Università di Pisa
LM Materials and Nanotechnology - a.a. 2016/17

Spectroscopy of Nanomaterials II sem - part 3

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Pushing conventional optics to the maximum performance: the confocal approach

OUTLOOK

We are still going into the details of conventional optical methods for spectroscopy at the nanoscale

We know that:
- moving to the nanoscale implies facing effects like scattering and interference
- conventional optics ha evolved very much
- refined configurations and instrumentation have been developed to meet many requirements

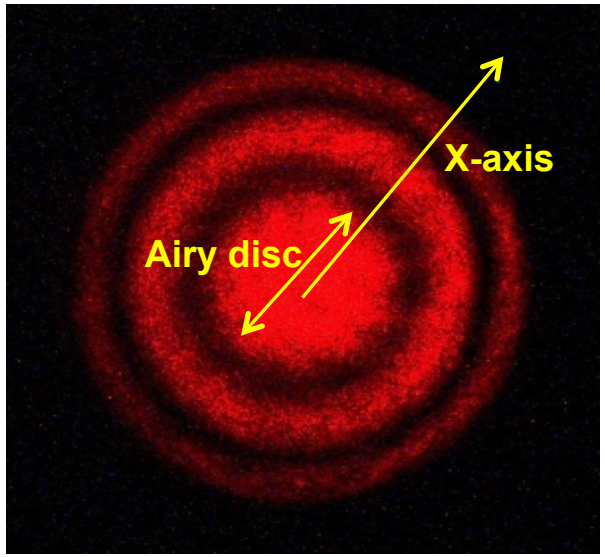
Today's menu (fast food):
- Starters of definitions for resolving power
- Main course of confocal microscopy with side dish of beautiful and colourful results
- No dessert

DIFFRACTION AND "DIFFUSION CONE"

For a circular aperture with diameter *a*
(that is, for a circular opaque scatterer with diameter *a*)

$$I(\theta) = I_0 \frac{J_1^2(\alpha)}{\alpha^2}$$

$$\alpha = \frac{a}{\lambda} \sin\theta \ ,$$

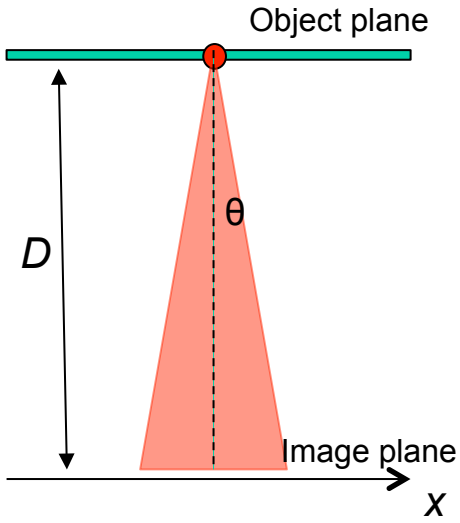


Airy disc (first dark fringe) is found for
$\sin\theta = 1.22\ a/\lambda$

BUT

$\sin\theta \sim tg\theta \sim x/D$

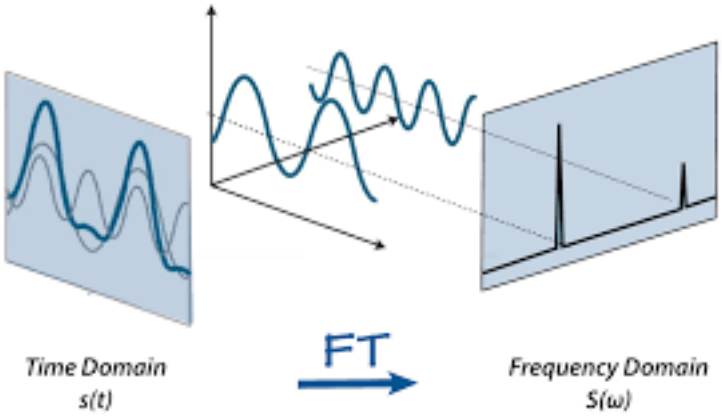
→ apparently, resolution depends on distance *D*



Diffraction sets a fundamental limitation to the attainable speatial resolution:
we need an approach to make it quantitative and find such a maximum resolution

The approach is based on Fourier trasnform and transfer function concepts

REMINDERS OF FOURIER

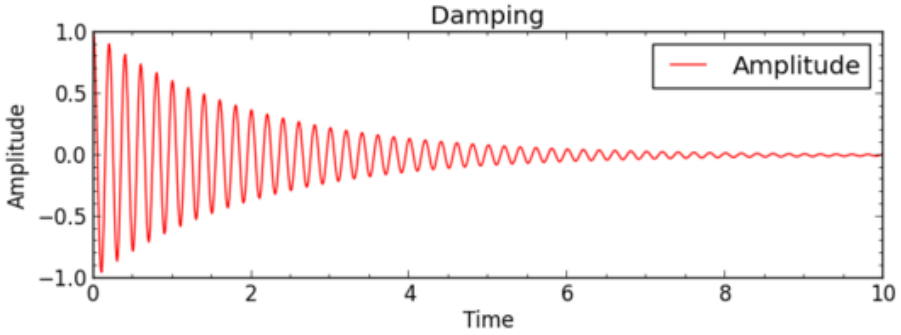


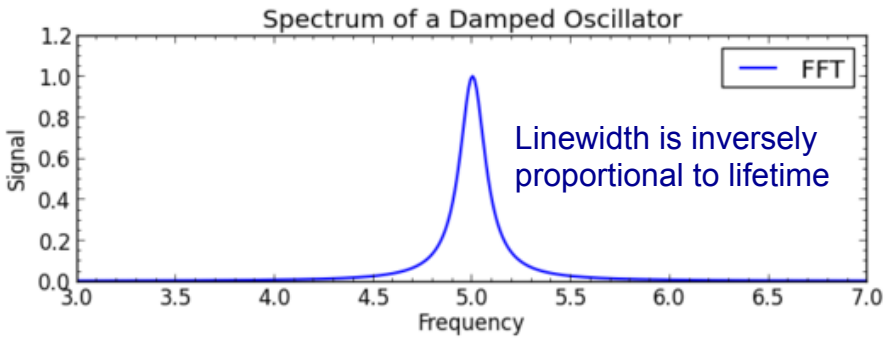
$$\mathcal{F}\{g(t)\} = G(f) = \int_{-\infty}^{\infty} g(t)e^{-i2\pi ft}\,dt$$

$$\mathcal{F}^{-1}\{G(f)\} = g(t) = \int_{-\infty}^{\infty} G(f)e^{i2\pi ft}\,df$$

f x t is a number (argument of sine/cosine)

