The Royal Swedish Academy of Sciences has decided to award the

2014 NOBEL PRIZE IN CHEMISTRY

to:

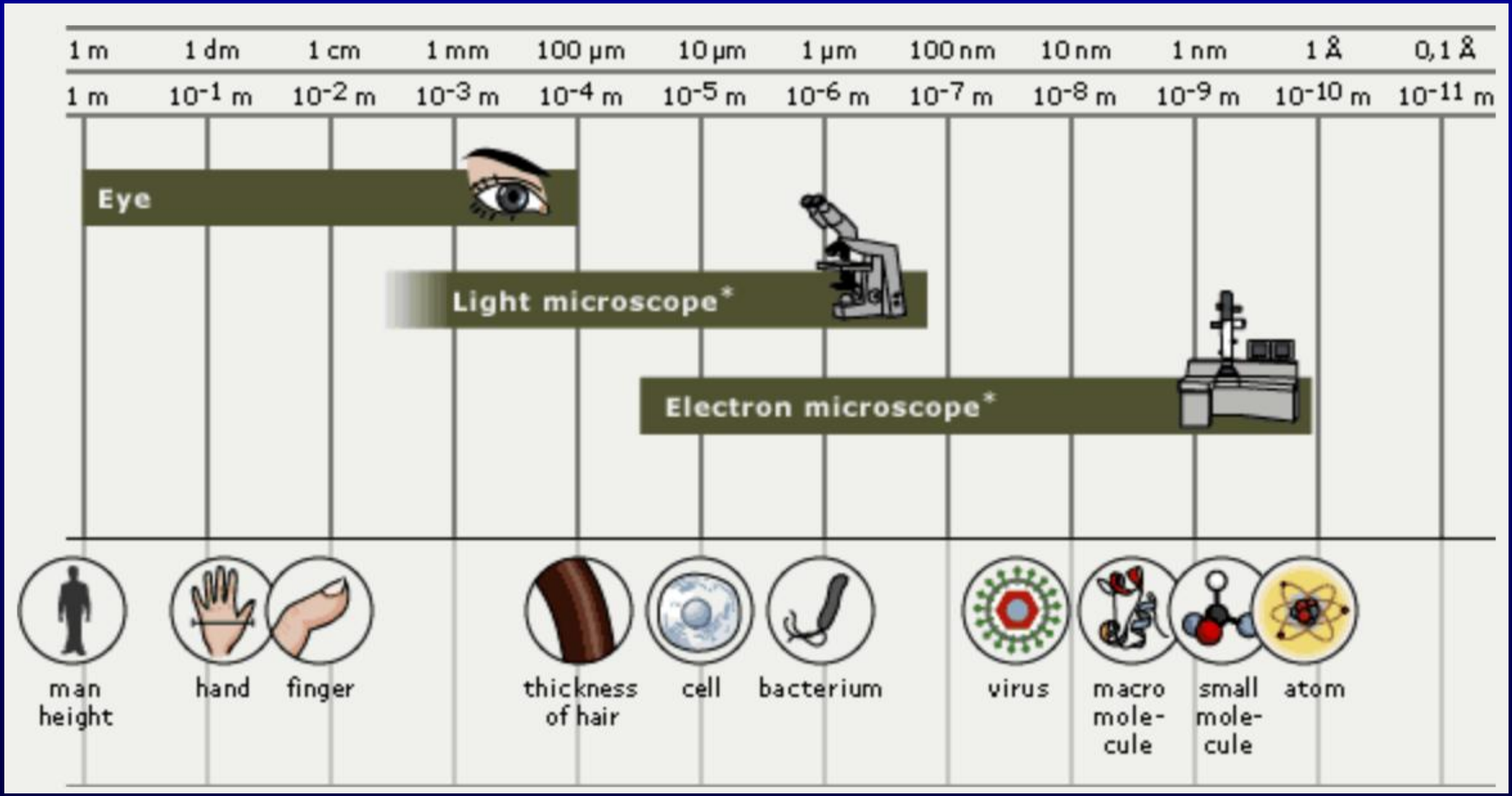
Eric Betzig, Stefan W. Hell and William E. Moerner

"for the development of super-resolved fluorescence microscopy"

Surpassing the limitations of the light microscope

For a long time optical microscopy was held back by a presumed limitation: that it would never obtain a better resolution than half the wavelength of light. Helped by fluorescent molecules the Nobel Laureates in Chemistry 2014 ingeniously circumvented this limitation. Their ground-breaking work has brought optical microscopy into the nanodimension.

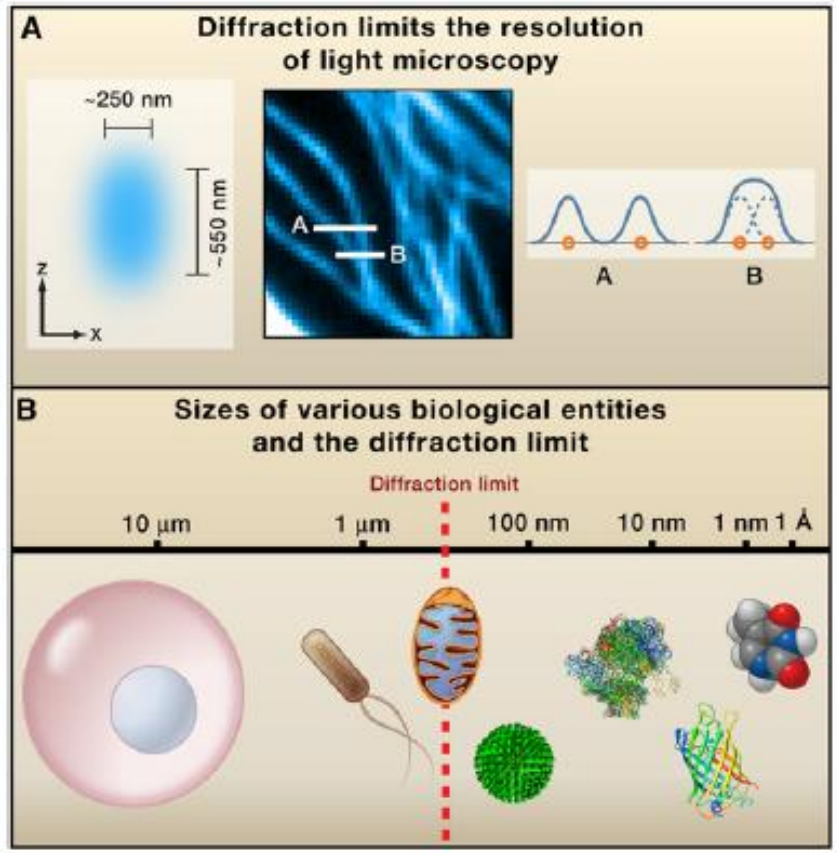
Circumventing the diffraction barrier

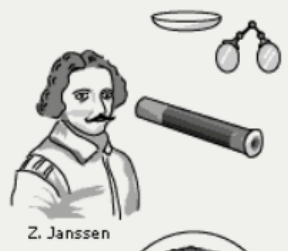


Resolution limit

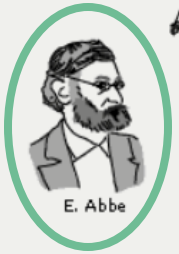
POINT SPREAD FUNCTION PSF

$$D \approx \lambda / n \cdot \sin\alpha$$




Z. Janssen


A. van Leeuwenhoek


E. Abbe


R. Zsigmondy


F. Zernike


E. Ruska


G. Binnig and H. Rohrer

14th century – The art of grinding lenses is developed in Italy and spectacles are made to improve eyesight.

1590 – Dutch lens grinders Hans and Zacharias Janssen make the first microscope by placing two lenses in a tube.

1667 – Robert Hooke studies various object with his microscope and publishes his results in Micrographia. Among his work were a description of cork and its ability to float in water.

1675 – Anton van Leeuwenhoek uses a simple microscope with only one lens to look at blood, insects and many other objects. He was first to describe cells and bacteria, seen through his very small microscopes with, for his time, extremely good lenses.

18th century – Several technical innovations make microscopes better and easier to handle, which leads to microscopy becoming more and more popular among scientists. An important discovery is that lenses combining two types of glass could reduce the chromatic effect, with its disturbing halos resulting from differences in refraction of light.

1830 – Joseph Jackson Lister reduces the problem with spherical aberration by showing that several weak lenses used together at certain distances gave good magnification without blurring the image.

1878 – Ernst Abbe formulates a mathematical theory correlating resolution to the wavelength of light. Abbes formula make calculations of maximum resolution in microscopes possible.

1903 – Richard Zsigmondy develops the ultramicroscope and is able to study objects below the wavelength of light.
The Nobel Prize in Chemistry 1925 »

1932 – Frits Zernike invents the phase-contrast microscope that allows the study of colorless and transparent biological materials.
The Nobel Prize in Physics 1953 »

1938 – Ernst Ruska develops the electron microscope. The ability to use electrons in microscopy greatly improves the resolution and greatly expands the borders of exploration.
The Nobel Prize in Physics 1986 »

1981 – Gerd Binnig and Heinrich Rohrer invent the scanning tunneling microscope that gives three-dimensional images of objects down to the atomic level.
The Nobel Prize in Physics 1986 »

What is resolution?

A microscope paints!

