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

Spectroscopy of Nanomaterials II sem – part 2

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Spectroscopy at the nanoscale with conventional tools: optical microscopy and some of its variants

OUTLOOK

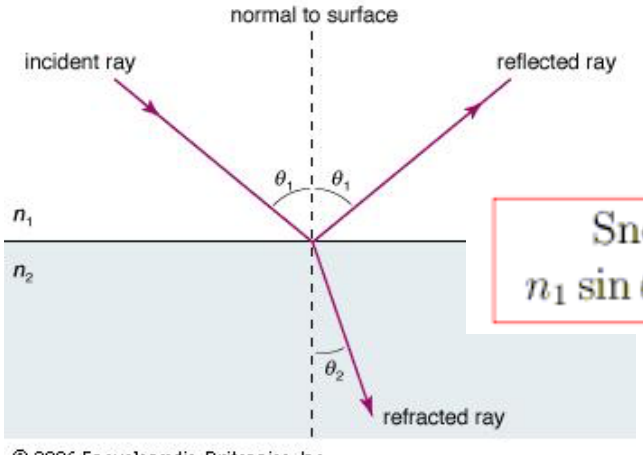
Thus far, we have seen:
- what's the typical size of the systems of our interest
- what we are eventually looking for in such systems (e.g., specific properties depending on size)
- what is the ultimate role of diffraction
- we have learned a few general aspects of nanoscale investigations by looking at some electron microscopy

Today's menu:
- Starters of problems, e.g., scattering, and possibilities
- Main course of (conventional) optical spectroscopy at the small scale and conventional optical microscopy
- Dessert and bitter: the wealth of configurations and methods in present optical microscopy (just a few samples, just to taste) and, to conclude, the rock problem of spatial resolution

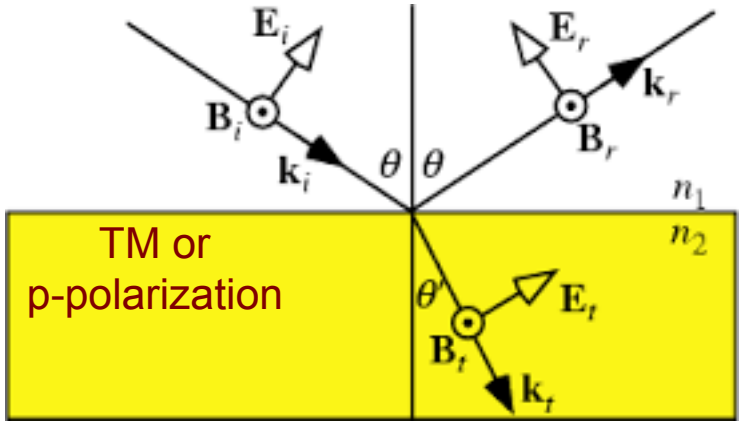
GENERAL OVERVIEW

In general terms, in the macroscopic + ideal world:

- e.m. radiation (light) is well described by rays;
- Snell, Fresnel and other geometrical rules hold and are sufficient to model phenomena (with dielectrics, for metals we will come back to the topic later on)



Snell's Law:
$n_1 \sin\theta_1 = n_2 \sin\theta_2$



$$r_s = \frac{n_1\cos\theta_i - n_2\cos\theta_t}{n_1\cos\theta_i + n_2\cos\theta_t} = t_s - 1,$$

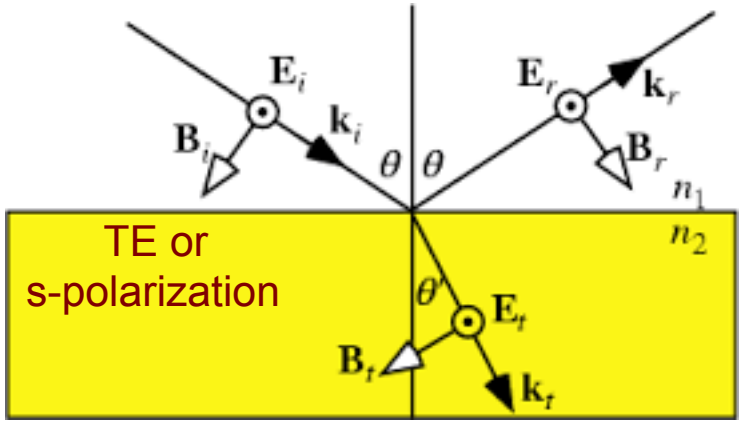
$$t_s = \frac{2n_1\cos\theta_i}{n_1\cos\theta_i + n_2\cos\theta_t} = r_s + 1,$$

TE or
s-polarization

$$r_p = \frac{n_2\cos\theta_i - n_1\cos\theta_t}{n_1\cos\theta_t + n_2\cos\theta_i},$$

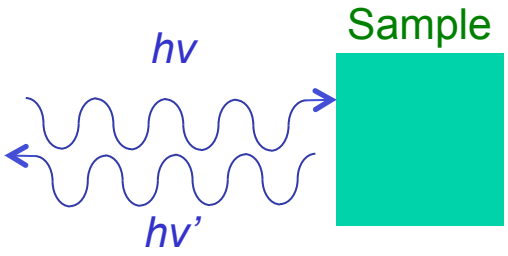
$$t_p = \frac{2n_1\cos\theta_i}{n_1\cos\theta_t + n_2\cos\theta_i}.$$

TM or
p-polarization

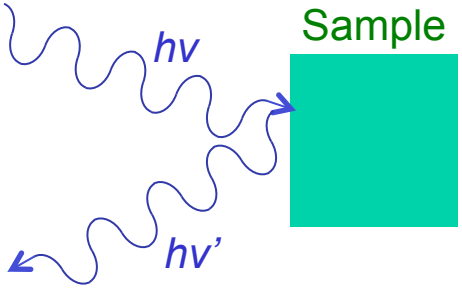


OPTICAL SPECTROSCOPY IN THE MACRO WORLD

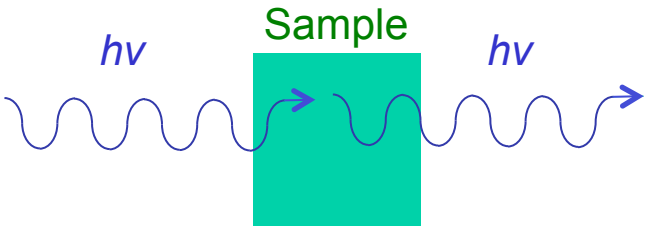
In the macroscopic + ideal world, different configurations can be envisioned for optical spectroscopy measurement, as, e.g.:



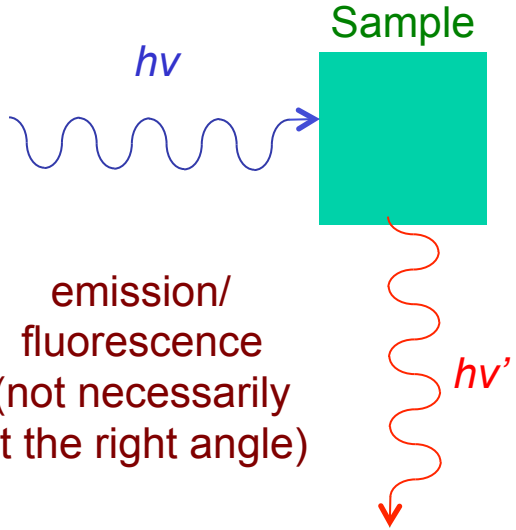
"reflection"
(at the right angle)



"reflection"
(at some angle)



transmission/absorption



emission/
fluorescence
(not necessarily
at the right angle)

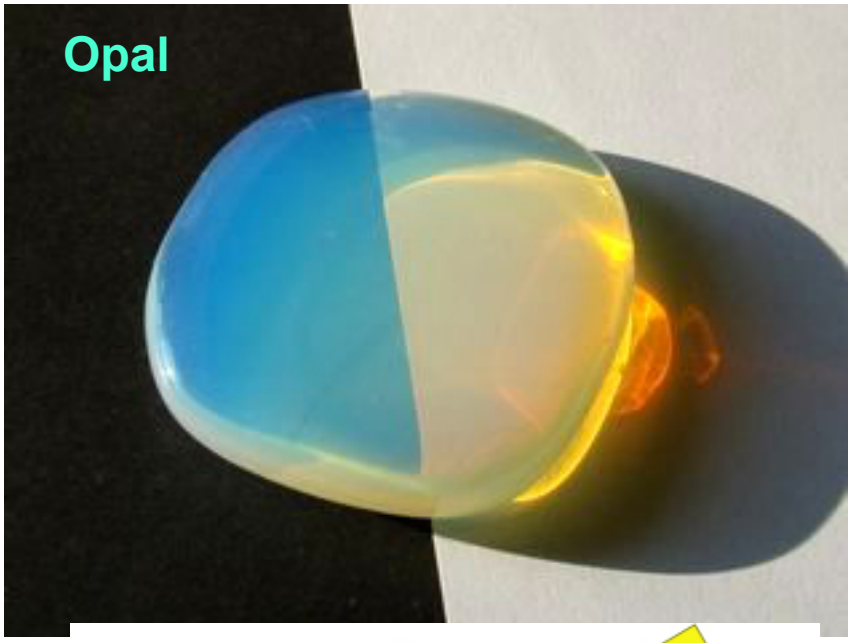
Elastic or inelastic processes can be involved in

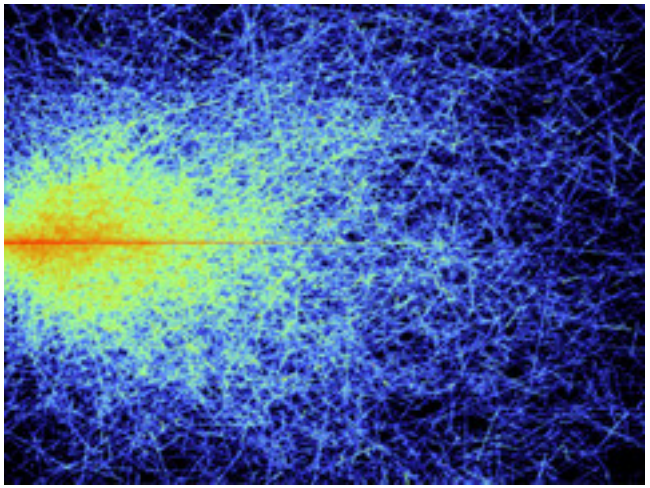
$hv' \cong hv$ $\quad hv' \neq hv$

$(hv' < hv)$

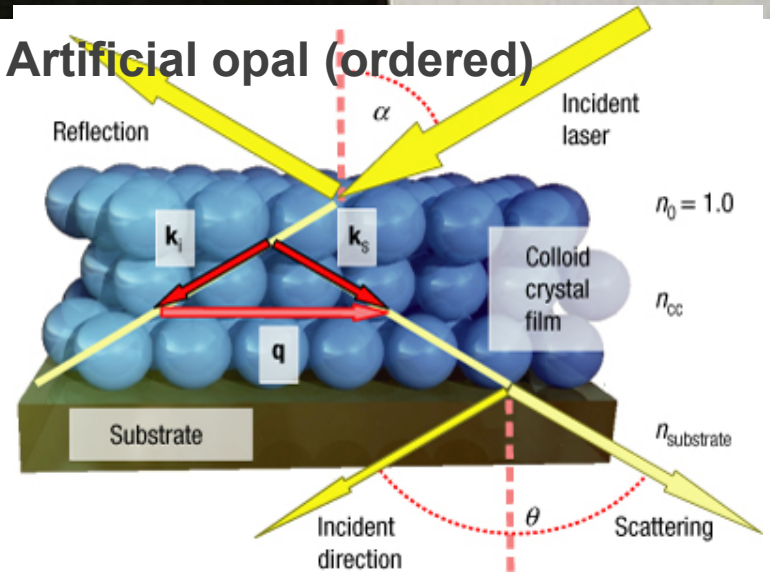
In the macroscopic world, there is freedom to setup configurations aimed at different purposes