Example: damped oscillator and Lorentz model for atomic emission





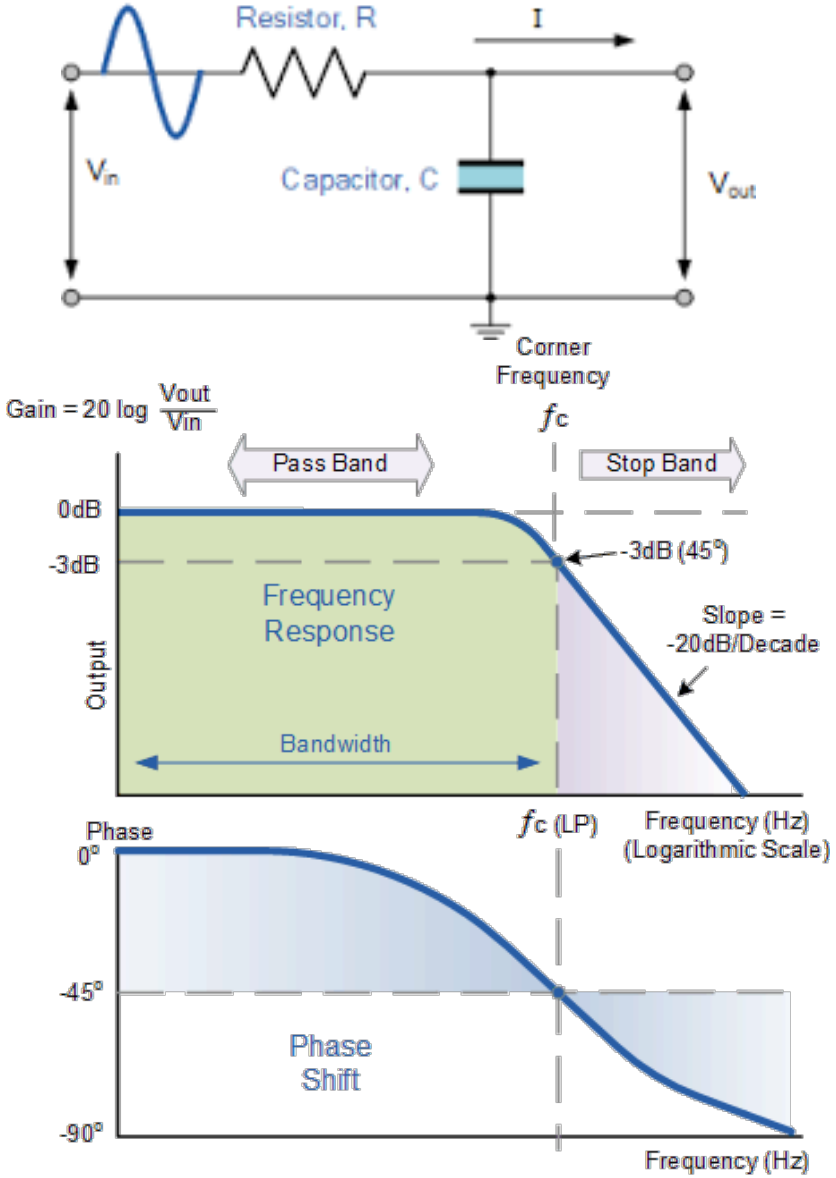
Fourier transformation applies to all "conjugate" pairs of variables, including, e.g., x k_x , with k_x x-wavenumber (projected wavevector in one direction)

$$G(k_x) = \int_{-\infty}^{+\infty} g(x)e^{-i2\pi k_x x}\,dx$$

$$g(x) = \int_{-\infty}^{+\infty} G(k_x)e^{i2\pi k_x x}\,dk_x$$

k_x called "spatial frequency"

REMINDERS OF TRANSFER FUNCTIONS



For sinusoidal signals, input and output (both written as complex functions) are related trough a (complex) **transfer function** *T(f)*

Example: a low-pass *RC* filter $T(f) = \frac{1}{\sqrt{1+\left(\frac{f}{f_T}\right)^2}}$ $f_T = \frac{1}{2\pi RC}$

The transfer function represents the response of any "linear" (causal) system to a sinusoidal perturbation

In case of non sinusoidal perturbation, individual response to any "harmonic" (to any frequency) can be accounted for through Fourier

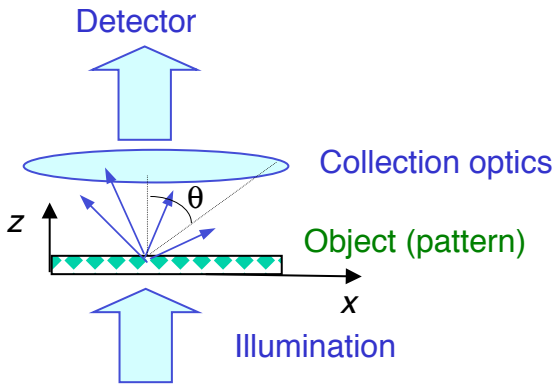
$$g_{out}(t) = \int_{-\infty}^{+\infty} T(f) G_{in}(f) e^{i2\pi ft}\, df$$

$$G_{in}(f) = \int_{-\infty}^{+\infty} g_{in}(t) e^{-i2\pi ft}\, dt$$

FOURIER OPTICS I

A microscope objective is a “linear” system connecting input (the object) to output (the image)

It can be described by a transfer function $T(k_x k_y)$ in the $k_x k_y$ space



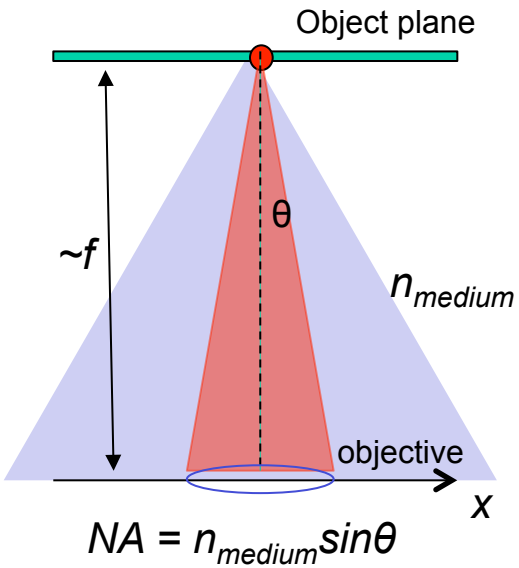
$$g_{out}(x,y) = \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} T(k_x,k_y) G_{in}(k_x,k_y) e^{i2\pi(k_x x + k_y y)}\, dk_x\, dk_y$$

$$G_{in}(k_x,k_y) = \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} g_{in}(x,y) e^{-i2\pi(k_x x + k_y y)}\, dx\, dy$$

$g_{in}(x,y)$ is a function describing the observed object (e.g., local brightness, or opacity)
$g_{out(}(x,y)$ is a function representing the obtained image

For instance:
- for a periodical pattern (e.g., a diffraction grating) g_{in} is a periodical function in the real space (usually in one coordinate) and G_{in} is a delta function centered on the spatial frequency of the pattern
- for a dot-like object, g_{in} is a delta function and G_{in} is a continuous function spanning over all the spatial frequency range

FOURIER OPTICS II



The finite diameter of the objective sets an upper limit to the $k_x = k\sin\vartheta = (2\pi/\lambda)\, n \sin\vartheta$ that can be collected and transferred to the output (the image)

Wavevector components out of the acceptance cone are not transferred to the image plane

The integrals over the $k_x\, k_y$ space are limited up to $k_{max} = (2\pi/\lambda)NA$

$$g_{out}(x,y) = \int_{-k_{max}}^{+k_{max}} \int_{-k_{max}}^{+k_{max}} T(k_x,k_y) G_{in}(k_x,k_y) e^{i2\pi(k_x x + k_y y)}\, dk_x\, dk_y$$

A microscope objective behaves like a *low-pass filter* in the spatial frequency domain

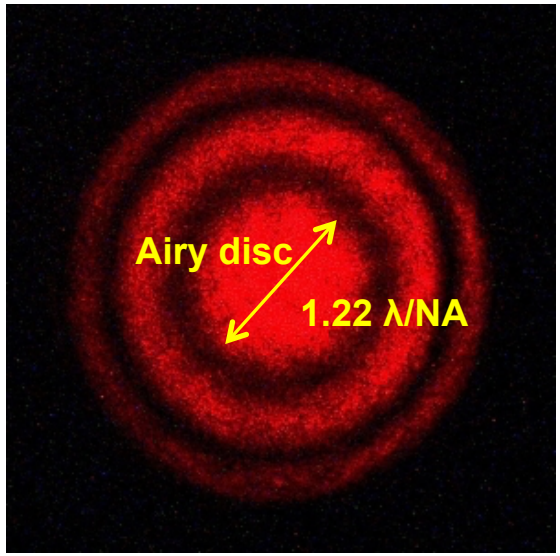
Nanosized details require high spatial frequency components to be reconstructed
→ diffraction blurs sharp details and nanosized objects

