF should be invariant to translation (perfect flatness-of-field, no refractive index mismatch). Calculated PSFs can be adapted to continuos deformation along the z-axis..
4. Measured PSFs should ideally be recorded right before image acquisition, at +/- the same conditions.