SCATTERING IN THE REAL WORLD





Artificial opal (ordered)



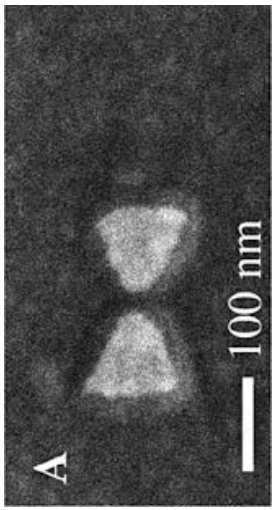
RAYLEIGH'S SCATTERING I

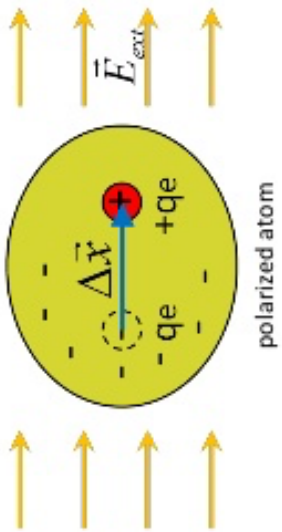
Note; we will restrict here to dielectric materials (mostly);
the specific behavior of metal nanoparticles will be discussed later on!

Background of general physics (Maxwell's equations):

an accelerated electric charge is source of e.m. radiation
→ an oscillating current is source of e.m. radiation

in dielectrics, polarization charge can be displaced by an electric field
→ an **oscillating dipole** is source of e.m. radiation (**antenna**)

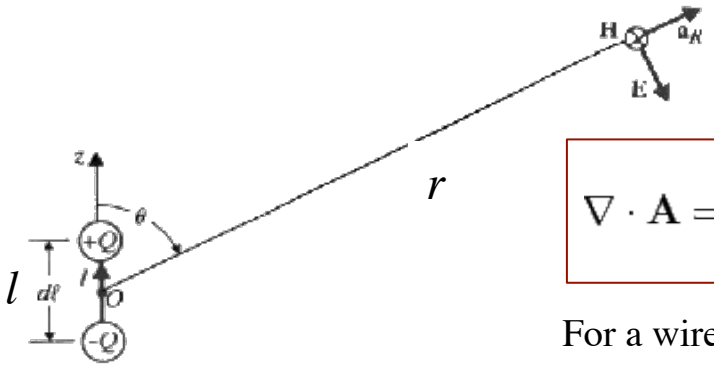




Antenna size is related to the wavelength

OSCILLATING DIPOLE EMISSION I

Note: we look at the "far-field" solution



$$I(t) = \frac{dq}{dt} = I_0 \cos(\omega t)$$

$$\nabla \cdot \mathbf{A} = -\epsilon_0 \mu_0 \frac{\partial \phi}{\partial t}$$

$$\mathbf{A}(\mathbf{r},t) = \frac{\mu_0}{4\pi} \int \frac{[\mathbf{j}]}{|\mathbf{r}-\mathbf{r}'|}\, d^3\mathbf{r}'$$

$$[f] = f(\mathbf{r}', t - |\mathbf{r}-\mathbf{r}'|/c)$$

For a wire of negligible thickness, we can replace $\mathbf{j}(\mathbf{r}', t-|\mathbf{r}-\mathbf{r}'|/c)\, d^3\mathbf{r}'$ by $I(\mathbf{r}', t-|\mathbf{r}-\mathbf{r}'|/c)\, dz'\, \hat{\mathbf{z}}$. Thus, $\mathbf{A}(\mathbf{r},t) = A_z(\mathbf{r},t)\, \hat{\mathbf{z}}$, and

$$A_z(\mathbf{r},t) = \frac{\mu_0}{4\pi} \int_{-l/2}^{l/2} \frac{I(z', t-|\mathbf{r}-z'\,\hat{\mathbf{z}}|/c)}{|\mathbf{r}-z'\,\hat{\mathbf{z}}|}\, dz'.$$

$$|\mathbf{r}-z'\,\hat{\mathbf{z}}| \simeq r$$

$$t - |\mathbf{r}-z'\,\hat{\mathbf{z}}|/c \simeq t - r/c$$

$$A_z(\mathbf{r},t) \simeq \frac{\mu_0}{4\pi} \int_{-l/2}^{l/2} \frac{I(z', t-r/c)}{r}\, dz' \simeq \frac{\mu_0}{4\pi} \frac{l\, I(t-r/c)}{r}$$

See, e.g.: http://farside.ph.utexas.edu/teaching/em/lectures/node92.html

OSCILLATING DIPOLE EMISSION II

$$\nabla \cdot \mathbf{A} = -\epsilon_0 \mu_0 \frac{\partial \phi}{\partial t}$$

$$\nabla \cdot \mathbf{A} = \frac{\partial A_z}{\partial z} \simeq \frac{\mu_0 \, l}{4\pi} \frac{\partial I(t - r/c)}{\partial t} \left(-\frac{z}{r^2 \, c} \right) + O\left(\frac{1}{r^2} \right)$$

$$\phi(\mathbf{r}, t) \simeq \frac{l}{4\pi \, \epsilon_0 \, c} \frac{z}{r} \frac{I(t - r/c)}{r}$$

$$\begin{aligned} \mathbf{E} &= -\frac{\partial \mathbf{A}}{\partial t} - \nabla \phi, \\ \mathbf{B} &= \nabla \times \mathbf{A}. \end{aligned}$$

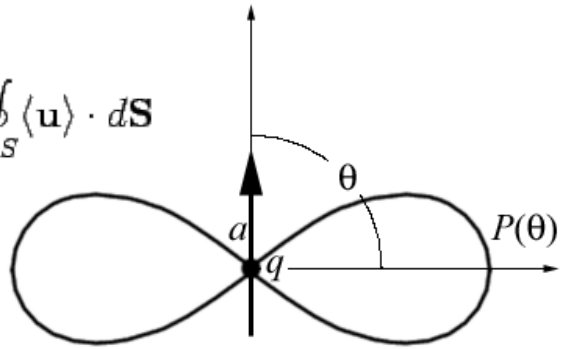
$$\mathbf{E} \simeq -\frac{\omega \, l \, I_0}{4\pi \, \epsilon_0 \, c^2} \sin\theta \, \frac{\sin[\omega(t - r/c)]}{r} \, \hat{\boldsymbol{\theta}}$$

$$\mathbf{B} \simeq -\frac{\omega \, l \, I_0}{4\pi \, \epsilon_0 \, c^3} \sin\theta \, \frac{\sin[\omega(t - r/c)]}{r} \, \hat{\boldsymbol{\varphi}}$$

$$\mathbf{u} = \frac{\mathbf{E} \times \mathbf{B}}{\mu_0} = \frac{\omega^2 \, l^2 \, I_0^2}{16\pi^2 \, \epsilon_0 \, c^3} \sin^2[\omega(t - r/c)] \frac{\sin^2\theta}{r^2} \, \hat{\mathbf{r}}$$

$$\langle \mathbf{u} \rangle = \frac{\omega^2 \, l^2 \, I_0^2}{32\pi^2 \, \epsilon_0 \, c^3} \frac{\sin^2\theta}{r^2} \, \hat{\mathbf{r}}$$

$$P_{\text{rad}} = \oint_S \langle \mathbf{u} \rangle \cdot d\mathbf{S}$$



See, e.g.: http://farside.ph.utexas.edu/teaching/em/lectures/node92.html

RAYLEIGH'S SCATTERING II

$$\mathbf{p}(t) = p_0 \sin(\omega t)\,\hat{\mathbf{z}}$$

$$p_0 = q_0 l = I_0 l/\omega$$

$$\langle \mathbf{u} \rangle = \frac{\omega^4 p_0^2}{32\pi^2 \epsilon_0 c^3} \frac{\sin^2\theta}{r^2} \hat{\mathbf{r}}$$

Emitted intensity depends on:
- **fourth power of frequency ω**
- **squared sinθ**

- ✓ In Rayleigh's scattering, a dielectric nanoparticle illuminated by e.m. radiation is described in terms of the **induced** dipole moment
- ✓ Scattering is due to re-emission of radiation from the so-induced oscillating dipole
- ✓ **The approximation is valid for particles "small" compared to wavelength**

$$\frac{2\pi R}{\lambda} << 1$$

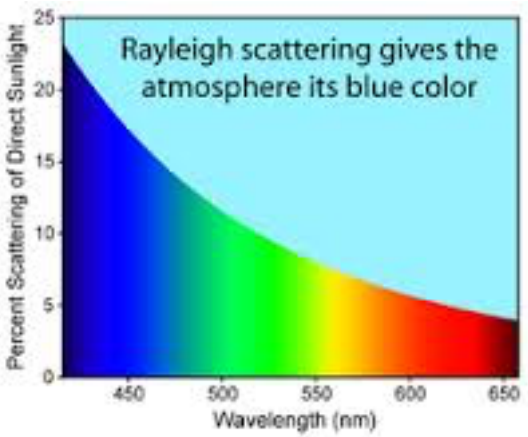
Typically: $R \leq \lambda/10$

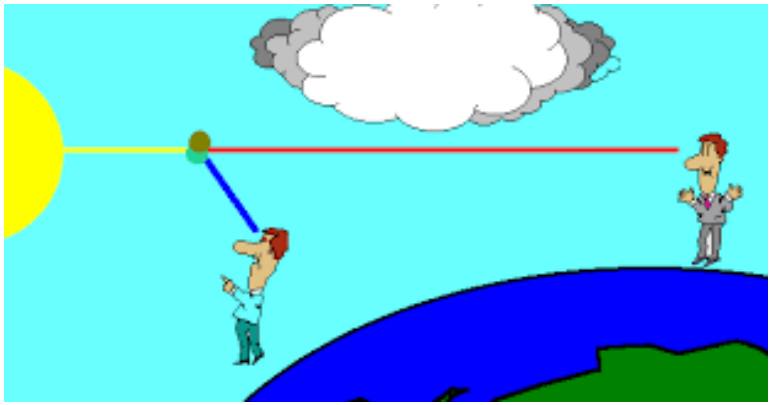
Now:
1. the induced polarization field is proportional to the electric field amplitude, that is, to the square root of the incident intensity;
2. the induced electric dipole moment is proportional to the polarization field times the volume;
3. the fourth power of the angular frequency is proportional to λ^{-4};
4. for a spherical dielectric particle, polarizability depends on the dielectric constant (macroscopic, see Clausius-Mossotti formula);

$$I_{scatt} \propto I_{inc} \left(\frac{2\pi}{\lambda}\right)^4 \left(\frac{\varepsilon_r - 1}{\varepsilon_r + 2}\right)^2 R^6 \frac{\sin^2\theta}{r^2}$$