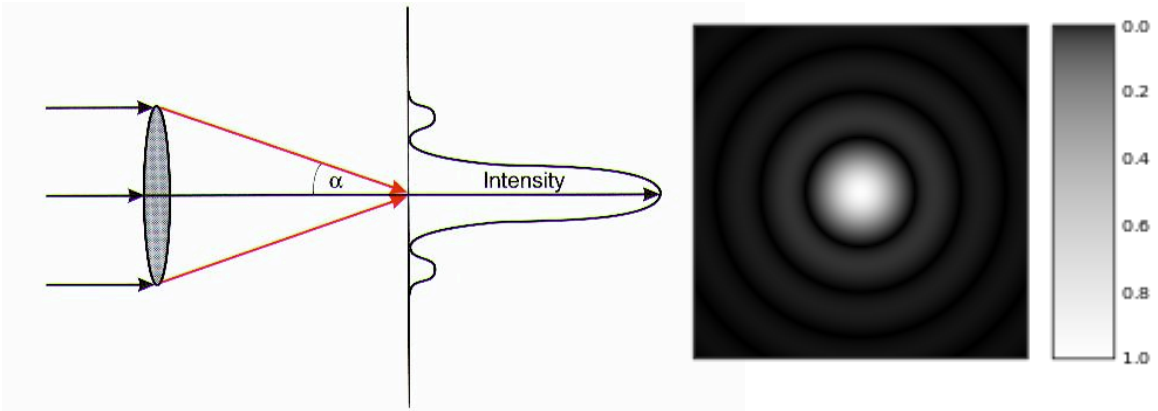
FOURIER OPTICS III

Nomenclature (not universal, yet):

- The complex transfer function of a real objective is called Optical Transfer Function (OTF)
- Its real part (the amplitude) is called Modulation Transfer Function (MTF)
- **The transfer function for a dot-like object is called Point Spread Function (PSF)**

The PSF for an **ideal objective** (i.e., **neglecting any "defect"** such as, chromatic and spherical aberration, astigmatism, response to polarization, etc.) is simply the **diffraction function in 2D** leading to Airy disc with diameter 1.22 λ/NA





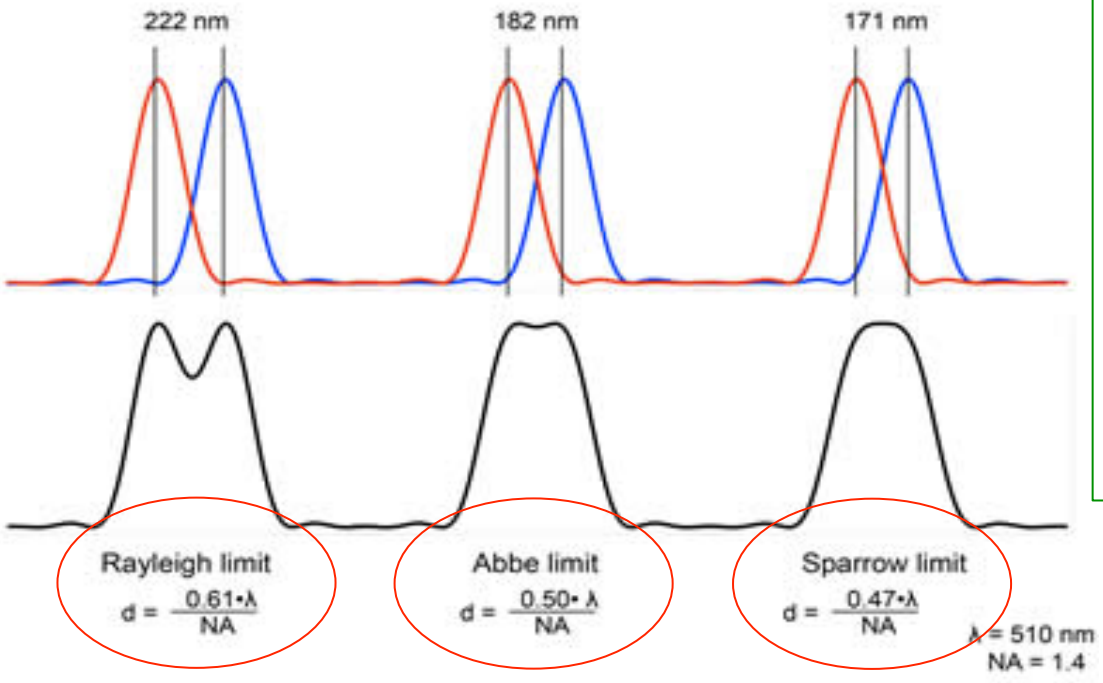
For reciprocity, this is also the diameter of the spot produced by the objective illuminated by a perfectly collimated beam (within the diameter there is more than 80% of the beam power)

RESOLVING POWER I

The performance of an optical system (intended to produce a magnified image of an object) is evaluated through the **resolving power:**

- The resolving power *d* is the minimum distance between two distinct, **dot-like** objects enabling their optical resolution, i.e., to distinguish them in the produced image
- The resolving power can be evaluated *theoretically* (i.e., in **ideal conditions**) once the PSF is known
- **For an ideal objective, whose PSF is described by the diffraction function, different criteria exist**

More precisely (with an example):



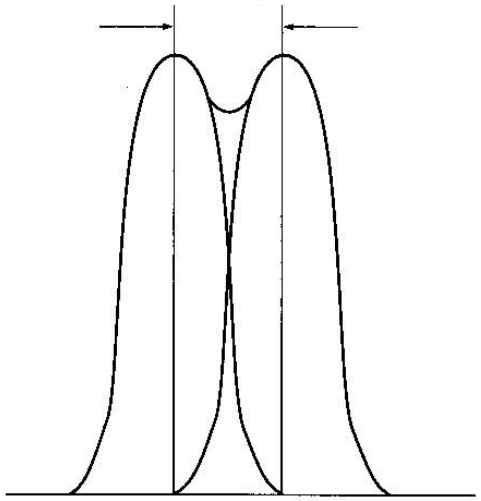
Rayleigh's criterion (the most frequently used):
Two dot-like objects are distinguished when the maximum of the diffracted intensity for one of them falls into the (first) minimum of the diffracted intensity for the other one
→ *Abbe limit*

Sparrow's criterion:
Two dot-like objects are distinugished when thier distance is equal to twice the fwhm of their diffraction intensity distribution