Image:

Object:

Brush:

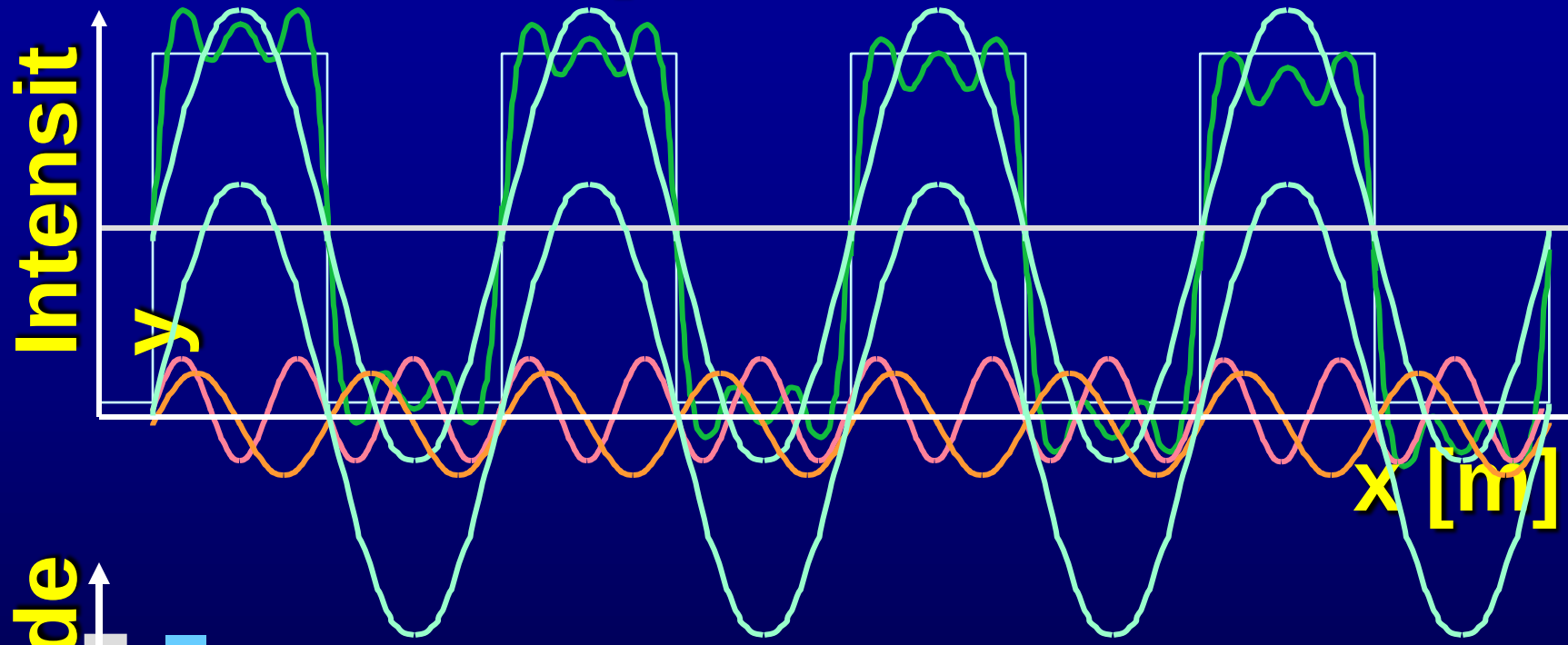
blurry

sharp

mathematically:
convolution of object with brush (point spread function)

Excurse: Spatial Frequencies

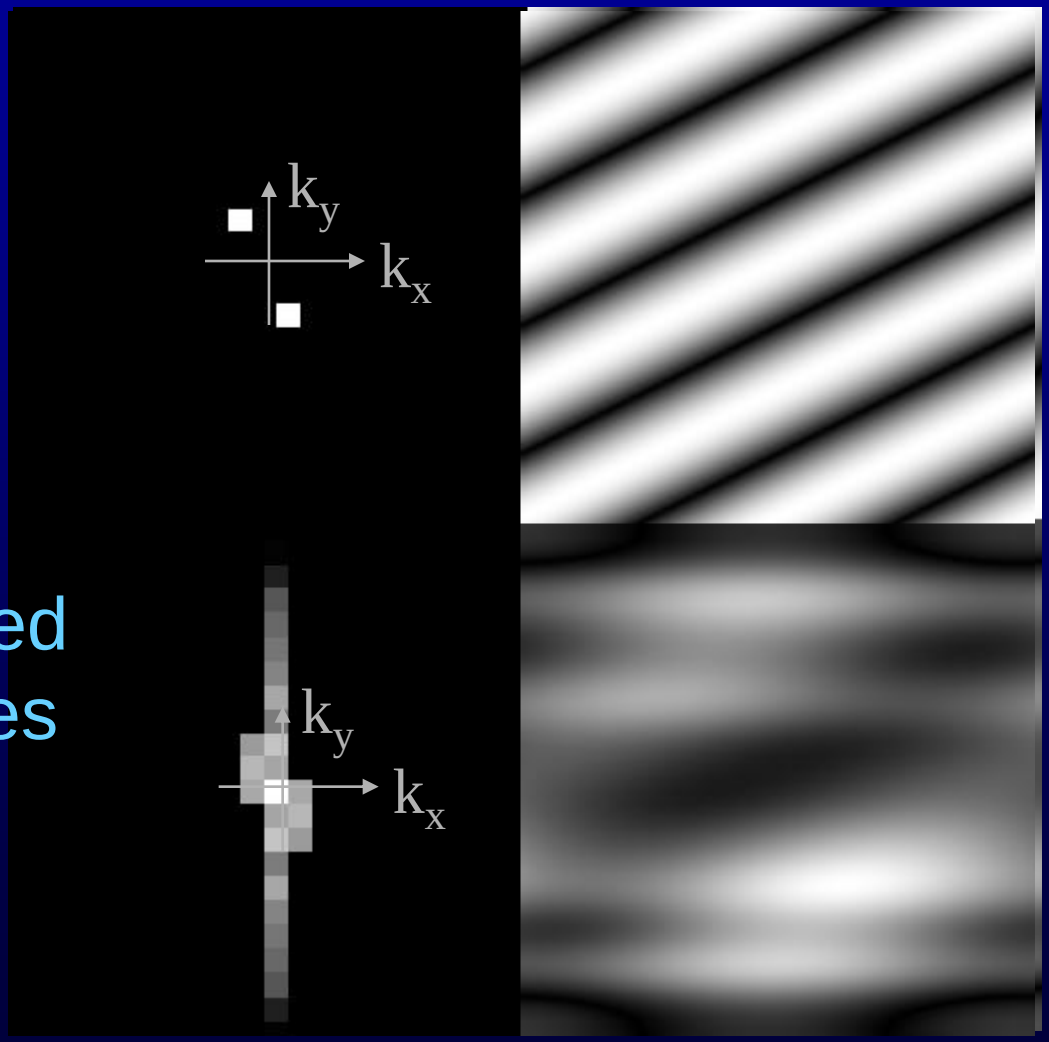
Real space:



Frequency space:

Constructing images from waves

Spatial Frequency



Corresponding Sine-Wave

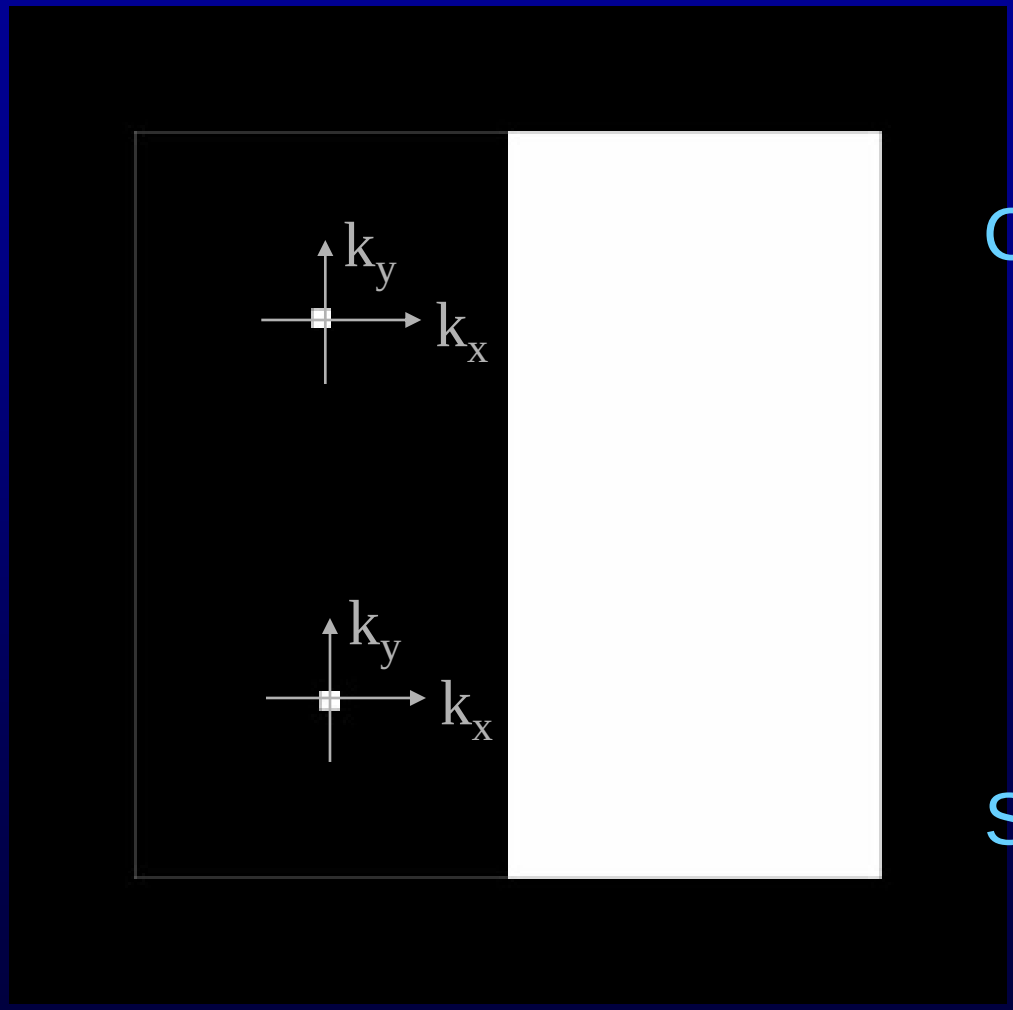
Accumulated Frequencies

Sum of Waves

Constructing images from waves

Spatial Frequency

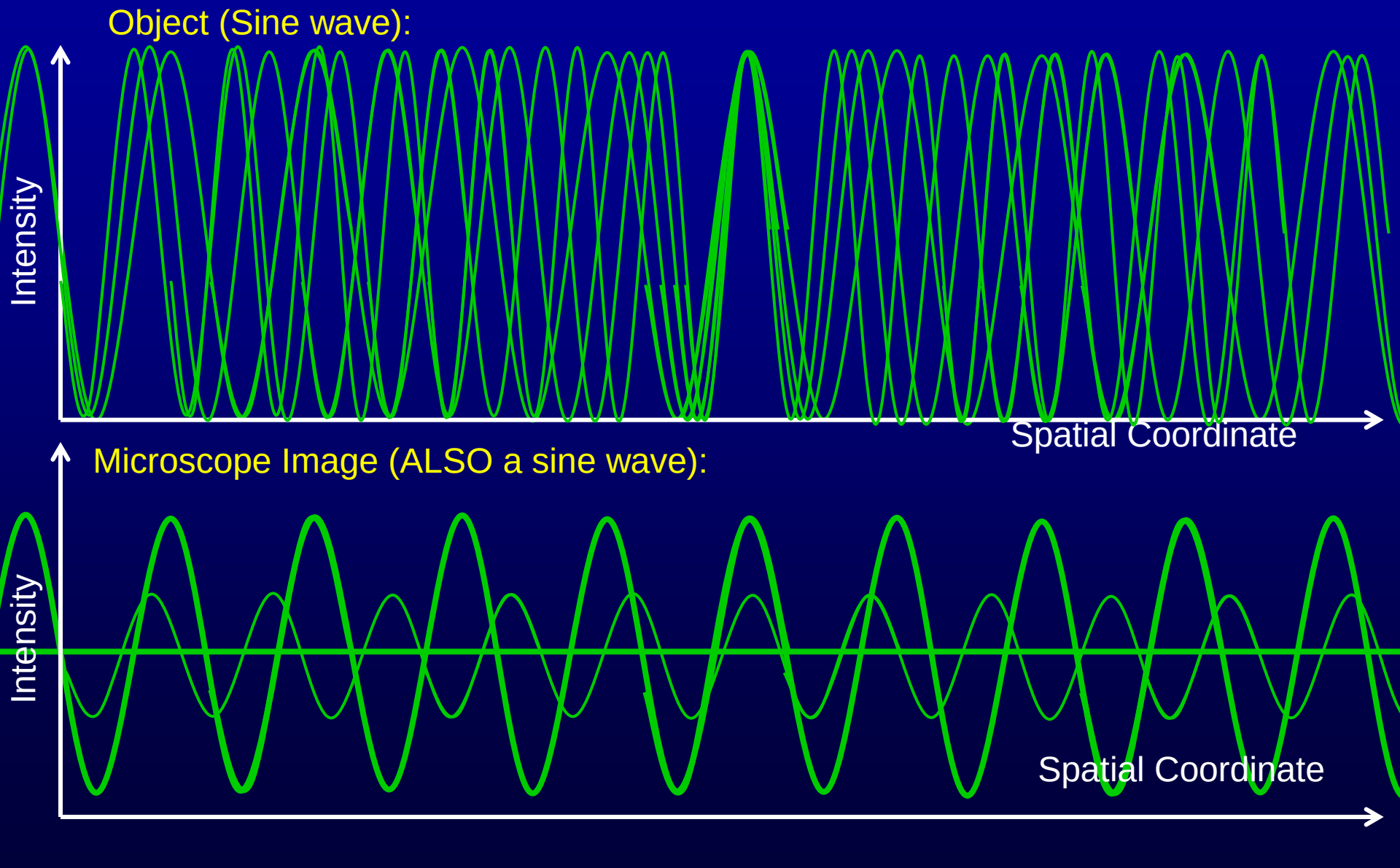
Corresponding Sine-Wave



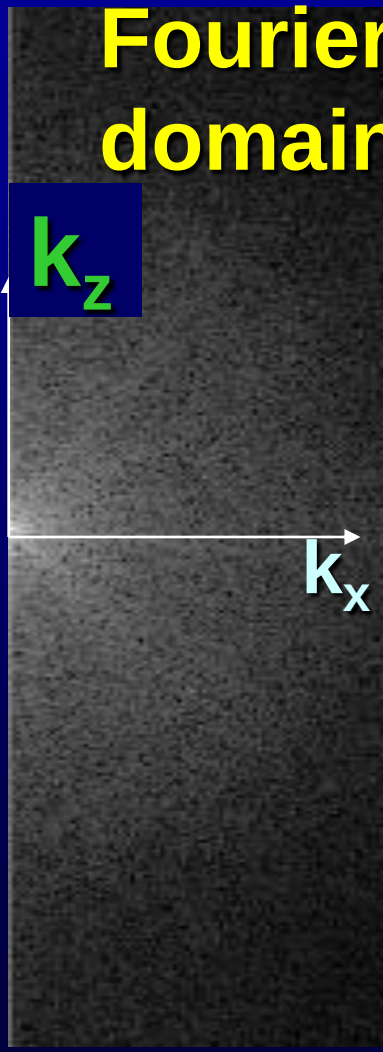
Accumulated Frequencies

Sum of Waves

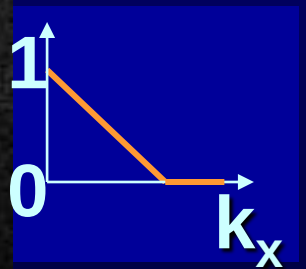
… Suppose sample is a sine-wave

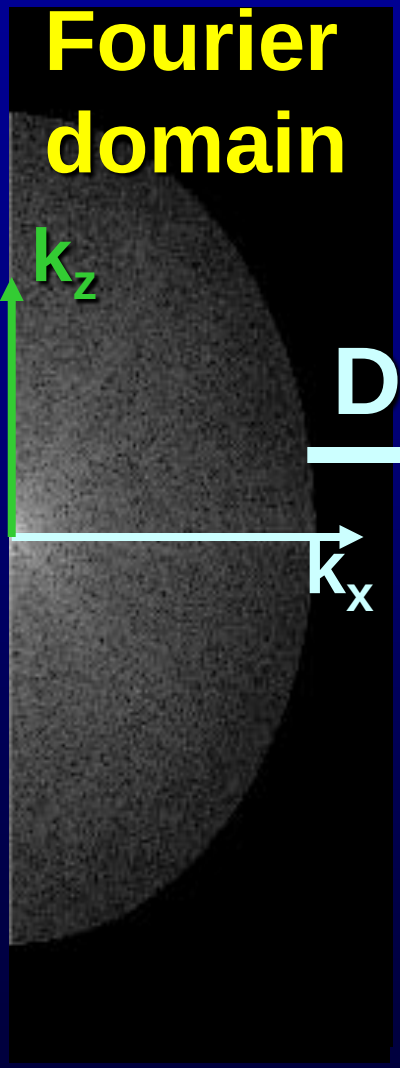


Fourier Filtering



suppress high spatial frequencies

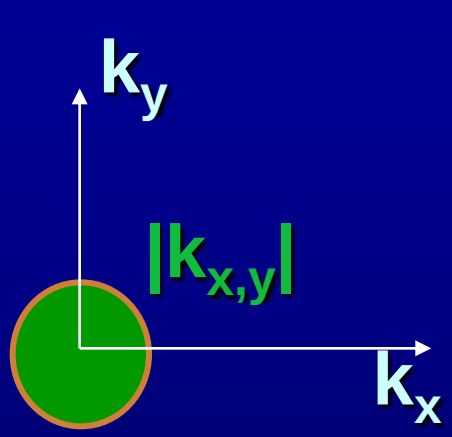


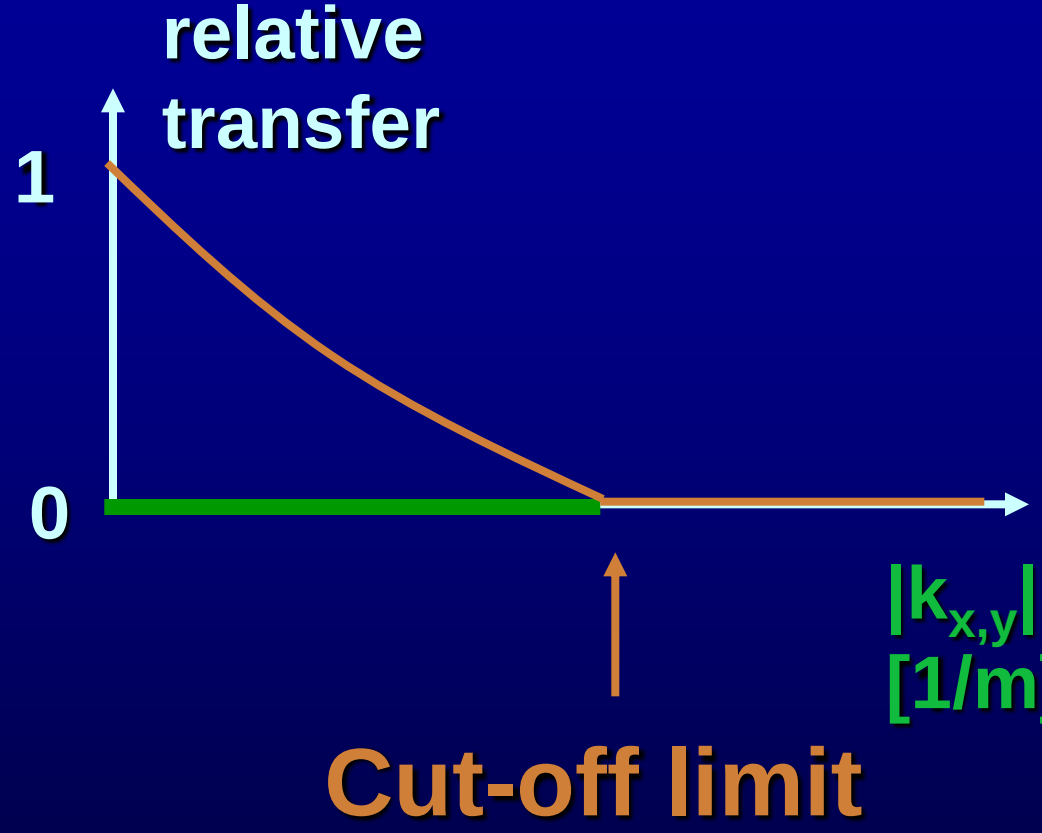


DFT

Real space

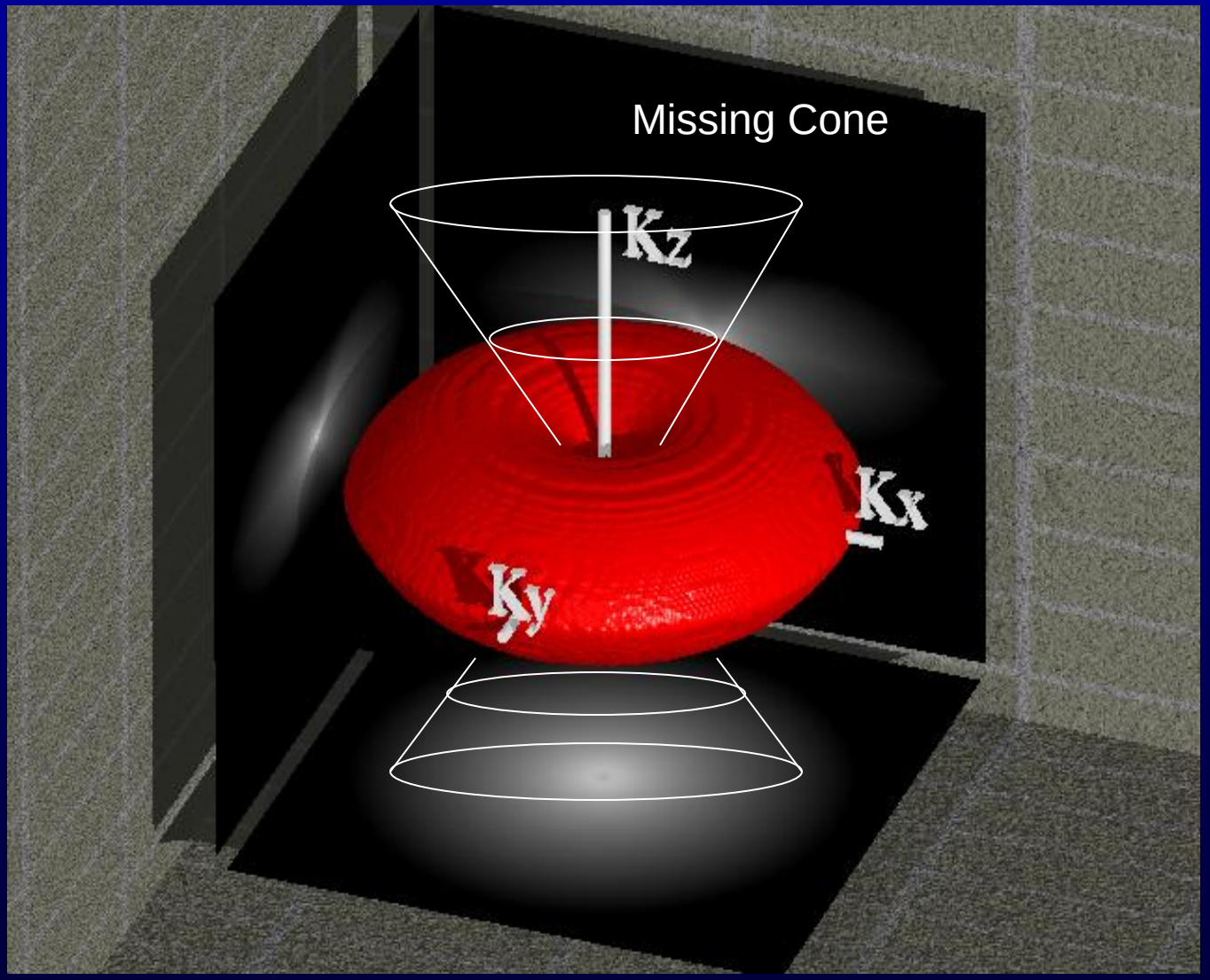
Optical Transfer Function





A microscope is a Fourier-filter!

Widefield OTF support

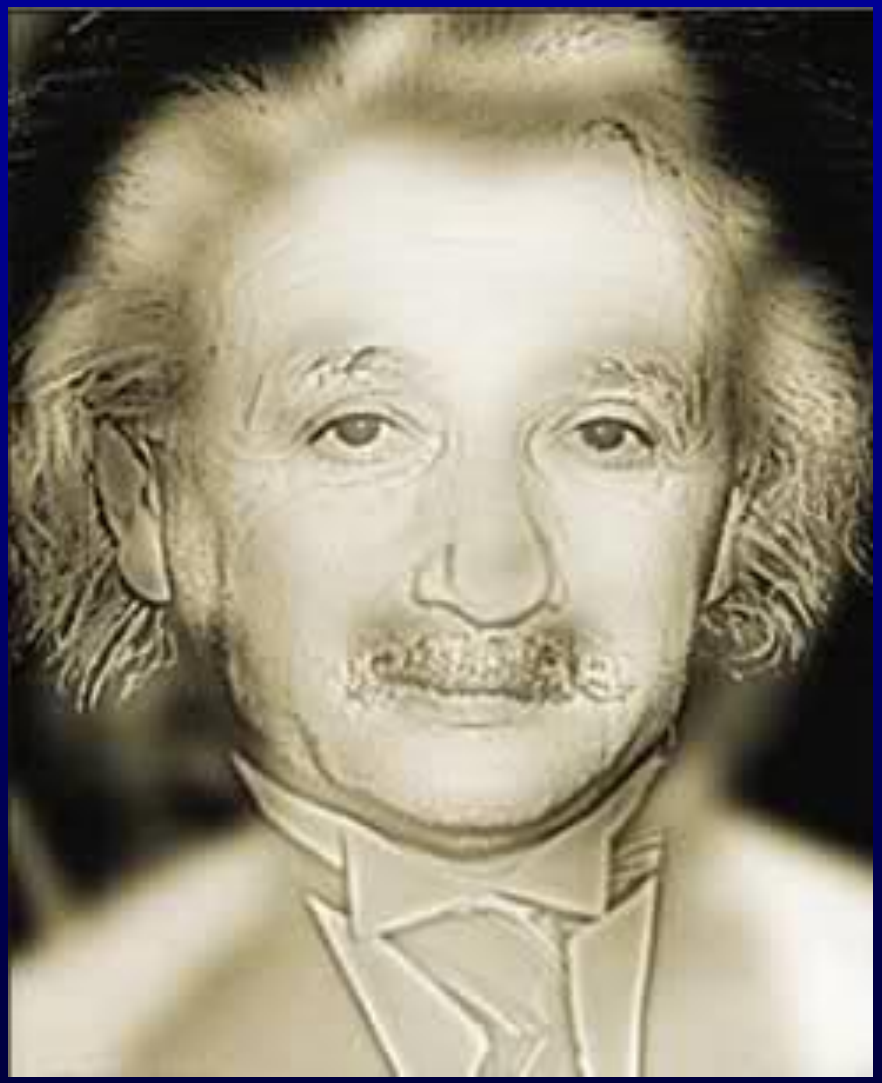


STED Microscopy

Stimulated Emission Depletion

physical limitation of the PSF