RAYLEIGH'S SCATTERING III

Rayleigh's scattering from molecule and very small aerosol particles explains the blue/red color of the sky at midday and the sunset





… but it cannot explain the white/grey color of the clouds (composed of larger size particles)

MIE'S SCATTERING I

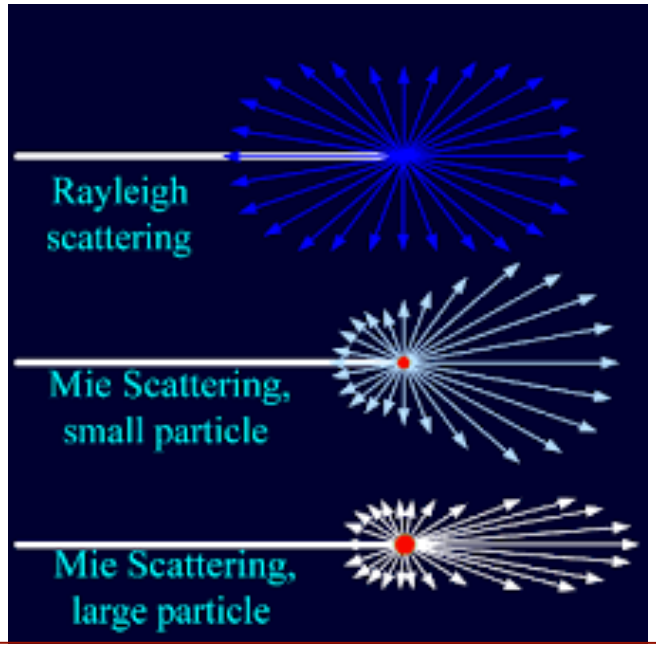
- ✓ For dielectric particles of a **larger size**, the actual particle shape affects the radiation pattern
- ✓ The so-called Mie approach is based on a multipole expansion of the oscillating electric distribution
- ✓ In the multipole expansion, each term is practically weighted by a factor containing λ^4

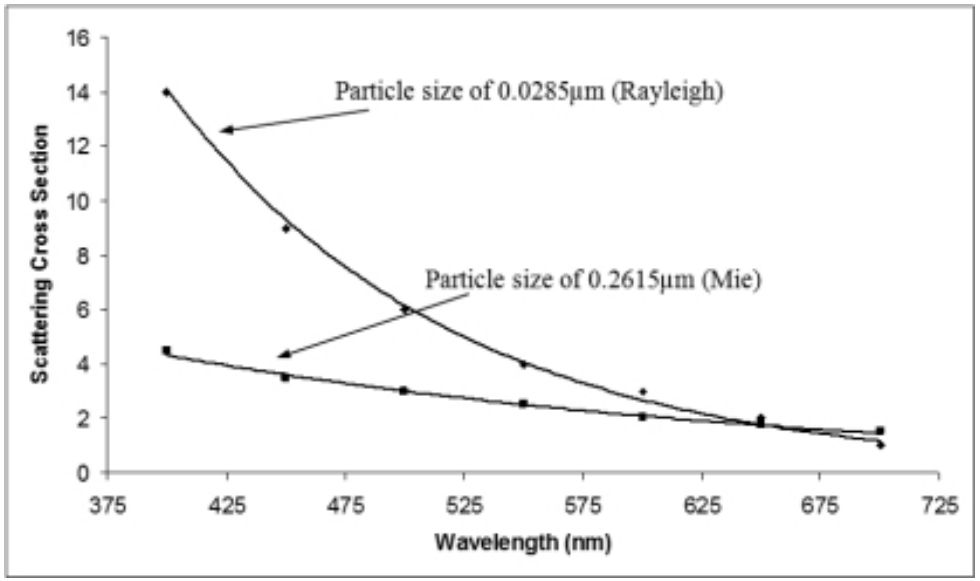
$$\frac{2\pi R}{\lambda} \leq 1$$

Typically: $R \leq \lambda$

The dependence on frequency/wavelength is much less relevant

The dependence on the angle is modified (no longer "symmetrical")

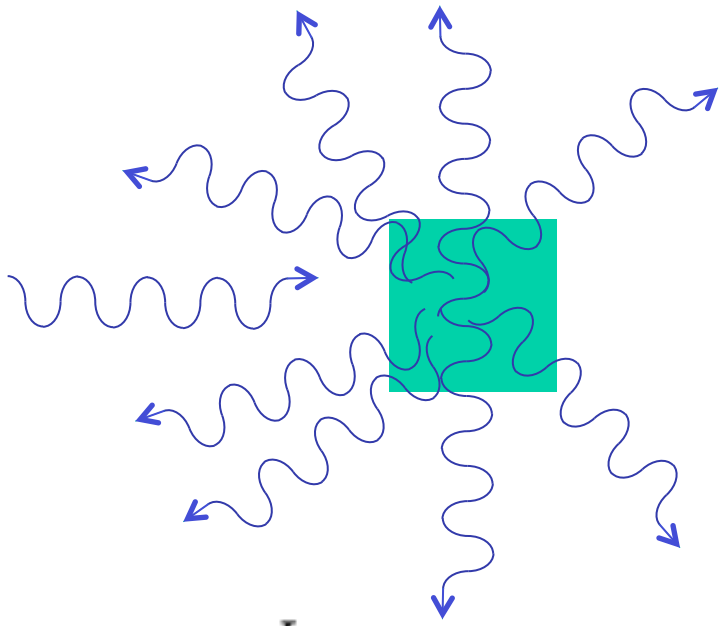




OPTICAL SPECTROSCOPY IN THE REAL WORLD

In any case, when nanostructured materials are involved, the occurrence of scattering (in either case) prevents “ideal” configurations

In the “real” macroscopic world, optical components (lenses, mirrors, etc.) are used to perform a “spatial integration” over all possible scattered directions



BUT

we are interested in optical features of strongly scattering entities (nanostructures, in general)

AND

we want to address individual, spatial resolved scattering entities (nanostructures, in general)

For instance: $\frac{I_1}{I_0} = \mathrm{e}^{-k_\lambda l} = T = \mathrm{e}^{-A}$

In transmission/absorption spectroscopy, even by assuming the ability to localize the “input” radiation, we cannot rule out multiple scattering inside the material

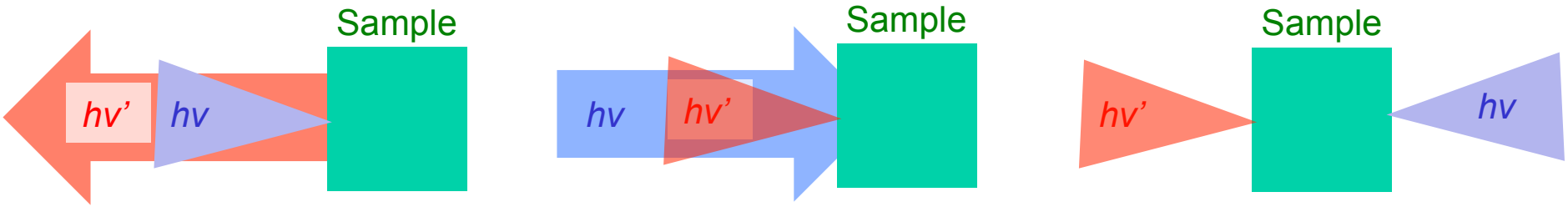
→ the material thickness cannot be determined

→ no quantitative information on the optical properties can be derived!

OPTICAL SPECTROSCOPY AT THE NANOSCALE

In very general terms:

- we localize the source (through a lens) and collect the scattered output
- we use a large source and collect locally the (scattered) output → **imaging**
- we do both (both "input" and "output" are localized)



Note:

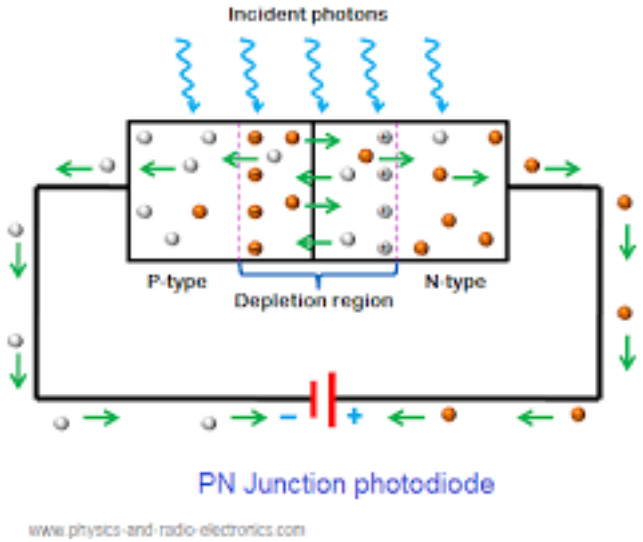
- anelastic processes, remarkably emission/fluorescence/photoluminescence, are preferred, owing to their inherent ability in distinguishing between "input" and "output"
- "directions" of "input" and "output" can be chosen and arranged at wish, when possible
- normally, condensed matter is considered (vapors and, less remarkably, liquids cannot ensure the nanostructure is steadily at the observation position for a sufficiently long time)
- it can be hard to identify the role of material volume against surface (unless very thin films are investigated)

A PRELIMINARY (GENERAL) TECHNICAL ISSUE

In the macroscopic world one can integrate over the volume

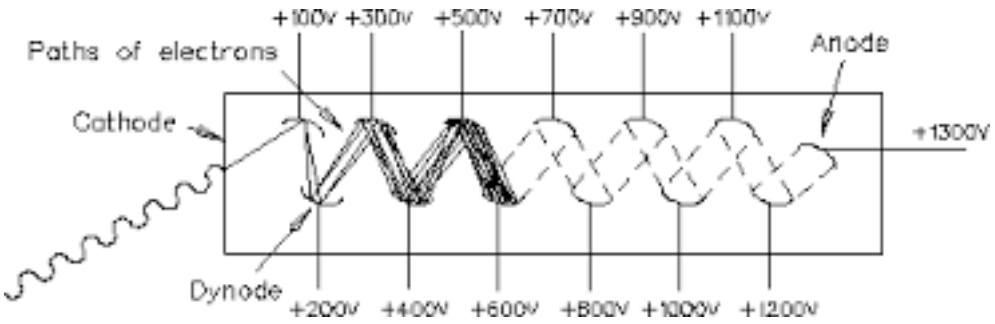
- → the *number* of concerned "material" (atoms/molecules, or whatever) can be increased
- → the *quantity* of output light can be made large (i.e., the number of produced photons per second)

Optical spectroscopy of nanomaterials quite often suffers from sensitivity issues
(especially in the case of fluorescence, where few emitting entities are addressed at once)