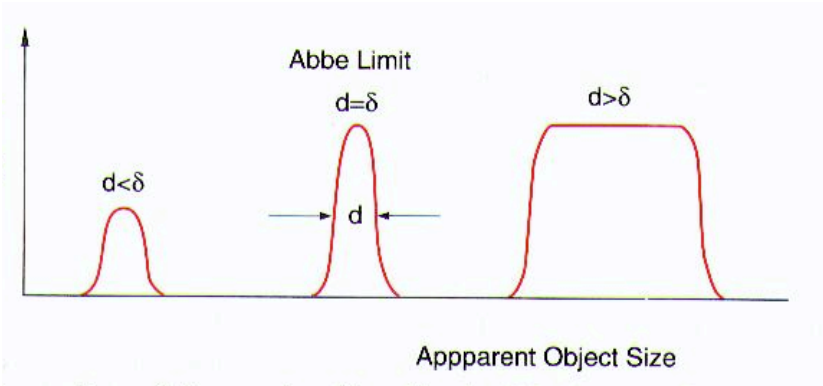
RESOLVING POWER II

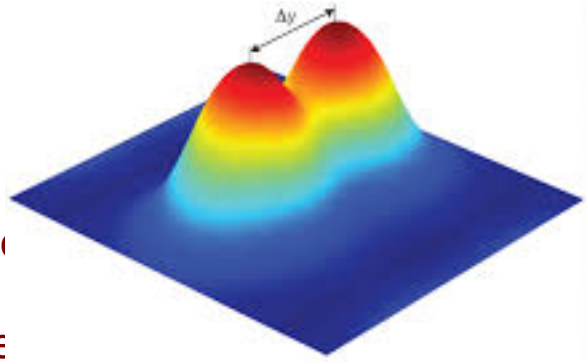
Abbe limit in practical terms

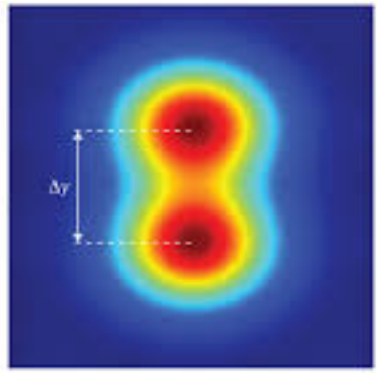
$$d = \frac{\lambda}{2n\sin\alpha} \sim \frac{\lambda}{2NA} \simeq \frac{\lambda}{2}$$



Diffraction-limited resolution (roughly λ/2) is often too much for the increasing demands of nanotechnologies!







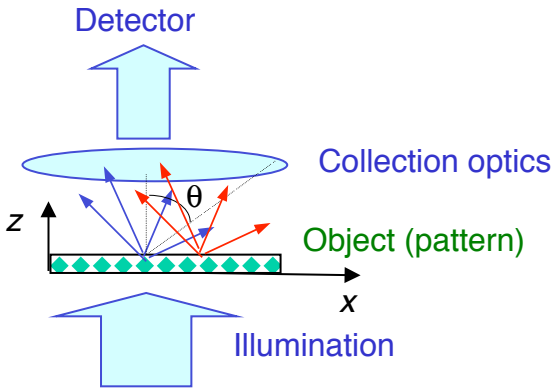
Note:

- The concept of spatial resolution is merged, som that of contrast
- An individual, isolated nanostructure may be dete conventional microscopes at the expenses of its “visibility”
- Signal-to-noise ratio must be kept at the maximum level in order to access diffraction-limited resolution

STRAY LIGHT AND BLURRING

Note, in addition, the following problem related to illumination of the sample**:**

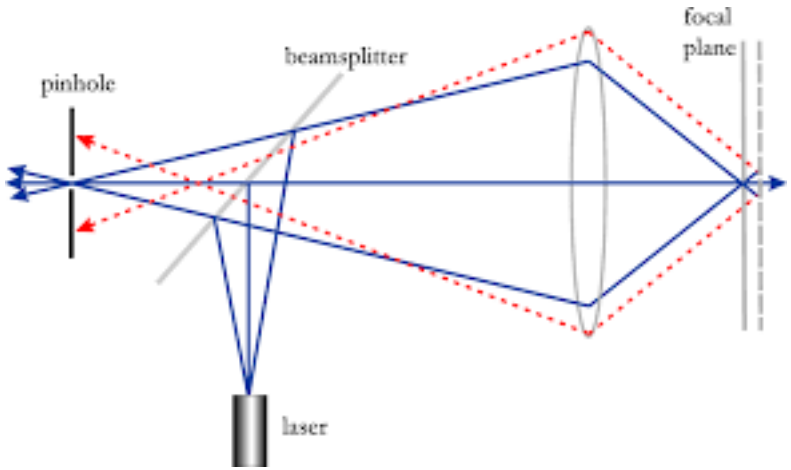
- In non point-like illumination schemes (conventional) rays emitted from different points of the surface can be collected
- Large numerical aperture l(eads inherently) to sensitivity to “stray light”
- Contrast falls down, signal-to-noise decreases and high space resolution is hampered



(dark field microscopy is an hystorically relevant, and still used, approach to limit stray light contribution, but by itself it is not powerful enough to meet the true and ultimate limit of resolution)

Diffraction-limited resolution (roughly λ/2) requires additional care besides short focal length, large NA and ideal objectives!

CONFOCAL MICROSCOPY/SPECTROSCOPY



- Illumination ("input") is accomplished only where collection ("output") is carried out, with a truly diffraction limited resolution
- Light scattered or emitted from out-of-focus planes is blocked by a pin-hole

Requires a **laser** (to achieve diffraction-limited spots) and a **scanning** approach

Offers strong sensitivity to the "vertical" direction

→ **Tomography capabilities**

Confocal Scanning Optical Microscope (CSOM/CLSM) provides the best spatial resolution in conventional instruments, in particular for fluorescent samples

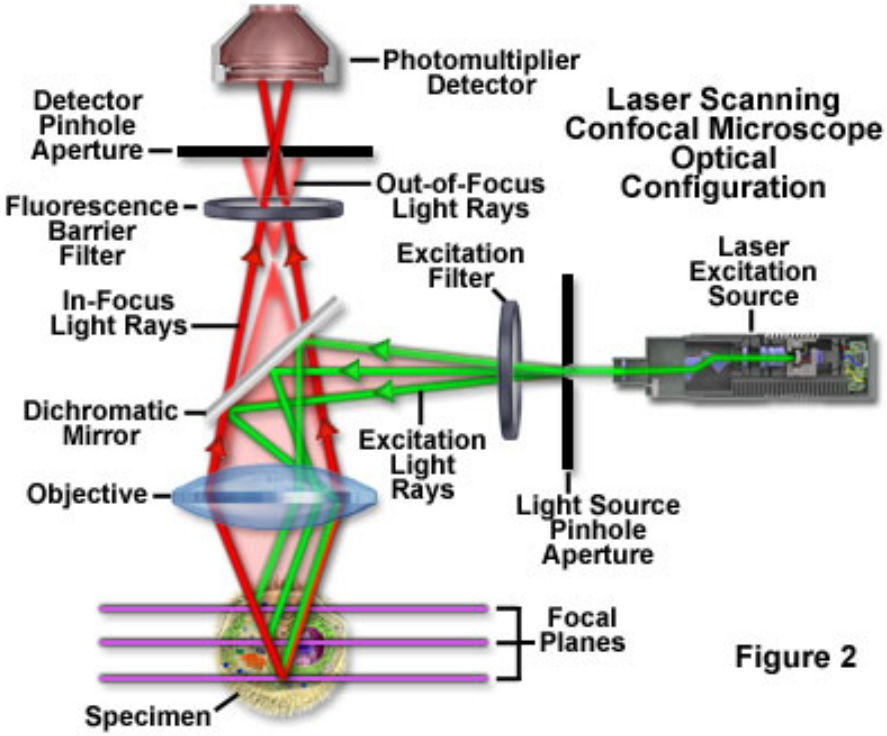


Figure 2

CSOM/CLSM

In perfectly ideal conditions, i.e., no stray light at all (and, obviously, ideal optical components and extreme sensitivity), the confocal approach allows “exploiting twice”, i.e., in illumination and collection, the resolving power

Theoretically (not very much used, though):

$$d_{CSOM} \simeq \frac{(0.61)^2 \lambda}{NA} = \frac{0.37\lambda}{NA}$$

In addition, the possibility to focus illumination and collection at a definite plane leads to a much reduced depth of focus and the associate tomographic capabilities, with a “vertical” resolving power theoretically given by