Resolution influences interpretation



http://www.filmigallery.com/mix-jokes/albert-einstein-marilyn-monroe-illusion-t26558.html

Detail (high freq.)

Detail (low freq. blurred)

In real space: PSF – Airy disc

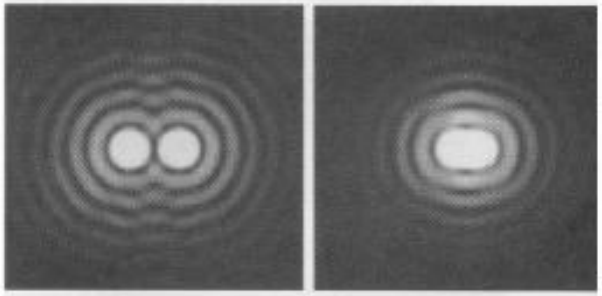


Figure 16-8 (a) Diffraction-limited images of two point objects formed by a lens. As long as the Airy discs are well separated, the images are well resolved. (b) Separated images of two incoherent point sources. In this diffraction pattern, the two images are well resolved. (c) Image of a pair of incoherent point sources at the limit of resolution. (Photos from M. Cagnet, M. Francon, and J. C. Thierr, *Atlas of Optical Phenomena*, Plate 16, Berlin: Springer-Verlag, 1962.)

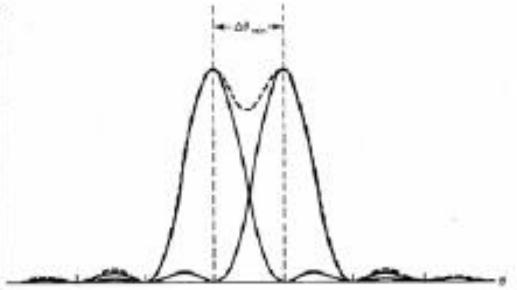


Figure 16-9 Rayleigh's criterion for just-resolvable diffraction patterns. The dashed curve is the observed sum of independent diffraction peaks.