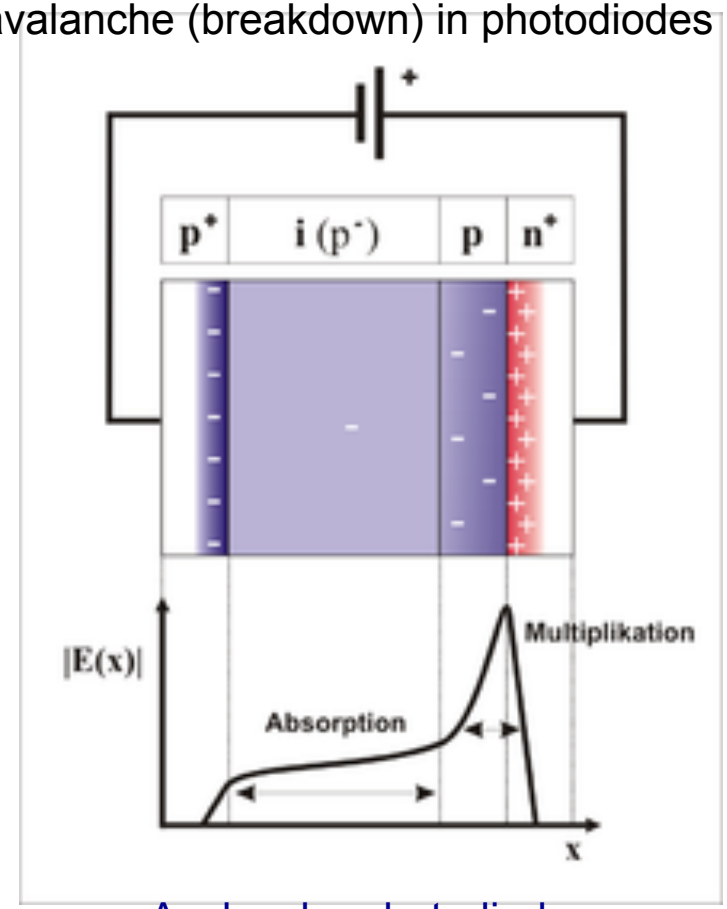
For instance, an ordinary photodiode can turn not adequate to measure the weak flux of photons:
In the best conditions, one photon leads to one electron-hole pair
For a fluorescent object, $\Phi_{phot} \approx \Gamma$ (spontaneous emsission rate)
$I_{phot} \approx \Gamma e = 1.6 \times 10^{-19} \Gamma$ [A] → *very weak current to be measured in conventional ways!*

HIGH SENSITIVITY DETECTORS



Photomultiplier

A nonlinear process is used to increase the number of produced charges, e.g., secondary electron emission in photomultipliers tube or avalanche (breakdown) in photodiodes



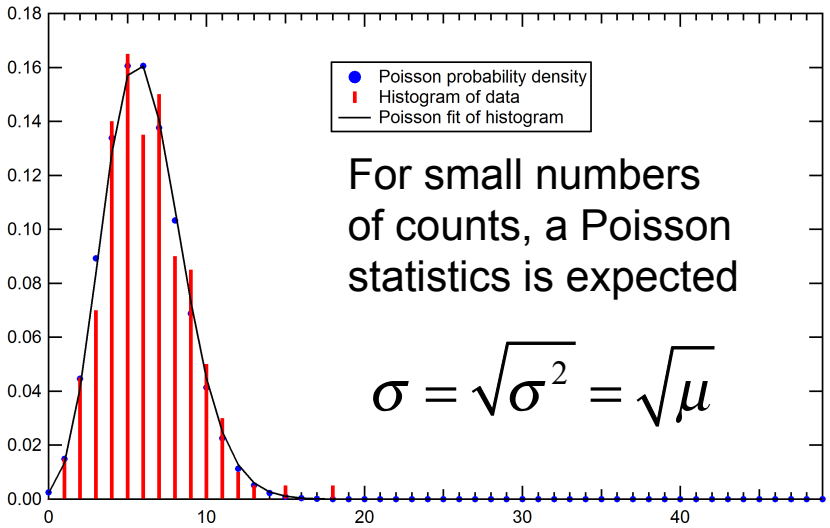
Avalanche photodiode

SENSITIVITY LIMITS

Gain can be very large (over 10^7 for a PMT), but noise is amplified as well (at room temperature, typical noise-equivalent-power - NEP ~ 0.1-1 fW, corresponding to roughly 10^3-10^4 photons/s)

Signal-to-Noise (S/N) ratio can be improved by operating in **counting mode**:

- a detected photon produces an electric pulse (voltage);
- electric pulses above a certain threshold are identified as arrival of single photons;
- the events are counted (in a digital way)



Upon assumption of Poisson statistics, the relative uncertainty is given by $1/\sqrt{\mu}$, μ being the average count number

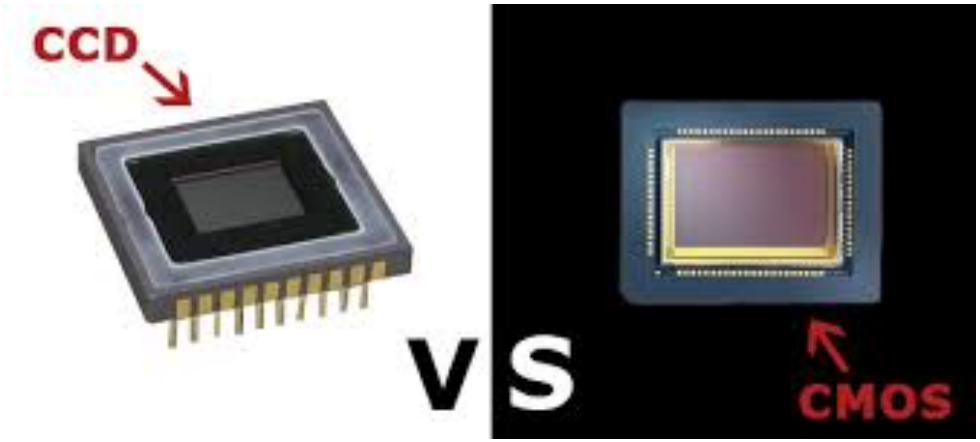
→ Collecting large data samples improve accuracy

"Single photon" detectors (based on thermolectrically-cooled avalanche photodiode with counting electroncs)

IMAGING DEVICES

In some cases, as we will see in the following, an **image** (e.g., fluorescence or scattering image in a microscope) is built, similar to direct eye observation

→ camera must be used, providing with an *almost* real-time 2D information

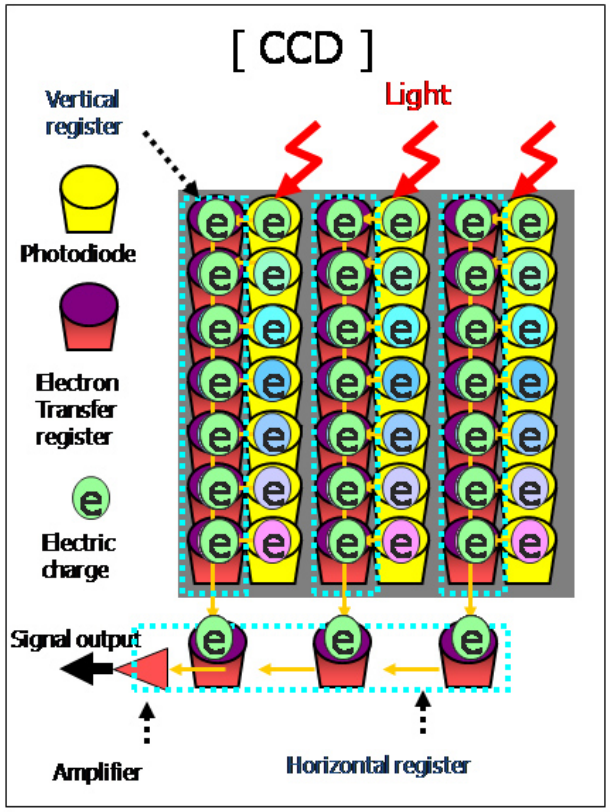


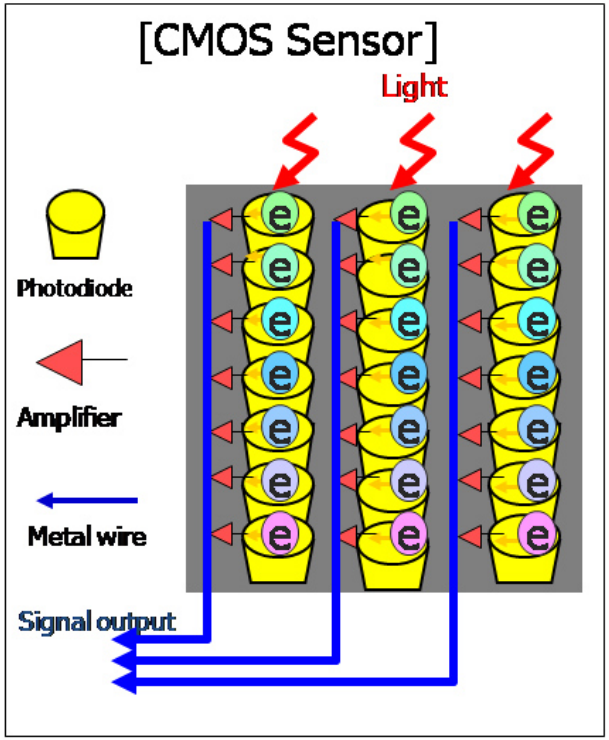
(Very) many individual, miniaturized photodetectors gathered together in a 2D array

Basically, each individual photodetector behaves similar to photodiode:

- Photons are converted into charge through electron-hole generation
- Charge is stored for a predefined duration in the individual device
- Charge is converted into a voltage, ready for further processing (analog-to-digital conversion, processing with a computer, visualization, storage, etc.)

CCD vs CMOS

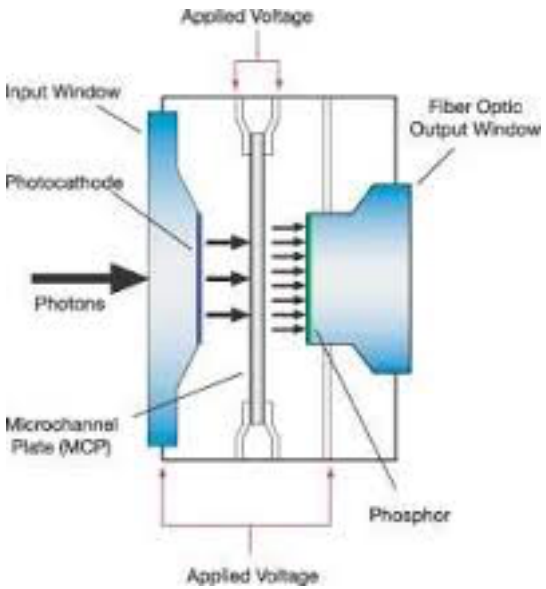


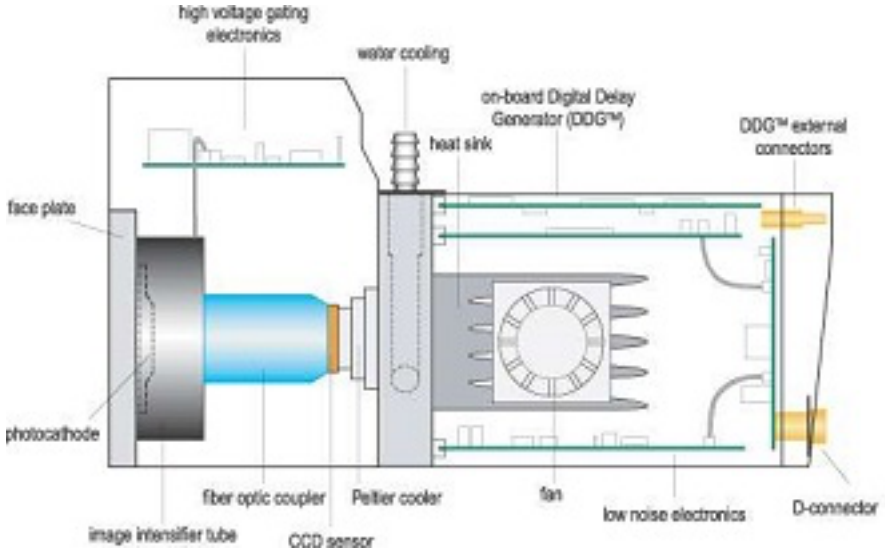


The main difference is in the readout strategy:
CCD less noisy and more dense (but slower), CMOS faster (but less sensitive and less resolved)