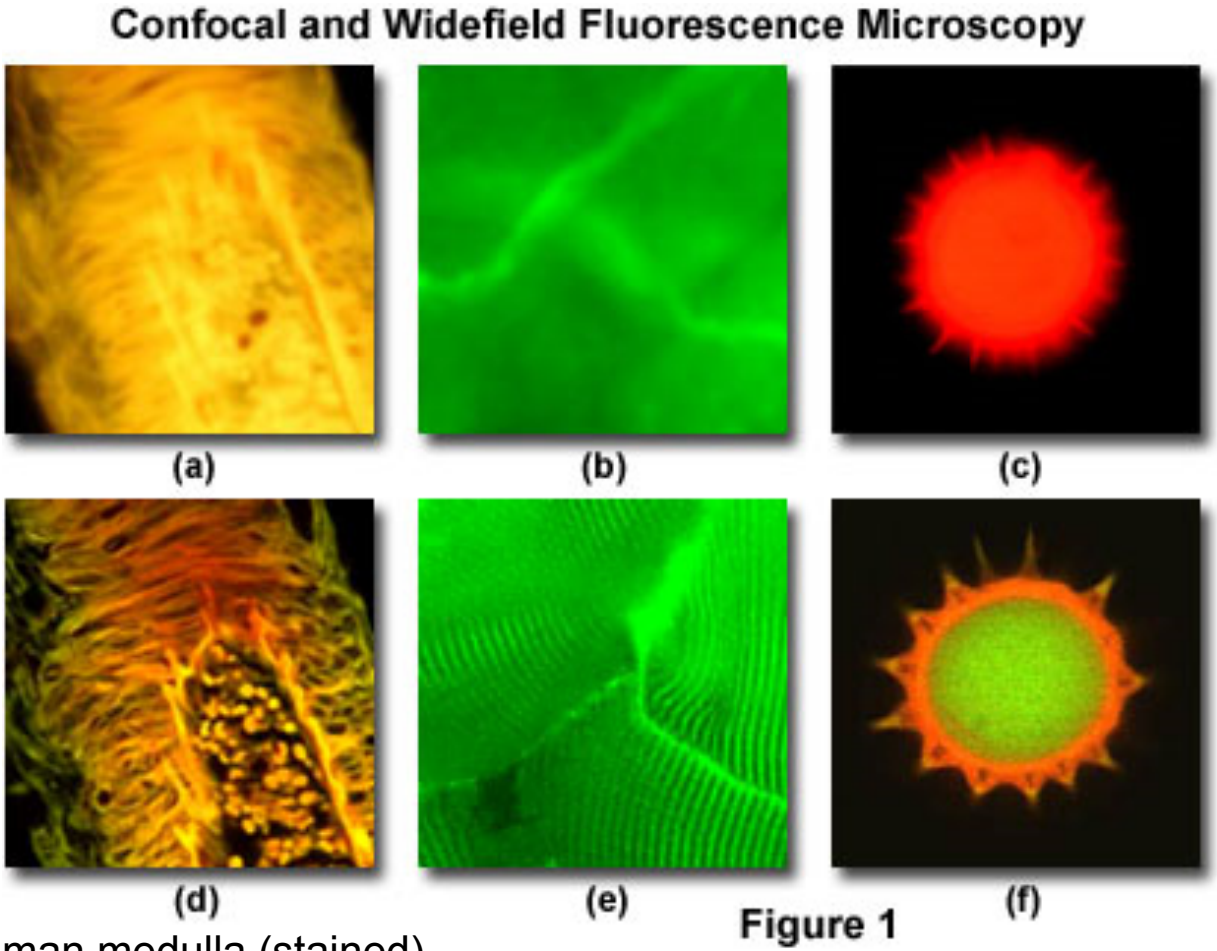
Theoretically (not very much used, though):

$$d_{z,CSOM} \simeq \frac{0.89\lambda}{NA^2}$$

Furthermore, the scanning technique allows improved signal-to-noise at the expenses of acquisition time (CSOM/CLSM does not provide with “real-time” imaging)

The use of monochromatic and coherent sources (laser, here the speckle problems is negligible thanks to the point-like illumination) allows contrast enhancement with fluorescent (stained, or autofluorescent) samples, thanks to the proven efficiency in wavelength filtering (i.e., discriminate effectively between emission and illumination radiation)

EXAMPLES I



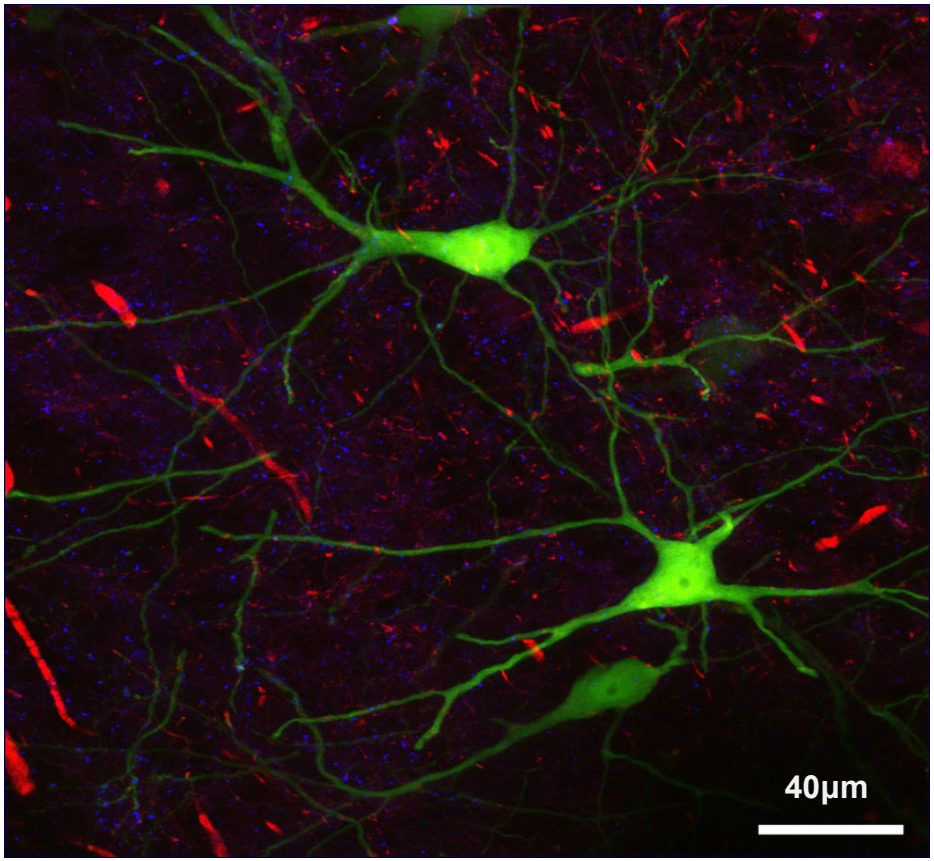
Human medulla (stained)

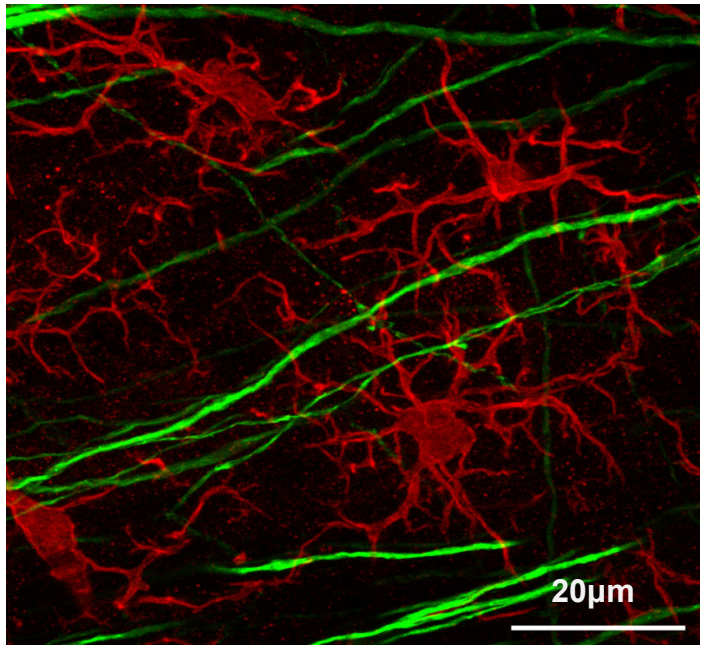
Rabbit muscle fiber (stained)