Figure 1. Top: Images of pinholes that are clearly resolved and right at the Rayleigh resolution limit. Bottom: The intensity profiles of the two images right at the Rayleigh limit. (From Pedrotti and Pedrotti, 2nd, ed.)

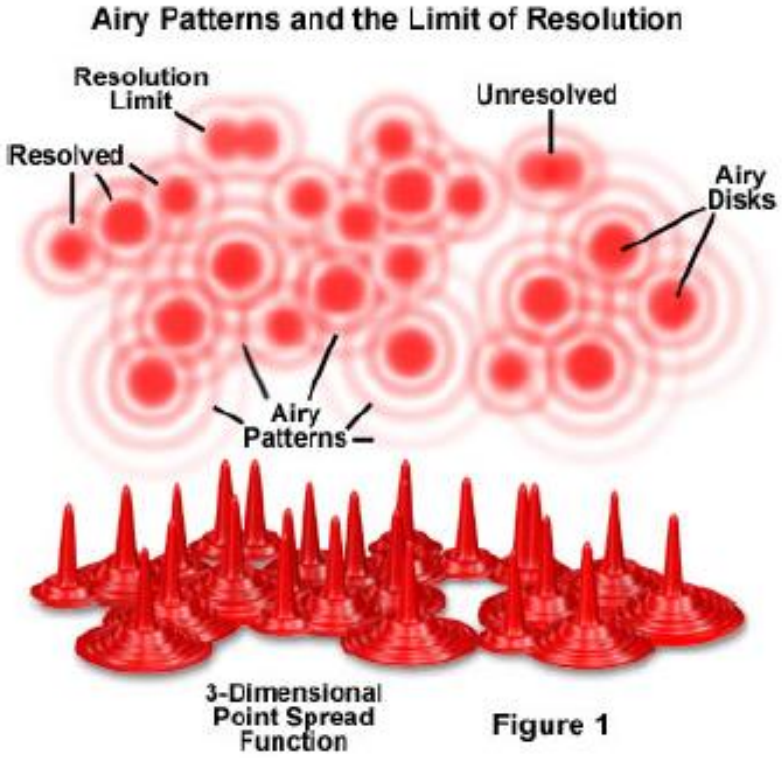
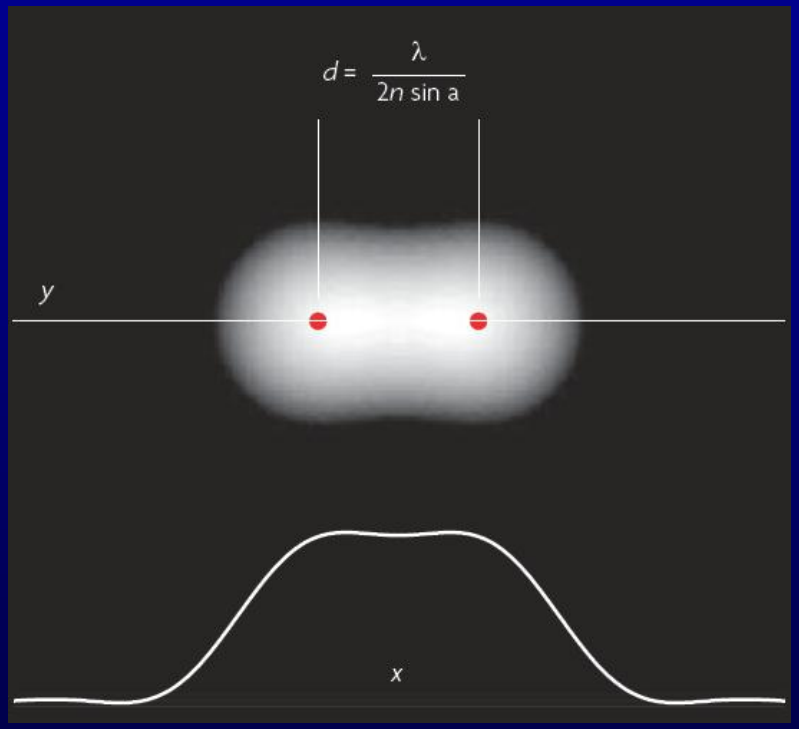


Figure 1

Increase the resolution

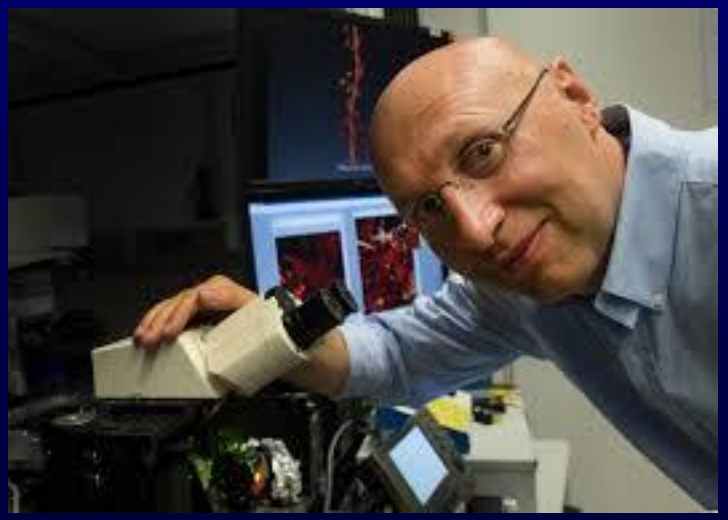
=

limit the PSF



REALIZED IN FLUORESCENCE MICROSCOPY

Two separate principles are rewarded. One enables the method *stimulated emission depletion (STED) microscopy*, developed by Stefan Hell in 2000. Two laser beams are utilized; one stimulates fluorescent molecules to glow, another cancels out all fluorescence except for that in a nanometre-sized volume. Scanning over the sample, nanometre for nanometre, yields an image with a resolution better than Abbe's stipulated limit.