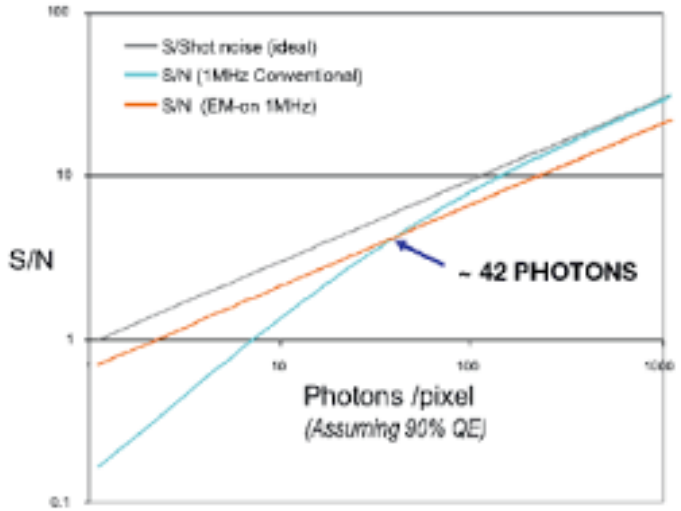
In addition: dynamical range can be a concern (a large-size pixel is needed to accomodate charge corresponding to strong illumination)

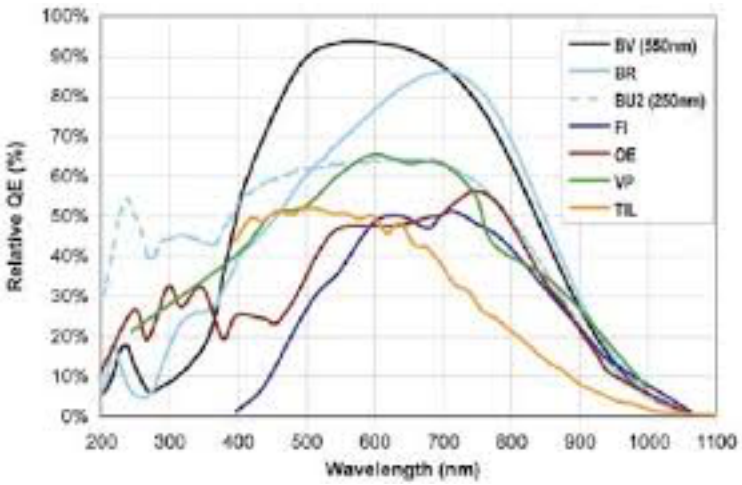
INTENSIFIED CAMERAS (EMCCD)





Photon-electron-multiplication + photon-electron conversion

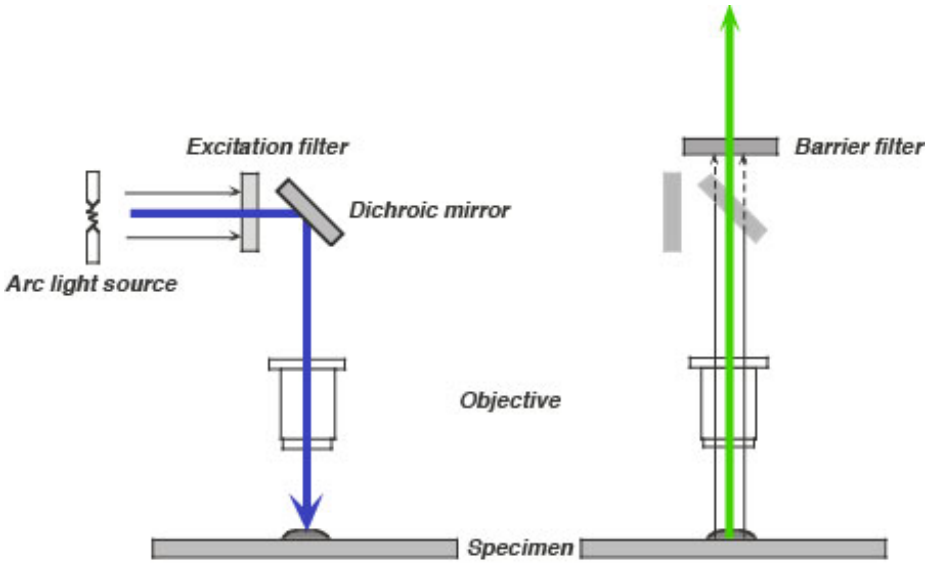




BACK TO OUR MAIN TOPIC!

In general terms, localization of illumination (input) or collection (output) requires optics, i.e., a **magnifying system**, as in an optical microscope

The microscope configuration specifically adopted, e.g., for fluorescence imaging, is usually called **epifluorescence**



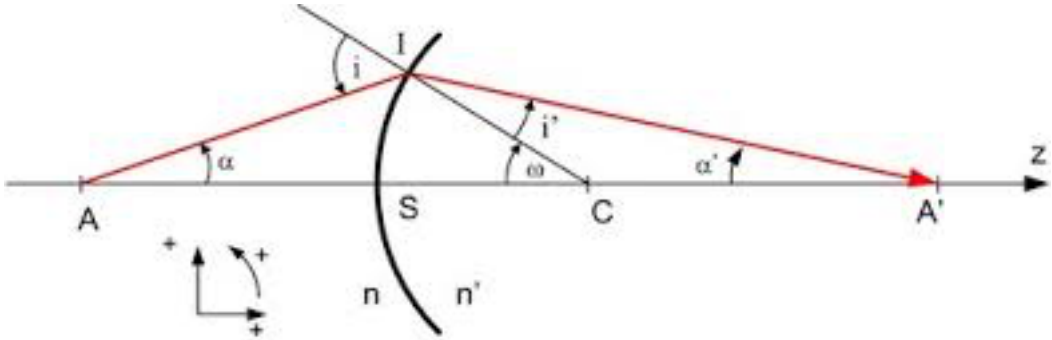
"input" "output"

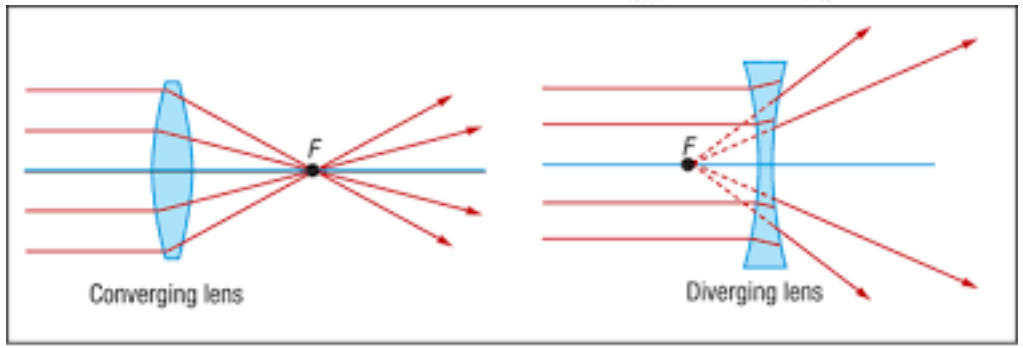
However, the basic potential (and limitations) of optical spectroscopy at the nanoscale can be well understood by considering, as first, generic optical microscopy

Optical microscopy is our first step towards spectroscopy of nanomaterials

GEOMETRICAL OPTICS

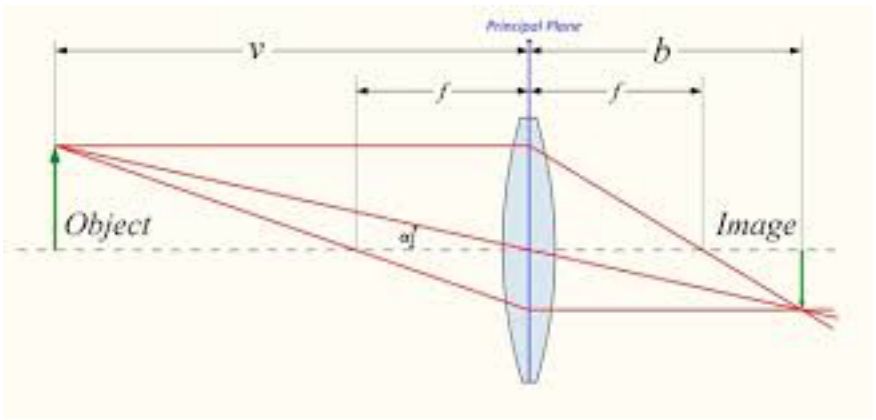
We go back again to ray (geometrical) optics, even though we know it is not a fully reasonable picture (e.g., scattering, diffraction!)



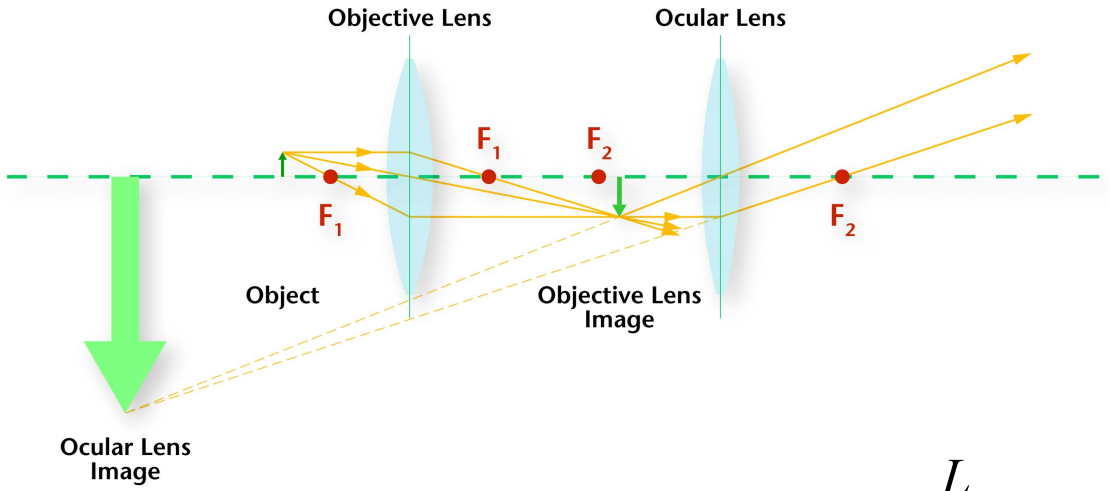


Imaging with a converging thin lens

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



MICROSCOPE (BASICS)



Magnification:

Objective $$M_{objective} = -\frac{d_{image}}{d_{object}}$$

Total $$M_{total} = M_{objective} M_{eyepiece}$$

$$M_{objective} = -\frac{L_{tube}}{f_{objective}}$$

L_{tube} is conventionally fixed (e.g., 200 or 250 mm)