Sunflower pollen (autofluorescence)

https://www.microscopyu.com

EXAMPLES II



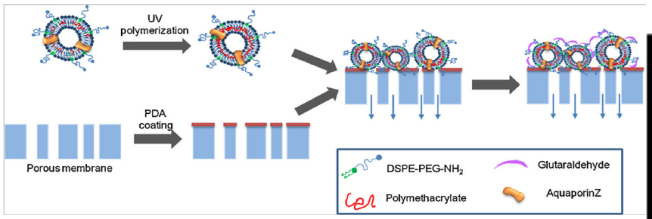


Mouse brain slice (3 dyes are used for selective staining of different parts, image results from merging of 3 subsequent scans)

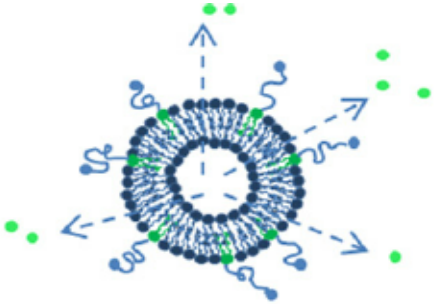
http://zeiss-campus.magnet.fsu.edu

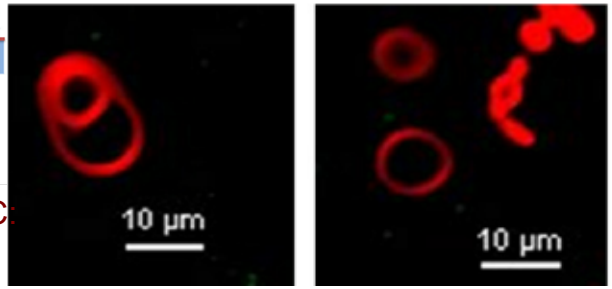
EXAMPLES III

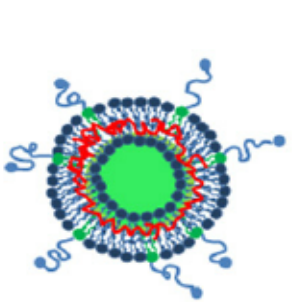
Polymer-coated lipid vescicles not-trapping or trapping fluorescein molecules (depending on the UV-induced growth of a sealing polymer network)

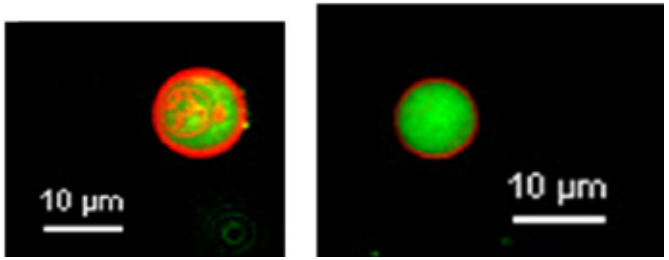


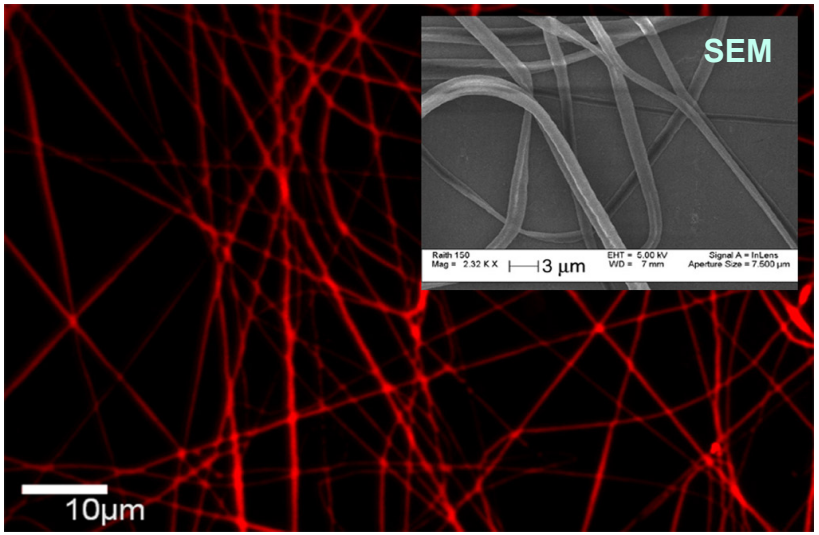
Colloids and surfaces B: Biointerfaces 102C: 466-471 · August 2012







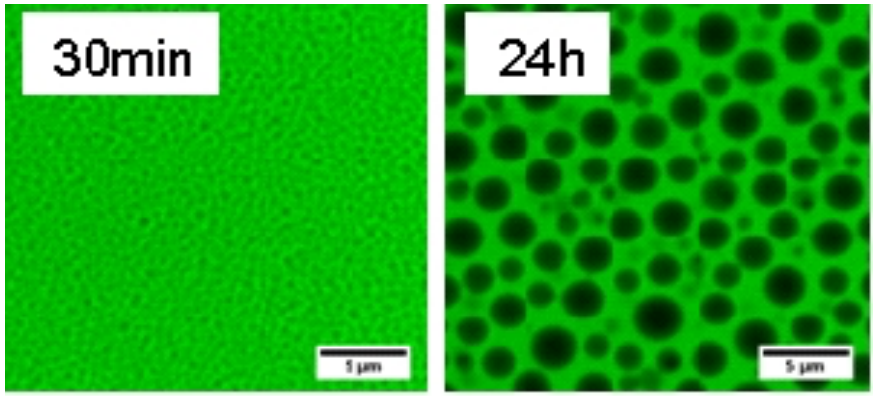




Network of polymer microfibers produced by electrospinning: fluorescence stems from the luminescence properties of the conjugated polymer (PPV) backbone and can be useful in nanophotonics applications

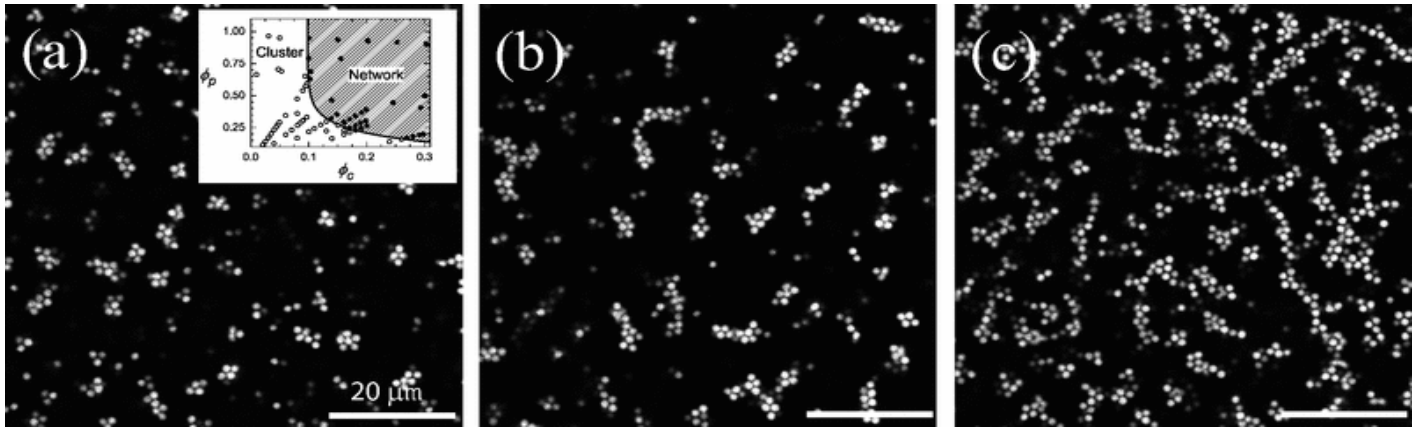
SPIE Newsroom
DOI: 10.1117/2.1201012.003450 (2012)