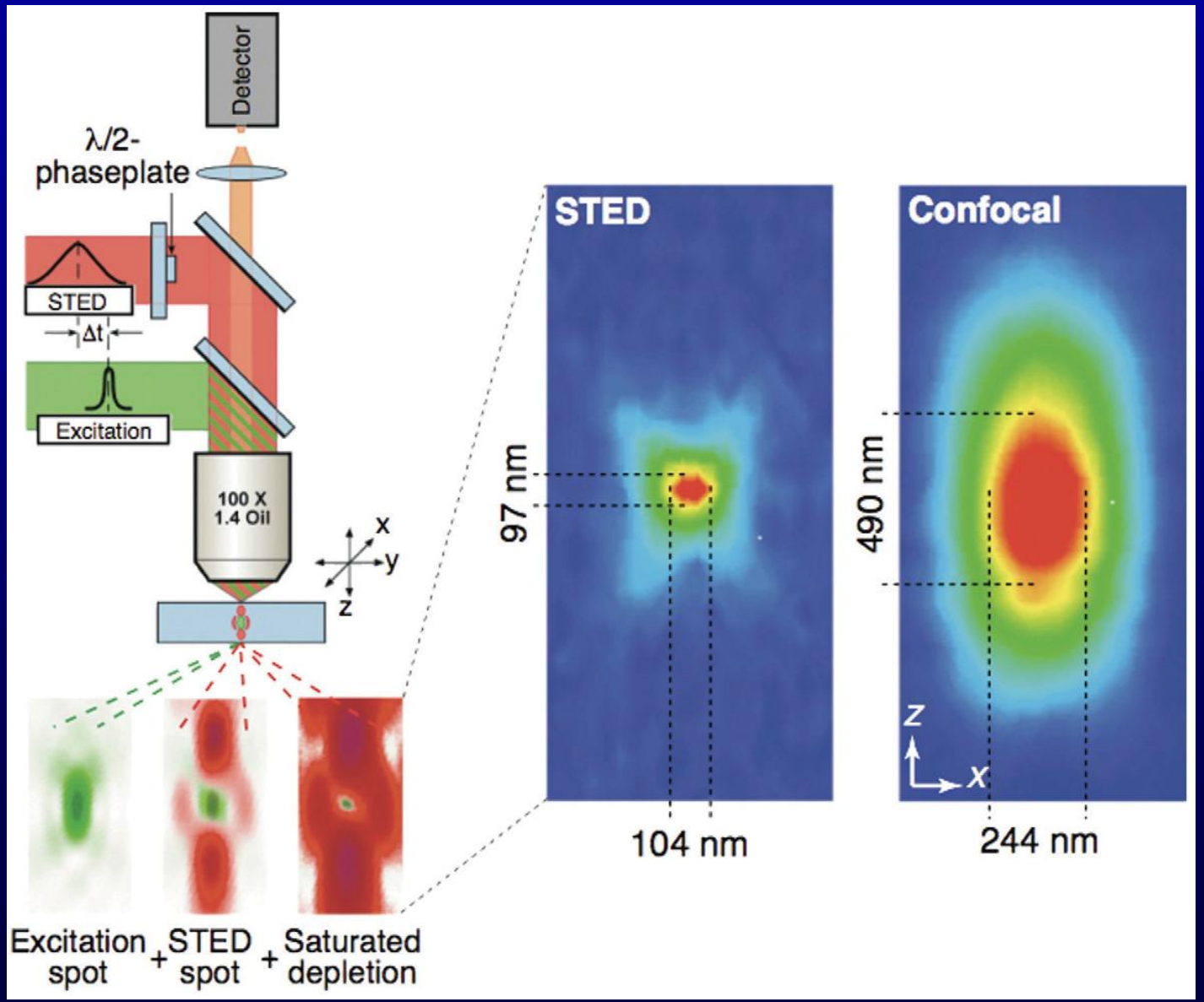


STED Microscopy

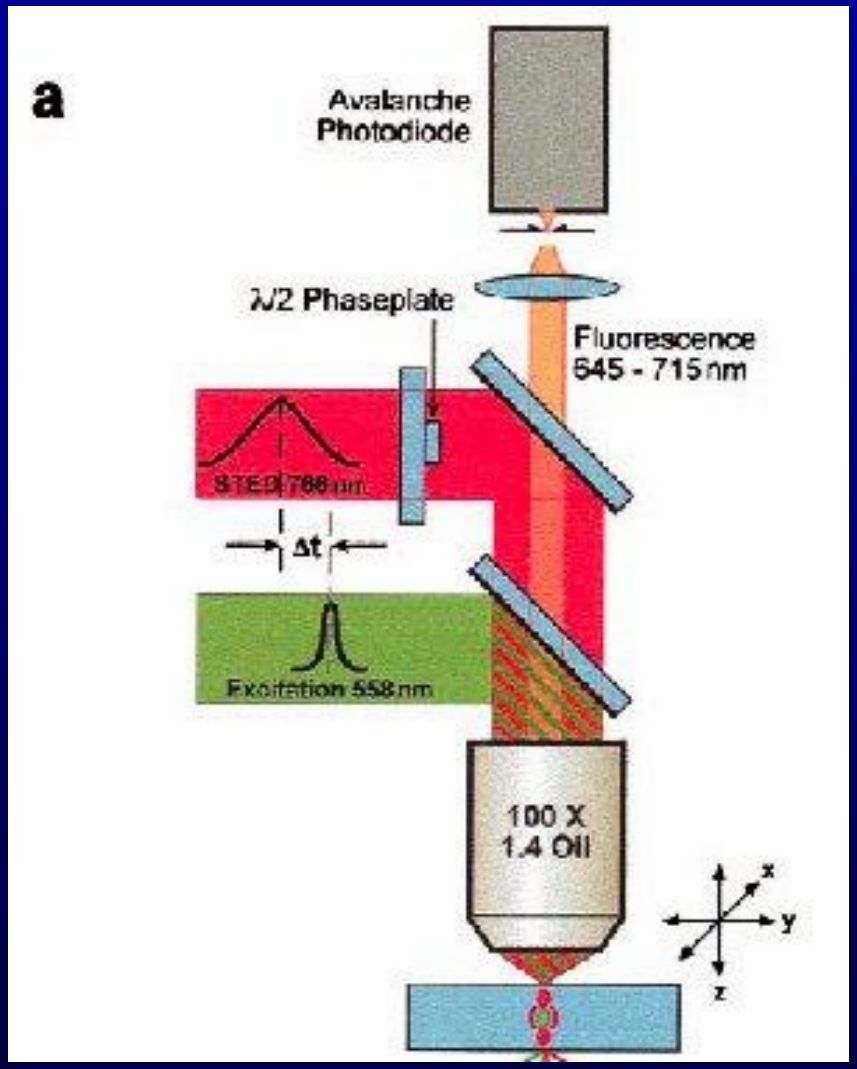
Stimulated Emission Depletion

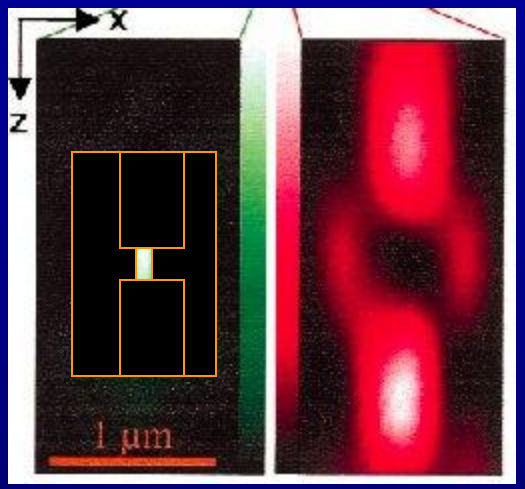
physical limitation of the PSF

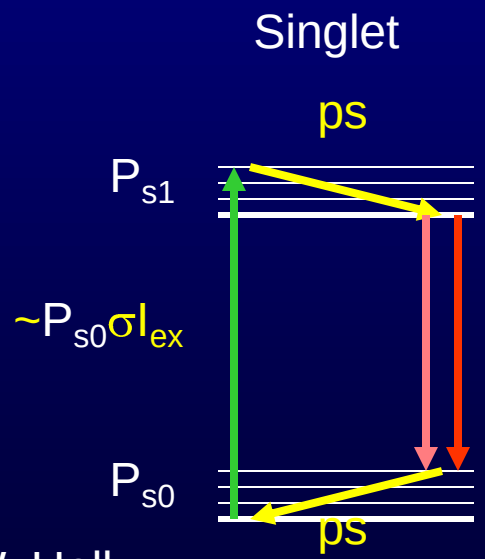
Stimulated Emission Depletion Microscopy (STED)



STED Microscopy







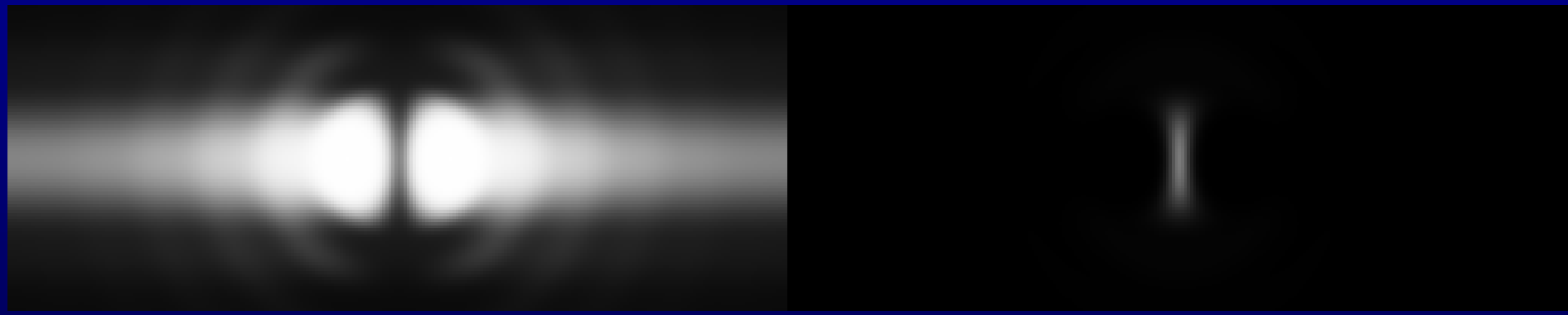
Stimulated Emission Depletion

Klar, T. A., S. Jakobs, M. Dyba, A. Egner and S. W. Hell (2000). Proc.Nat. Acad. Sc. U.S.A. 97(15): 8206-8210.