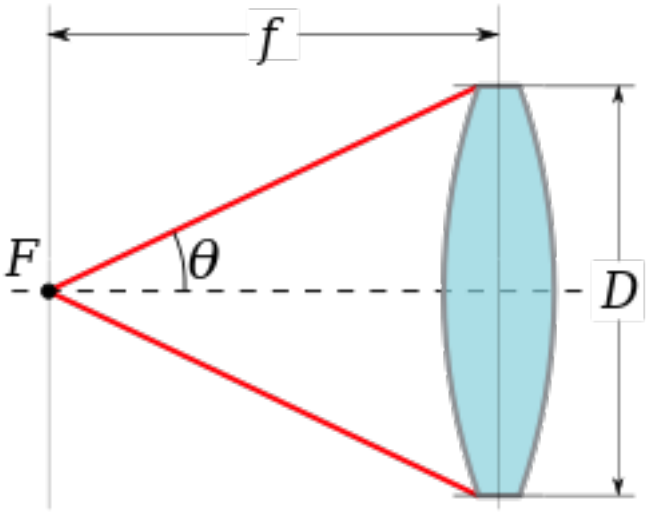
→ $M_{objective}$ depends *solely* on its focal length

Lens maker formula
(very rough, for thin lenses)

$$\frac{1}{f} = (n-1)\left(\frac{1}{R_1} - \frac{1}{R_2}\right)$$

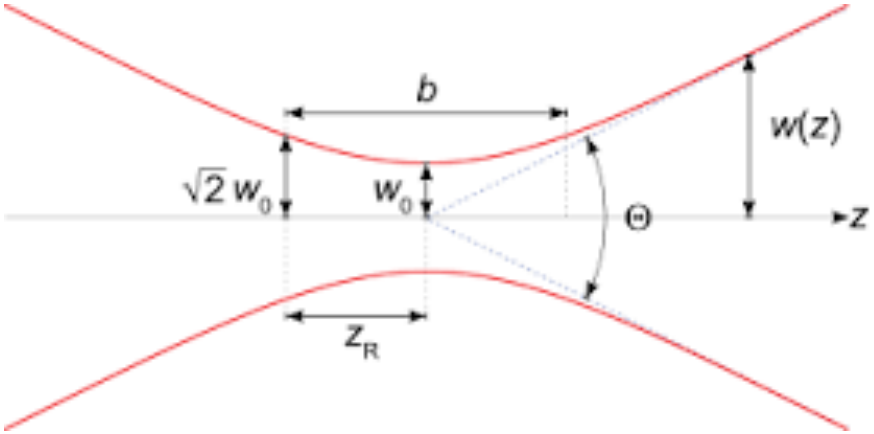
Virtually, geometrical optics allows for any magnification to be obtained with a converging lens (just playing with curvature radii)

NUMERICAL APERTURE AND DEPTH OF FOCUS



$$NA = n_{medium} \sin\theta$$

In case $D << f$, $sin\theta \sim tg\theta \sim f/(D/2)$



*Anticipating more accurate descriptions (**Gaussian optics**, works with coherent light):*

$$z_R = \frac{w_0}{NA}$$

*(for a purely Gaussian beam, **diffraction-limited**:*

$$w_0 \sim \frac{\lambda}{NA}$$

A MICROSCOPE OBJECTIVE RATHER THAN A LENS

Figure 1

CFI Apo SR TIRF 100X Oil

Mag	NA	WD
100×	1.49	0.12mm

Research, Confocal, TIRF, Laser Trapping /Laser Tweezers,

CFI Apo TIRF 100X Oil

Mag	NA	WD
100×	1.49	0.12mm

Research, Confocal, TIRF, Laser Trapping /Laser Tweezers,

CFI Apo TIRF 60X Oil

Mag	NA	WD
60×	1.49	0.12mm

Research, Confocal, TIRF, Laser Trapping /Laser Tweezers

CFI 4X

Mag	NA	WD
4×	0.1	30mm

Education, Clinical Laboratory

CFI 100X Oil

Mag	NA	WD
100×	1.25	0.23mm

Education, Clinical Laboratory, Laser Trapping /Laser Twe

CFI P 4X

Mag	NA	WD
4×	0.1	30mm

Education, Research

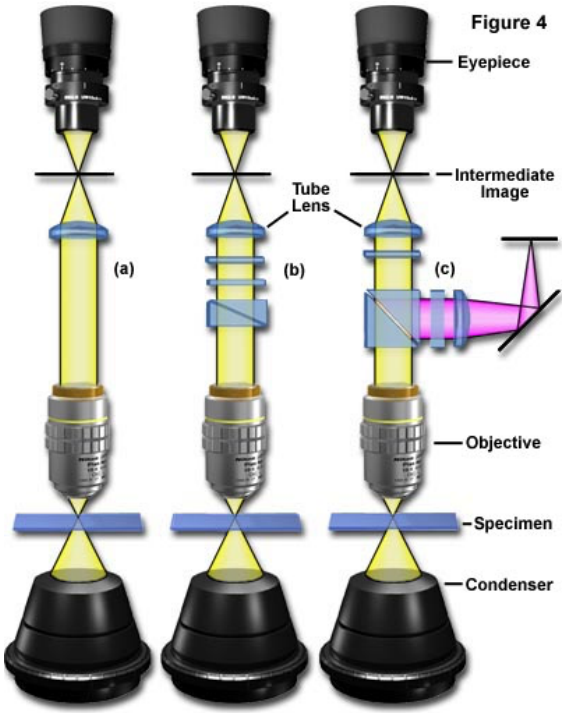
CFI P 100X Oil

Mag	NA	WD
100×	1.25	0.23mm

Education, Research

A huge variety of objectives are available, including immersion lenses ($n_{medium} > 1$)

A MICROSCOPE RATHER THAN A TOY



Typically, **infinity-corrected** objectives are currently used

Modern scientific microscopes are of the “inverted” type (larger stability, ease of specimen positioning, longer and more controlled optical path, etc.)

The parallel ray optical path enabled by infinity-corrected objectives allows for inserting optical components with negligible distortion of the wavefront

VARIETY OF IMPLEMENTED SPECTROSCOPIES

Microscopy is based on contrast, i.e., the measured quantity must bring a local information

The contrast mechanism is by itself based on some kinds of spectroscopy, ready to be used for quantitative information at the local scale (diffraction limited)

A few examples of variants:

- Dark field microscopy
  → attempt to reduce stray light contribution (a very low-cost version of confocal)

- Fluorescence microscopy (epi-fluorescence)
  → fluorescence emission at the local scale (also with tagging capabilities)

- Polarization microscopy
  → dichroism and birefringence measurements (also for device assessment)

- Differential Interference Microscopy
  → measurements of morfology/topography (e.g., optical profilometry)

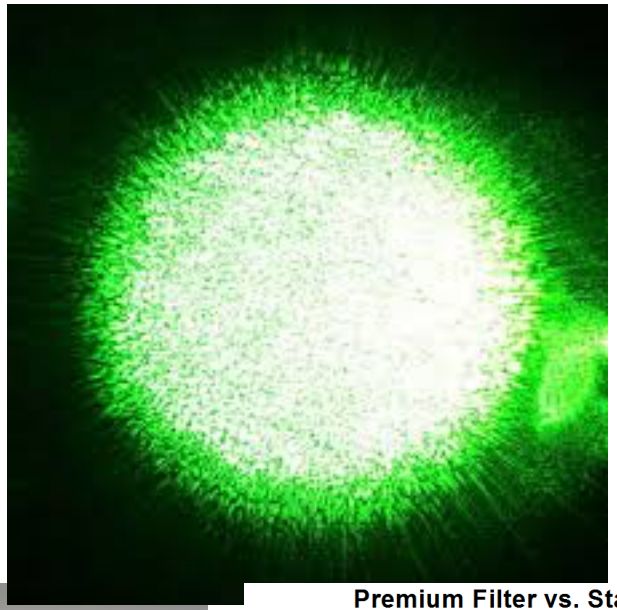
A rather comprehensive overview of microscopy techniques can be found in, e.g.:
https://www.microscopyu.com
http://www.olympusmicro.com
http://zeiss-campus.magnet.fsu.edu

ILLUMINATION AND RELATED PROBLEMS

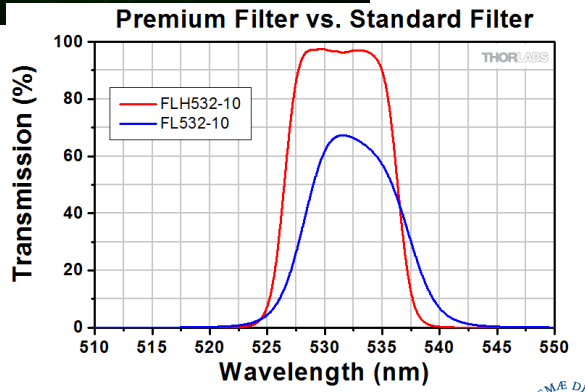
Illumination of the sample is necessary for any microscope configuration
It can in principle be accomplished by using either **incoherent** (e.g., lamps, LEDs, at some extent) or **coherent** (laser) sources

Unless strictly needed, in conventional optical microscopy incoherent sources are preferred to prevent speckles formation

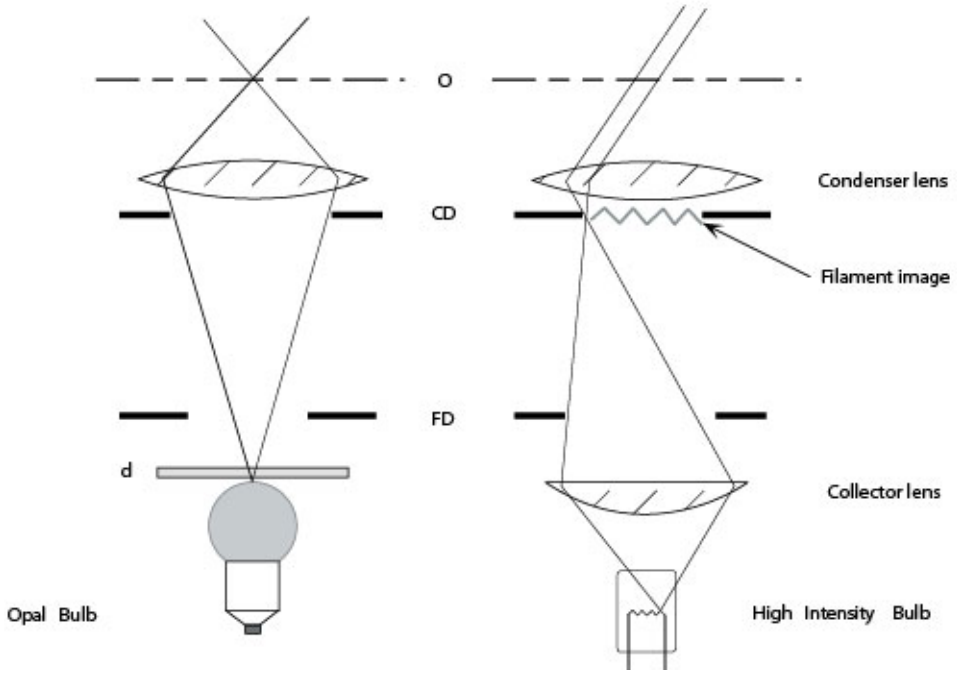
Speckles result from multiple interference between coherent light scattering from nanosized irregularities of a surface, and are a typical signature of laser light (see, e.g., the pattern obtained when shining a laser beam onto a rough surface such as, a painted wall or a paper sheet)



Whenever an almost monochromatic illumination is needed, for instance in epifluorescence, bandpass filters (interference filters, based on Bragg scattering, we will see more in the future) are used



CRITICAL vs KOEHLER ILLUMINATION



Critical Illumination. Conjugate planes are the illuminating bulb filament and sample plane (O). When adjusted correctly, the image of the filament is seen coincident with the sample image. A diffusing glass filter (d) is used to blur the filament image.