EXAMPLES IV



Phase separation in a polymer blend PM:PS 50:50 (polymethylphenylsilane: polystyrene); PS is labeled with a fluorescent chromophore and 100 C annealing is used

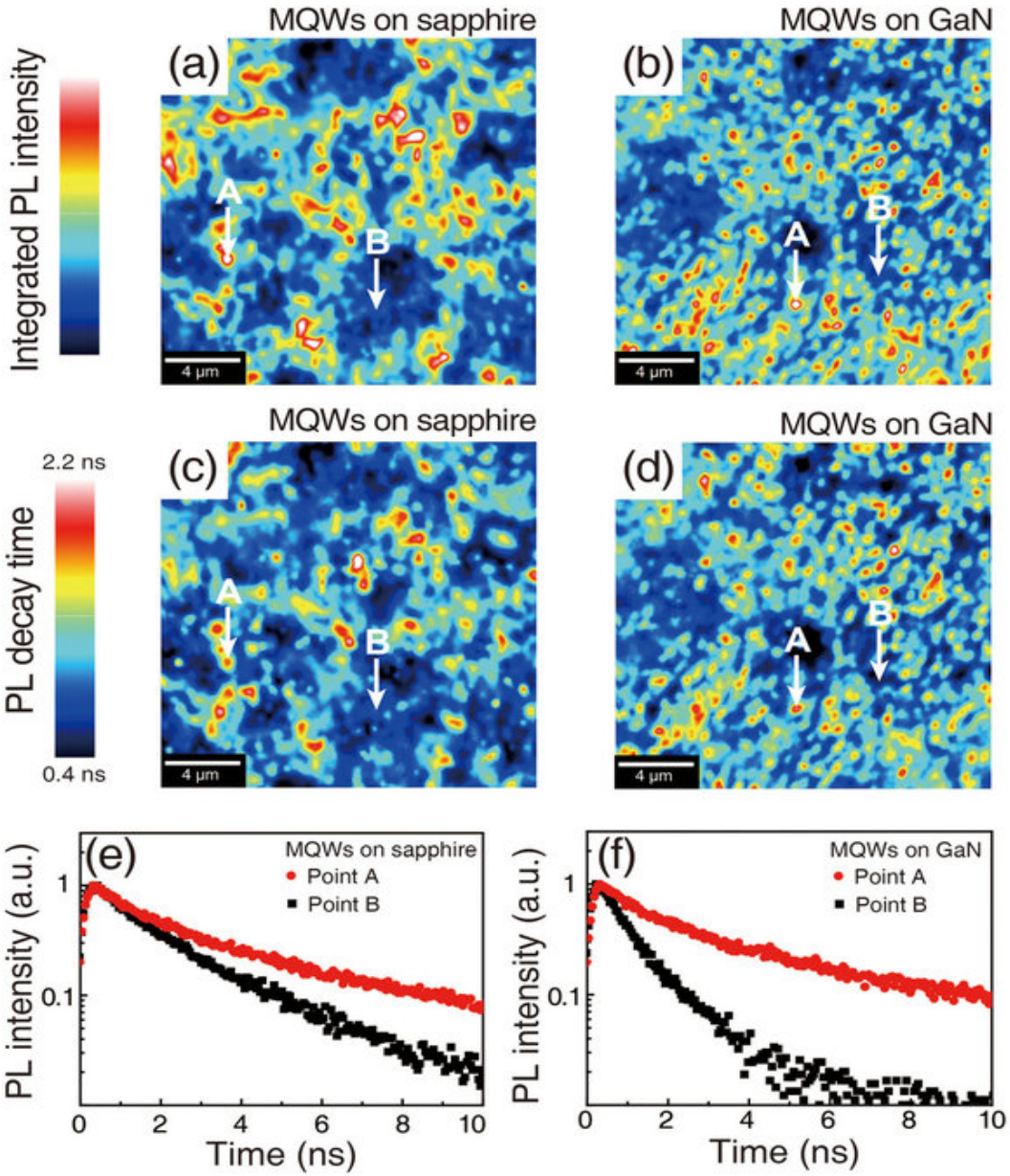
http://www.mpip-mainz.mpg.de/62534/Confocal_Microscopy



Interaction between polymer colloidal nanoparticles at different concentration ratios (ϕ_C) to investigate long range forces: fluorescent labeling is used for the colloidal nanoparticles