STED Microscopy

STED beam

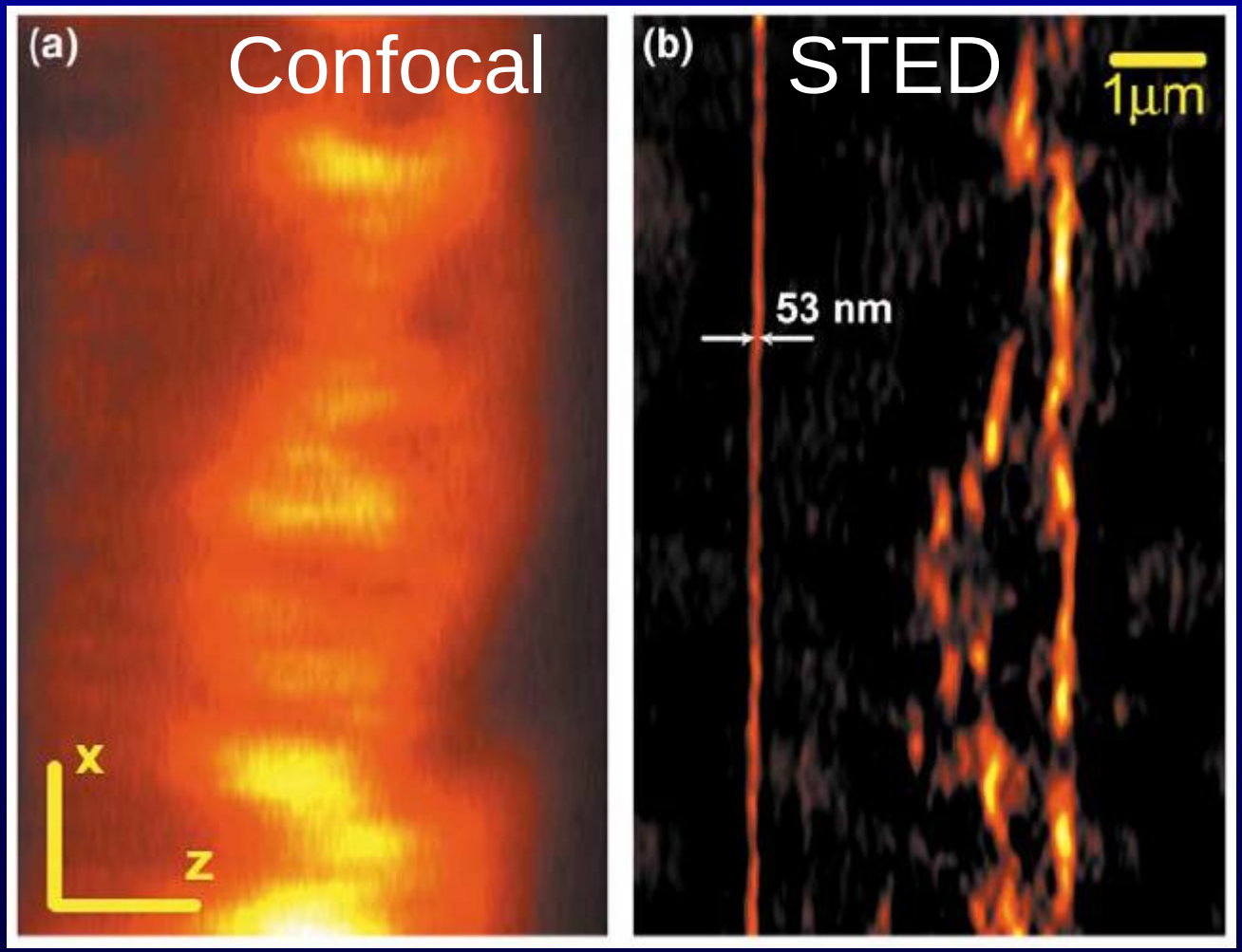
Resulting PSF



1 µm

NA 1.3, 10nm pixelsize, no background

STED Images

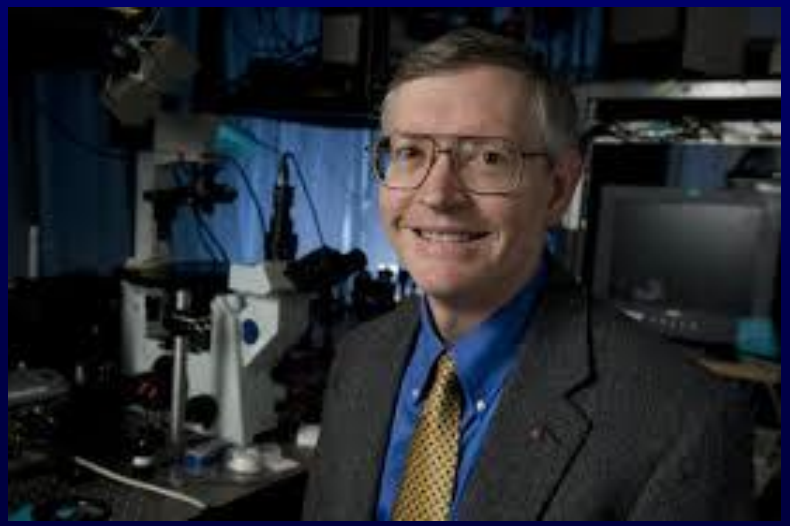


human embryonic kidney labeled with a red-emitting dye (MR 121SE)
Microtubules
Immunofluorescence

Current Opinion in Biotechnology 2005, 16:3–12

From micro to nano: recent advances in high-resolution microscopy; Yuval Garini, Bart J Vermolen and Ian T Young

Eric Betzig and William Moerner, working separately, laid the foundation for the second method, *single-molecule microscopy*. The method relies upon the possibility to turn the fluorescence of individual molecules on and off. Scientists image the same area multiple times, letting just a few interspersed molecules glow each time. Superimposing these images yields a dense super-image resolved at the nanolevel. In 2006 Eric Betzig utilized this method for the first time.



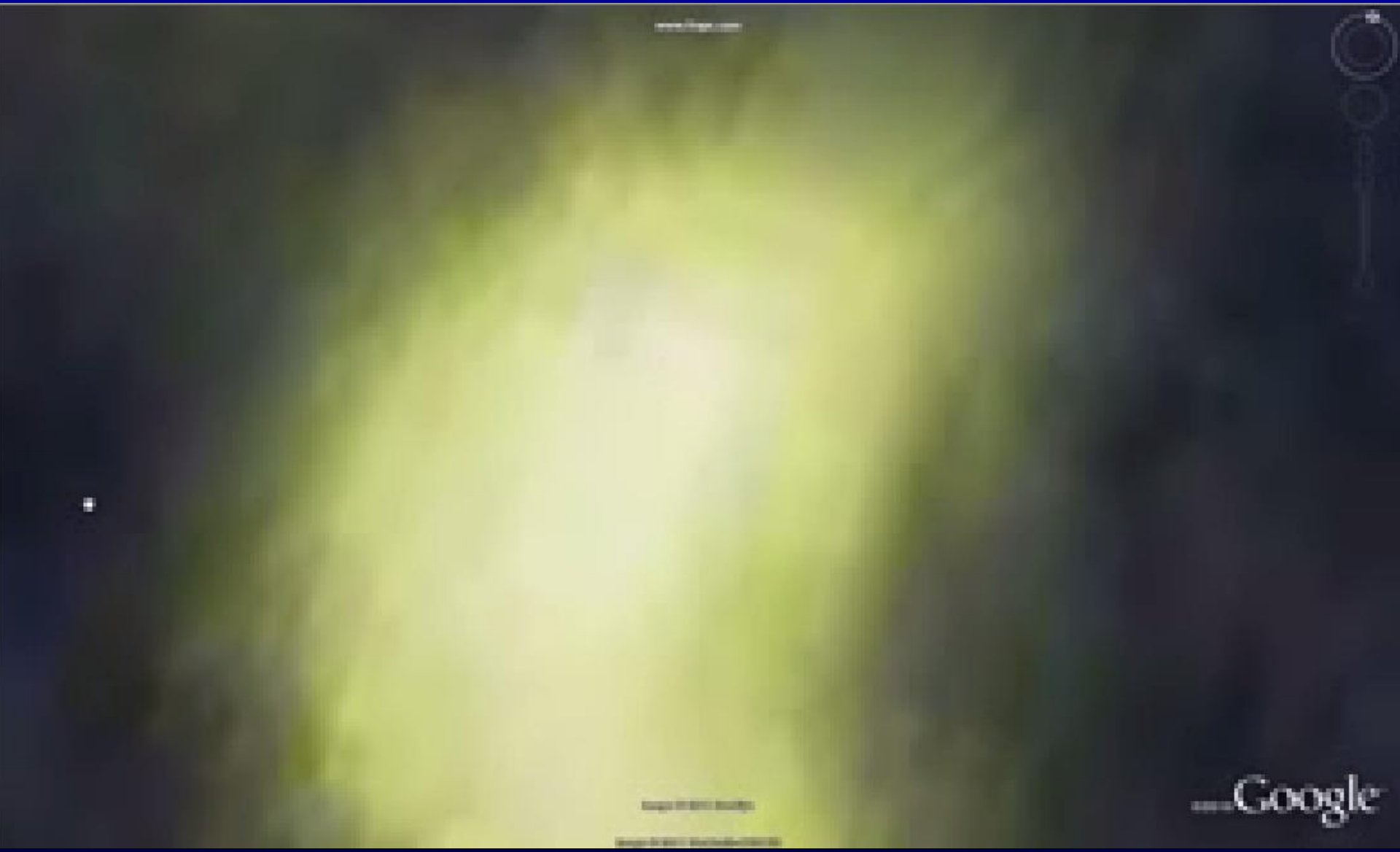
Pointillism

PALM & STORM

Image reconstruction based on a precise
localisation of the PSF centroids of individual molecules

The Night on Earth

Identification of individual light sources

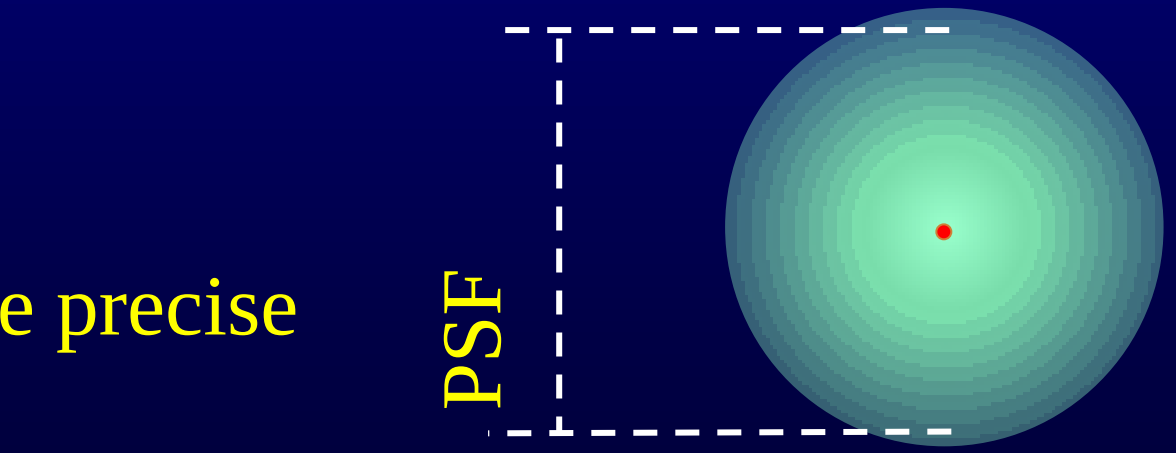


The Night on Earth

Localisation of individual houses:

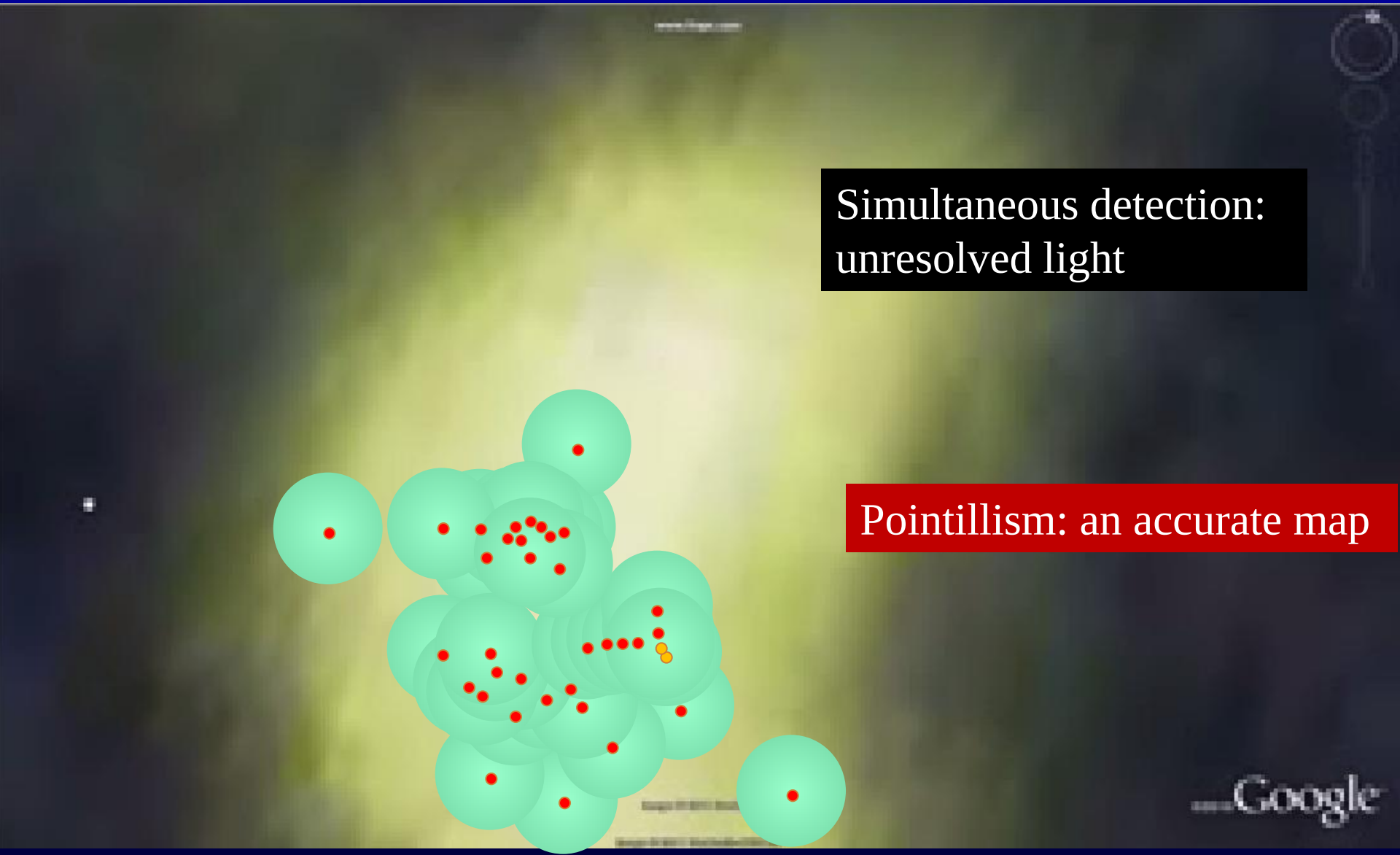
Switching on the ligths in each house according to a predetermined schedule

Localisation is more precise than the resolution!



Localization

Mapping


Simultaneous detection:
unresolved light
Pointillism: an accurate map
Google

Localization & Pointillism

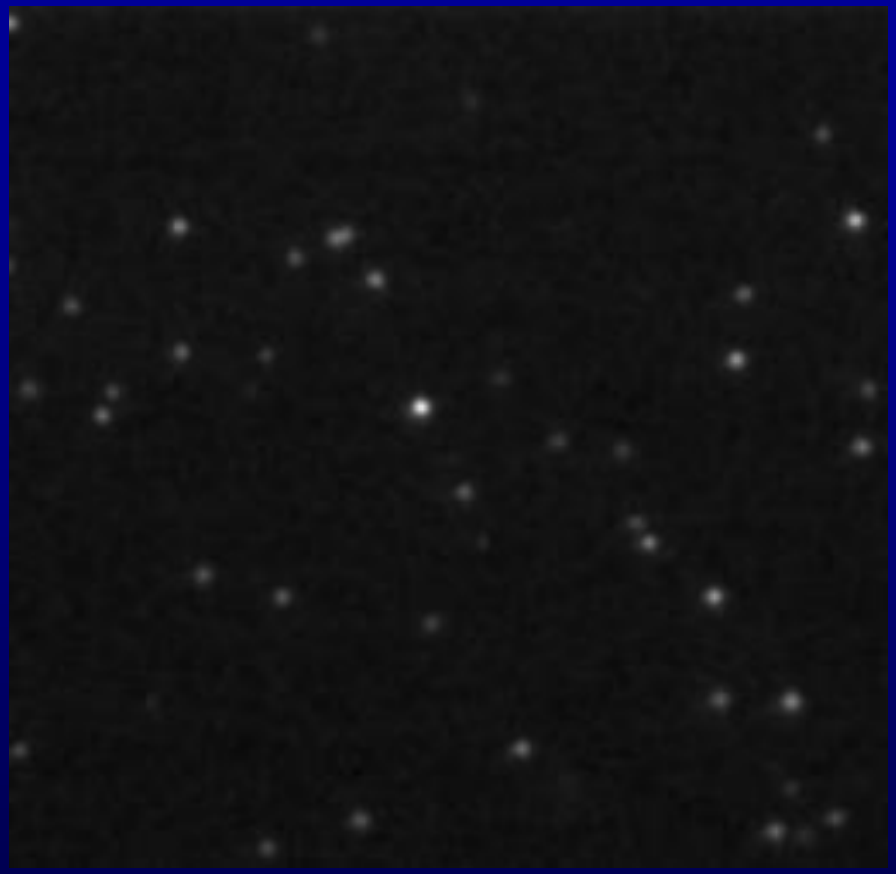
First localize all molecules,
and than reconstruct the image!

Seurat: Tigar

Douthwaite: Lewis Hamilton

When molecules are separated,
it is possible to determine their
positions with a high accuracy.

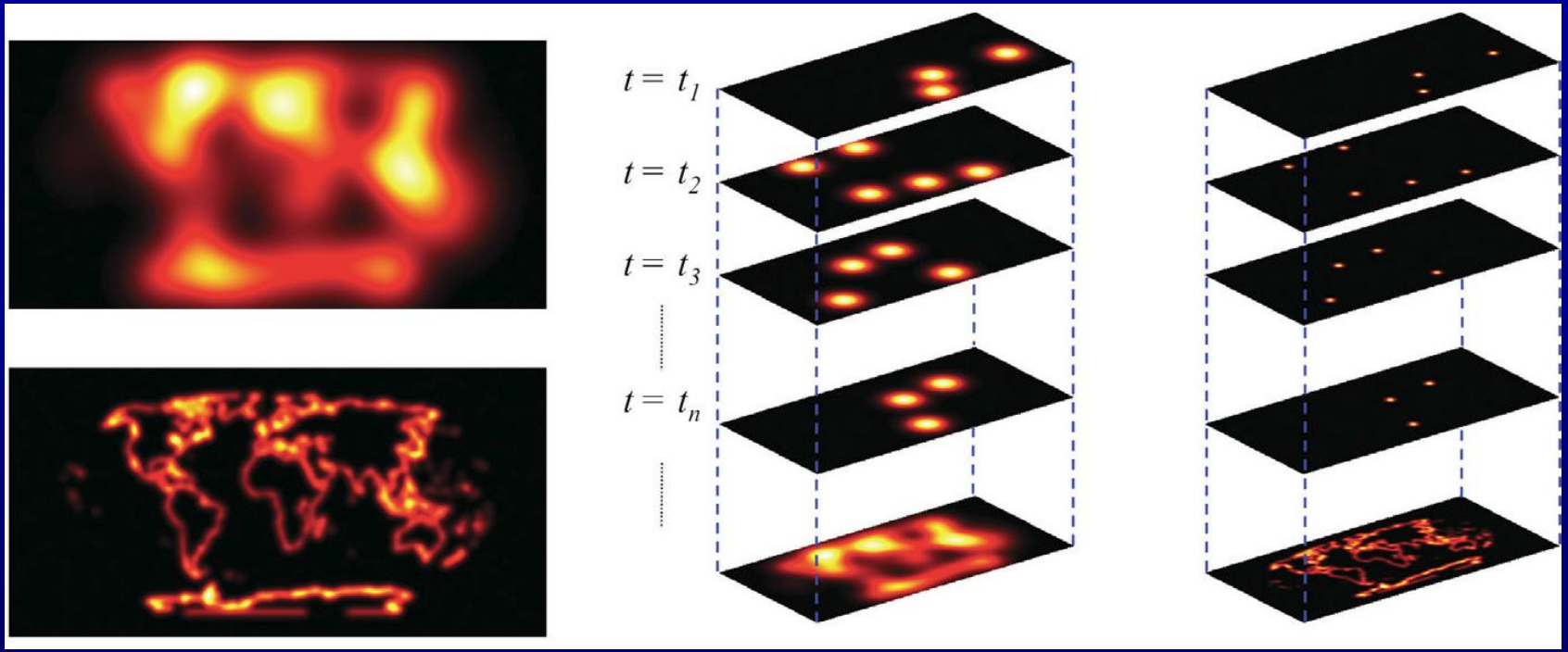
Blinking of individual quantum dots

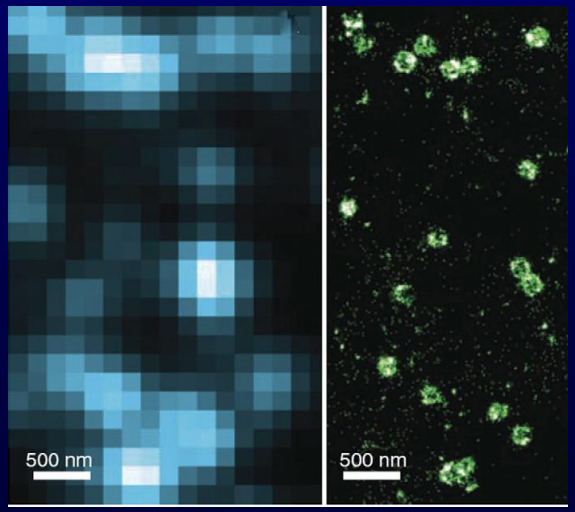


655 nm quantum dots

K.A. Lidke, B. Rieger, T.M. Jovin, R. Heintzmann *Optics Express* **13**, 7052-7062, 2005.

Super-Resolution Fluorescence Microscopy by Single-Molecule Switching





PhotoActivated Localization Microscopy (PALM)