FD: Field diaphragm
CD: Condenser diaphragm

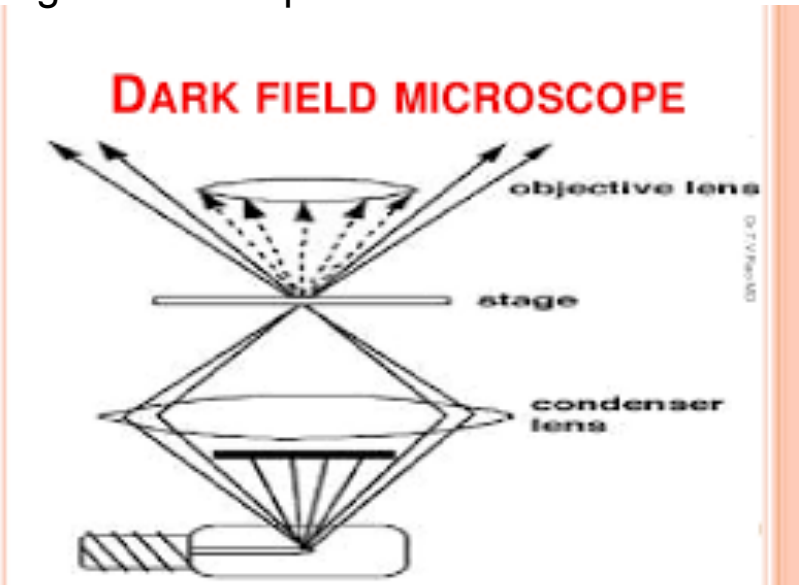
Köhler Illuminating: Conjugate planes are the illuminating bulb filament and Condenser diaphragm. Second conjugate planes are the Field diaphragm and the sample plane. When adjusted correctly, the image of the field diaphragm and the sample are coincident. The filament is out of the plane of focus, and thus uniformly diffuse.

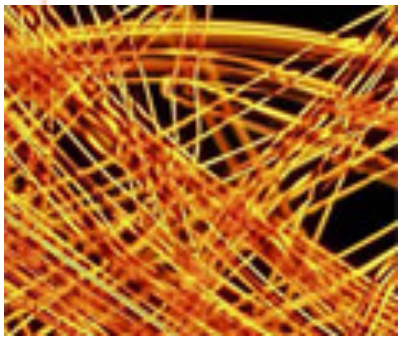
Koehler illumination scheme enables minimizing intensity variations over the illuminated region
Basically, it requires focusing the source on the back focal plane of the objective (illumination passes through the objective)

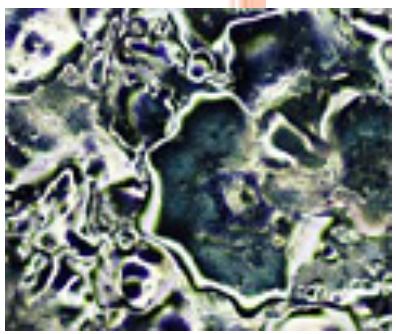
DARK FIELD MICROSCOPY

Dark field microscopy implements a variant of illumination aimed at reducing contribution of stray light (coming from out-of-focus planes) into the image formation

An example is **annular ring** (cardioid) illumination, achieved by stopping the central part of the source







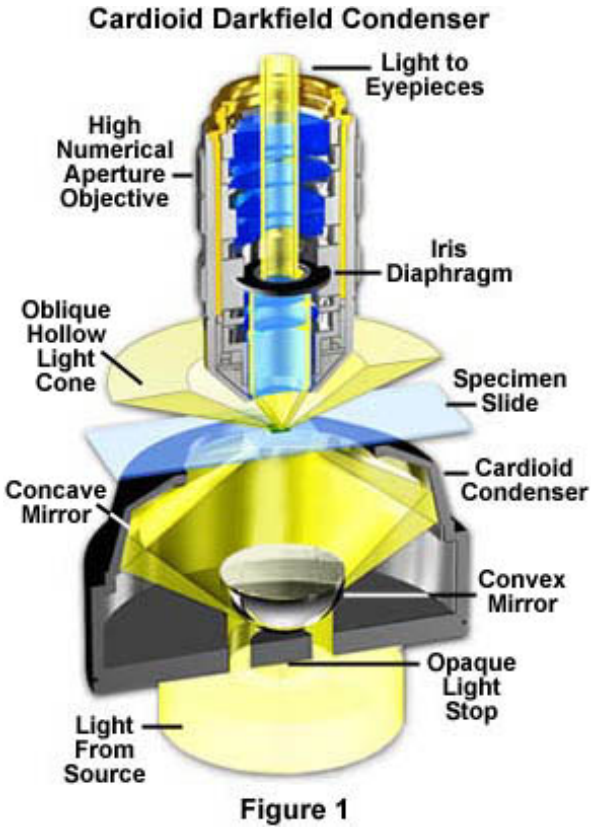
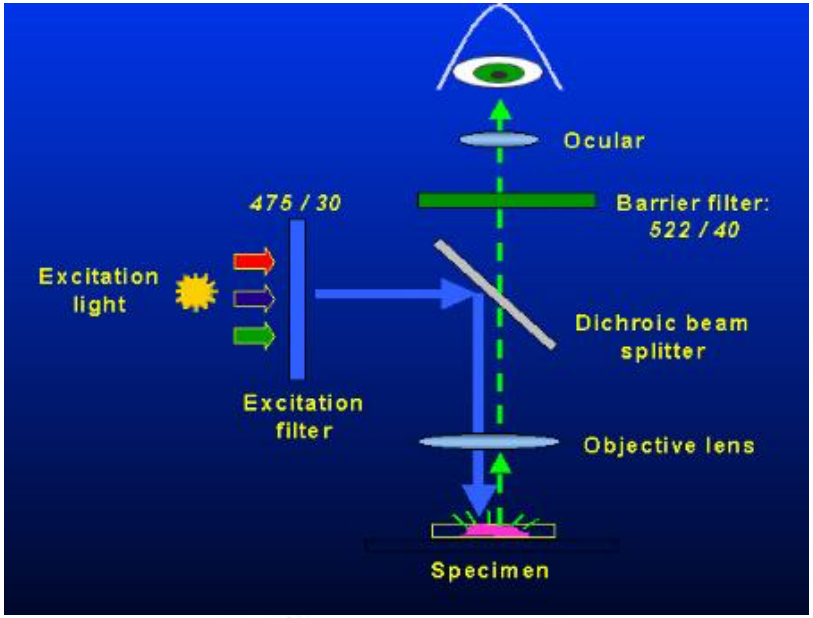


Figure 1

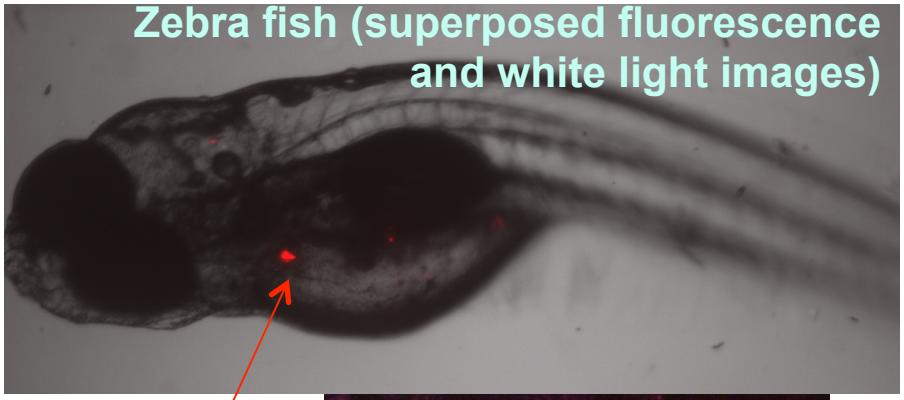
An increase of contrast is frequently achieved, leading to an apparent enhancement of details

EPI-FLUORESCENCE MICROSCOPY

Traditionally, very popular in life-science applications where it enables identifying previously stained (with chromophores/ dyes) portions of biological entities

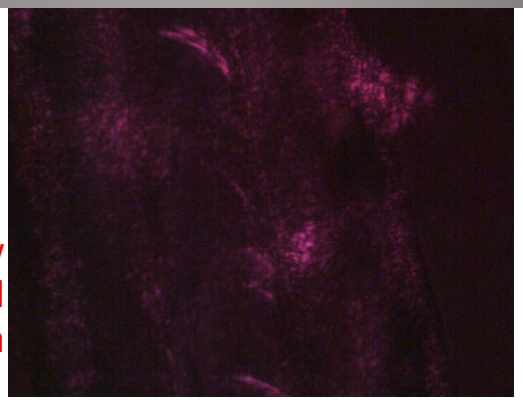


Excitation and emission filter assembly
("fluorescence cube")