Physical Review Letters 94(20):208301 · June 2005

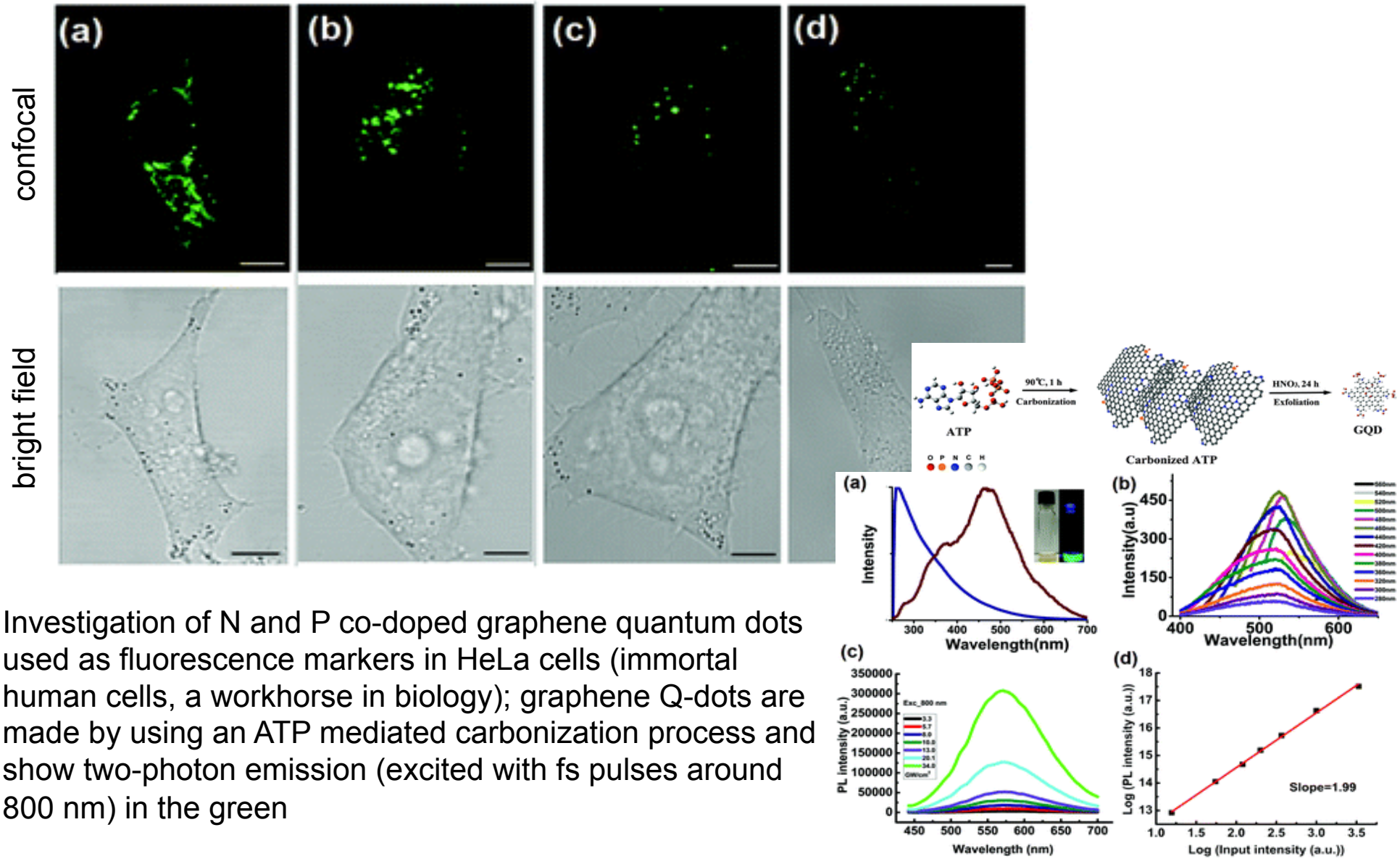
EXAMPLES V



Full spectroscopic analysis of In-rich InGaN/GaN multiple quantum wells (MQWs) grown on sapphire and GaN substrates: the photoluminescence (PL), i.e., the photon-excited fluorescence emission, is mapped and, being the experiment carried out with pulsed excitation (i.e., illumination), the decay time (i.e., the lifetime) is obtained pixel by pixel and mapped as well

Scientific Reports **5**, Article number: 9373 (2015)
doi:10.1038/srep09373

EXAMPLES VI



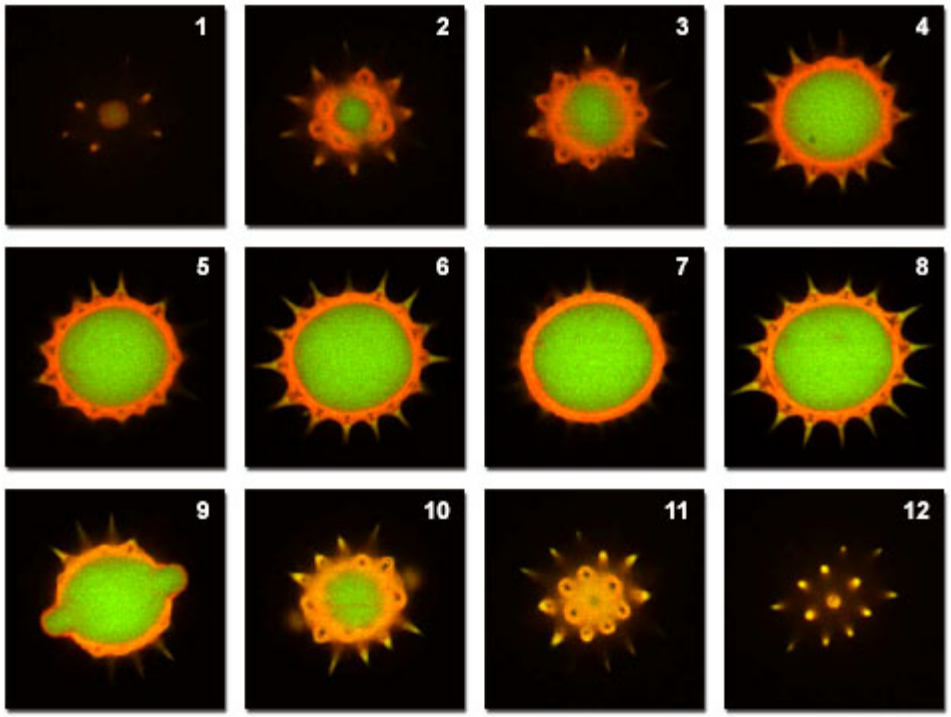
Investigation of N and P co-doped graphene quantum dots used as fluorescence markers in HeLa cells (immortal human cells, a workhorse in biology); graphene Q-dots are made by using an ATP mediated carbonization process and show two-photon emission (excited with fs pulses around 800 nm) in the green

DOI: 10.1039/C5NR01519G (Paper) Nanoscale, 2015, 7, 8159-8165

EXAMPLES VII

When observing relatively thick specimens, such as life-science samples, the tomography (sectioning or slicing) abilities of CSOM/CLSM can be very useful to recosntruct the 3-D shape of the object

Pollen Grain Serial Optical Sections by Confocal Microscopy



https://www.microscopyu.com

Figure 6

Multiple information stack

The Spectral Imaging Lambda Stack

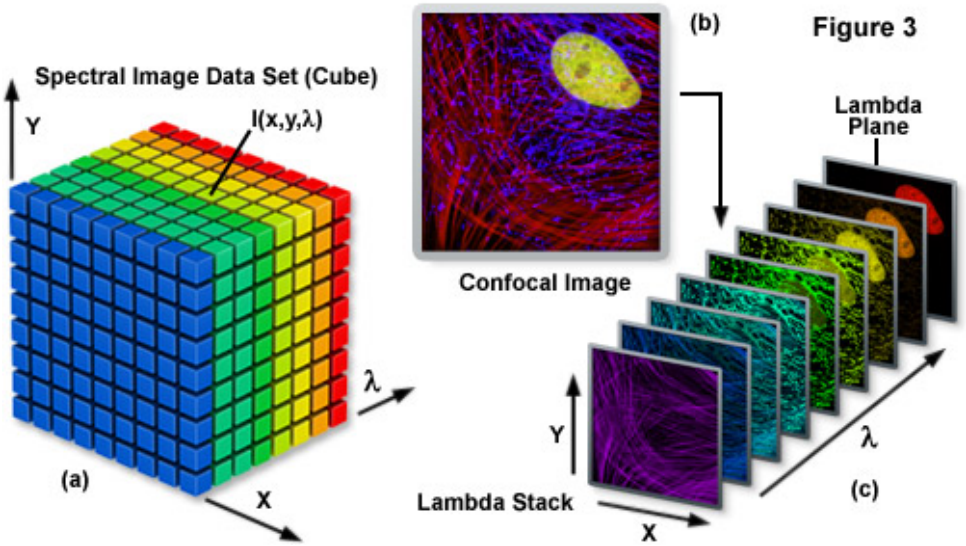


Figure 3

http://zeiss-campus.magnet.fsu.edu

Automated instruments exist able to perform multiple investigations (e.g., slicing and spectral analysis of the emission)
→ Increase of scan speed is continuously searched for, by using fast sample positioners (remind the scanning nature of the technique)

CONCLUSIONS

- ✓ Fourier optics (the very few elements mentioned here!) show how imaging sub-wavelength details requires trasferring high spatial frequency components from the object to the image planes
- ✓ Within this frame, optical resolution can be quantified through the resolving power
- ✓ Different criteria and limti formula can be outlined: they all agree, roughly, with the old-but-gold Abbe's rule: at maximum, details as small as half the wavelegth can be retrieved in optical microscopy/spectroscopy
- ✓ Meeting the Abbe's limit (or going a little bit beyond it) is a non trivial task: confocal approaches have to be used in order to achieve diffraction-limited resolution

However, the need for methods capable of a larger resolution still exists

We will see in the next lectures how to go beyond the diffraction limit

FURTHER READING

For more details on microscopy and confocal techniques:

T.R. Corle, G.S. Kino, Confocal Scanning Optical Microscopy and Related Imaging Systems, Academic Press, San Diego (1996). [Chapter 1, in particular]

For a well conceived review on confocal methods:

R.H. Webb, Confocal Optical Microscopy, Rep. Prog. Phys. 59, 427–471 (1996). [Open access]

For many interesting information regarding opitcal microscopy from the practical and fundamental points of view, including a large library of examples:

https://www.microscopyu.com
http://www.olympusmicro.com
http://zeiss-campus.magnet.fsu.edu