(Small) feature
of interest

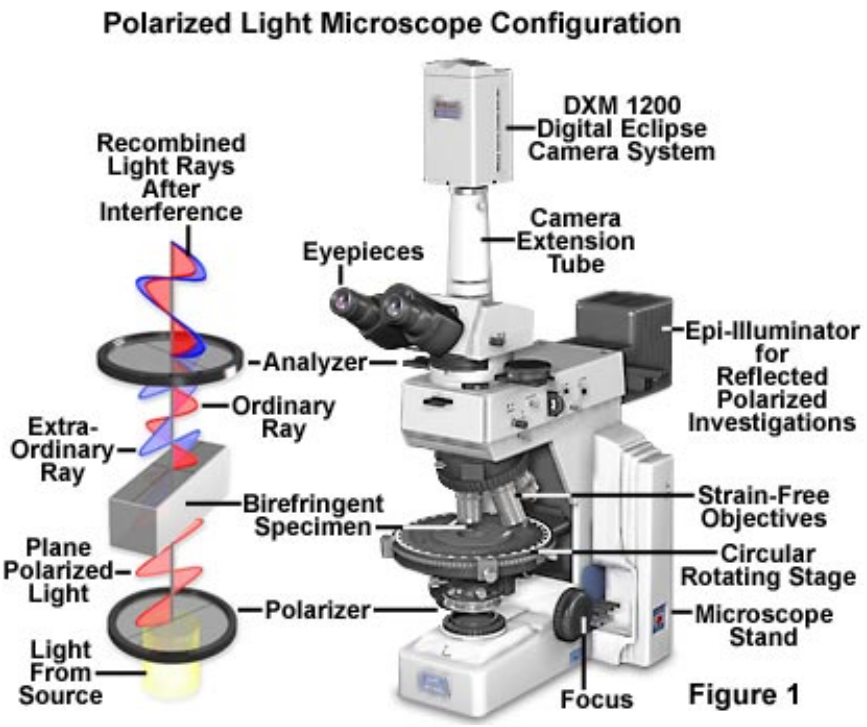
Maginified view
of the stained
region

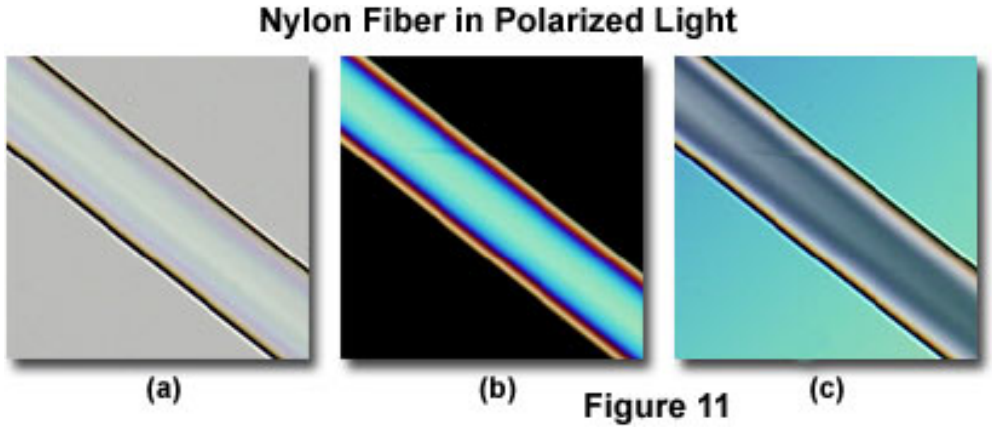


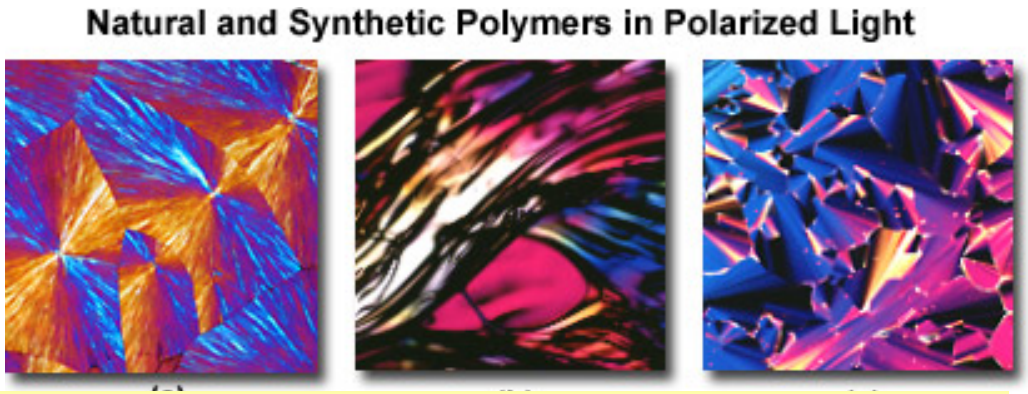
As we will see, breaking the diffraction limit (super-resolution) has been demonstrated mostly with fluorescence microscopes (from confocal to STED)

POLARIZATION MICROSCOPY

As we will better see in the future, some material system can be sensitive to the polarization of the illumination light
→ Polarization analysis can offer a powerful contrast mechanism able to enhance the apparent spatial resolution

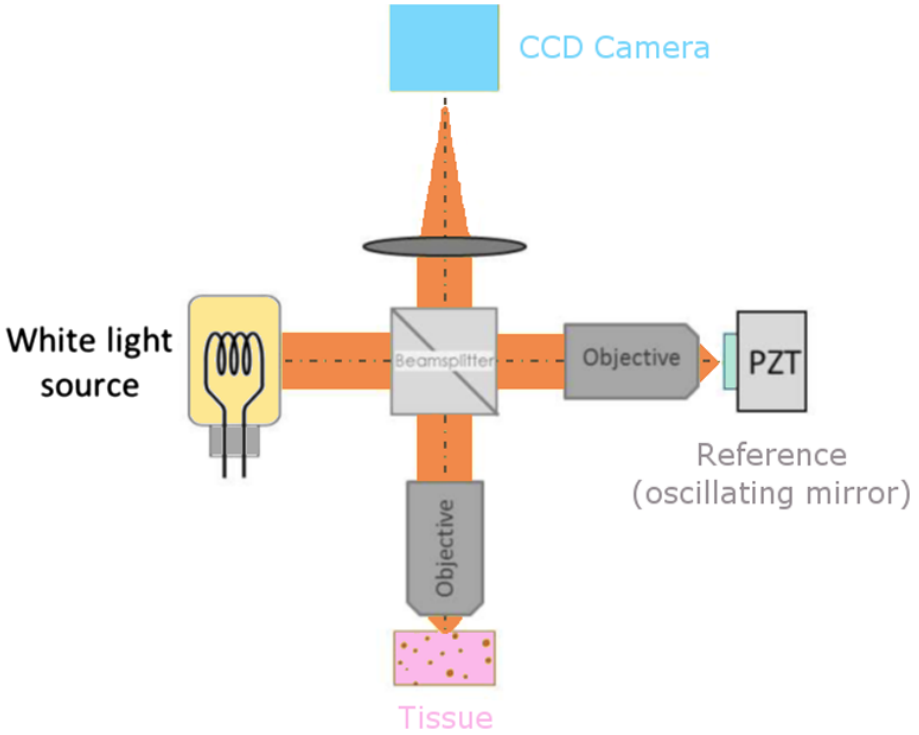


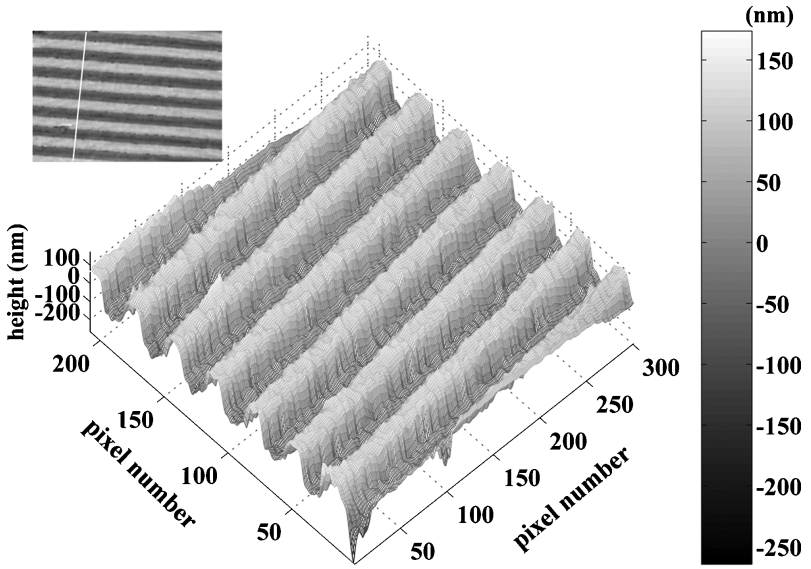




Otherwise invisible details can be captured by using polarization microscopy, i.e., by selecting the polarization state of the input light and/or analyzing the polarization at the output

DIFFERENTIAL INTERFERENCE MICROSCOPY





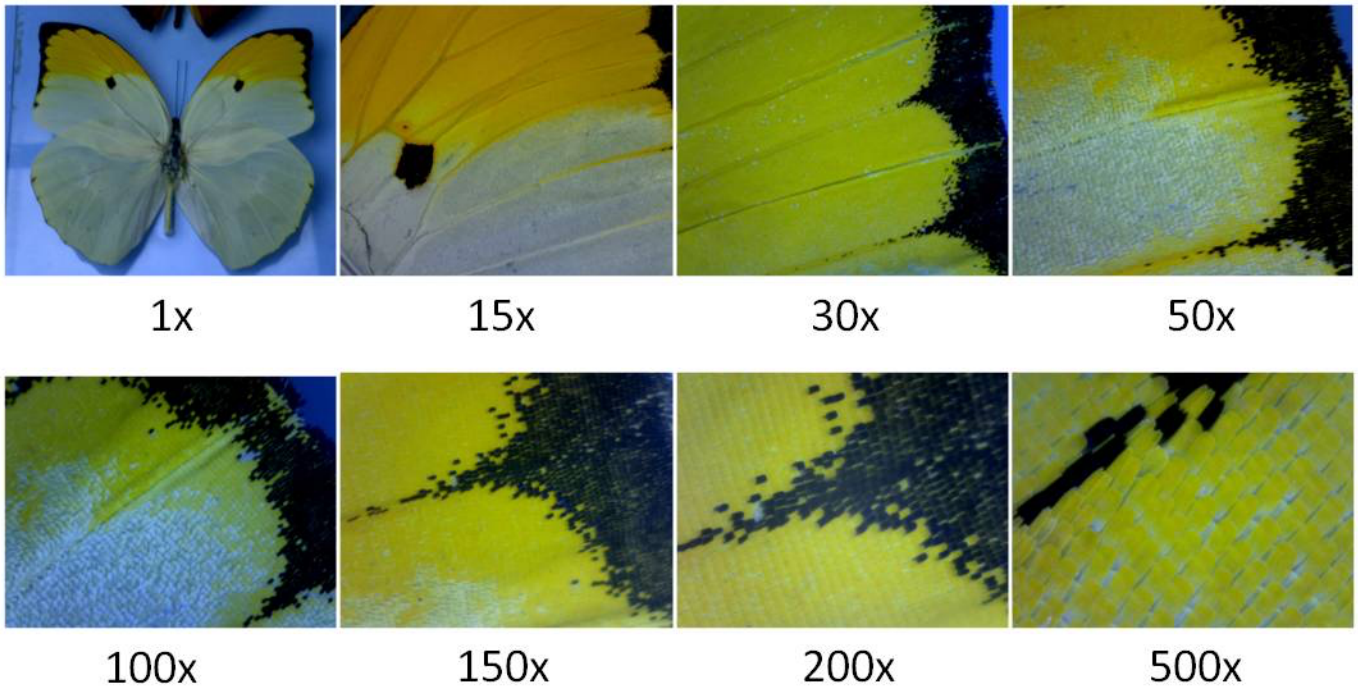
The image is reconstructed from the interference between the scattered light from the sample and the one going through a controlled path, like in a Michelson interferometer

White light (incoherent) can be used thanks to the possibility of precisely matching the optical paths (no need for long coherence length)

Once treated by specific algorithms, looking at the phase modifications as a function of the position, a (rather reliable) map of the surface topography can be be built

Optical profilometry (non contact(can be carried out with sub-wavelength vertical accuracy

BACK TO SPATIAL RESOLUTION



No matter the method or configuration:

because of diffraction, spatial resolution cannot be increased at wish …

CONCLUSIONS

- ✓ Spectroscopy of nanomaterials naturally entails adding spatial resolution to conventional (macro-scale) techniques
- ✓ In the optical regime, this is the basic goal of (conventional, optical) microscopy
- ✓ In the micro (and nano!) world, effects like scattering, diffusion, interference can occur, which are superposed or in competition with genuine imaging
- ✓ Optics has evolved very much, providing researchers with sophisticated instrumentation and, mostly, with a multitude of configurations and methods able to capture details and enhance the contrast, hence the apparent resolution

However, no matter the method or the instrument: diffraction still survives playing the role of a fundamental limitation to spatial resolution

Therefore, we have still to meet the ultimate performance allowed by conventional optics, in order to move towards more refined approaches (next lectures)!

FURTHER READING

For more details on optics, including scattering:

E.Hecht, Optics, Pearson, Harlow (2014).

For many interesting information regarding opitcal microscopy from the practical and fundamental points of view:

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