Scuola di Dottorato Leonardo da Vinci – a.a. 2012/13

LASER: CARATTERISTICHE, PRINCIPI FISICI, APPLICAZIONI

Versione 4 – July 13 – http://www.df.unipi.it/~fuso/dida

Part 8
Applications of lasers to metrology, microscopy and nanoscopy

OUTLOOK

- Coherence, in general terms, is probably the most striking feature of laser radiation:
  - *How can coherence be exploited in (advanced, high end) applications?*
  - *How can laser provide with an added value in pushing spatial resolution of "coventional techniques" to the levels required for nanotechnology?*

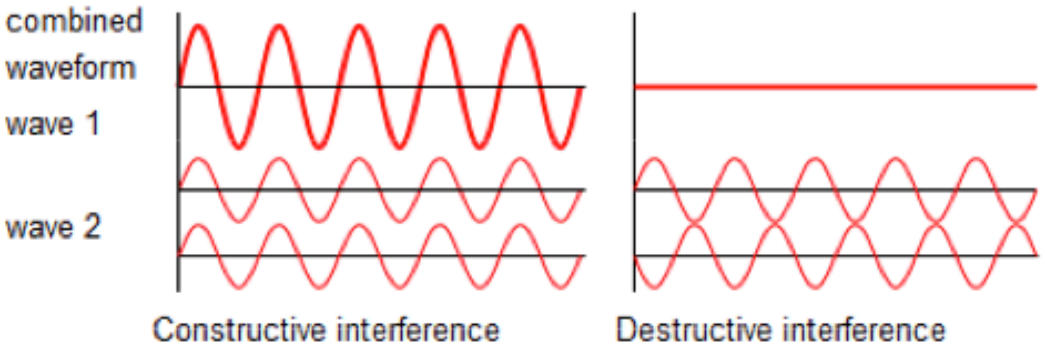
- Back to interference and the need for coherence:
  - Applications of coherence for metrological non-contact analysis
  - Optical profilometry

- Back to microscopy and the helpful adevnt of lasers:
  - Limitations of conventional microscopy
  - The STED for ultimate spatial resolution
  - Using extreme diffraction to gain spatial resolution: the SNOM

Objective : to see how lasers have allowed a substantial revival (with large improvements!) of various optical techniques

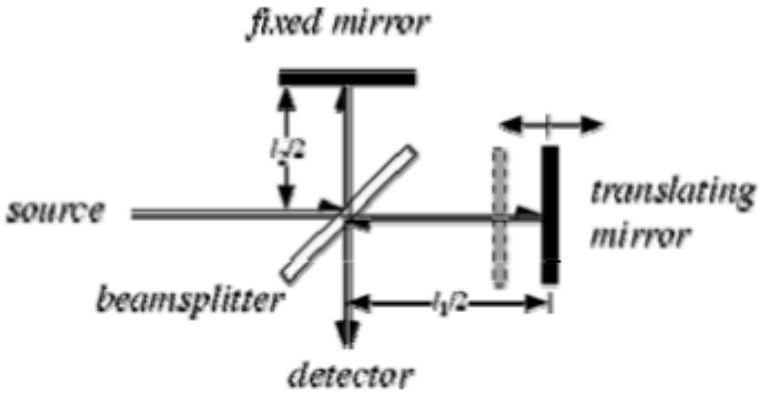
BACK TO INTERFERENCE

The principle of superposition of waves states that when two or more waves are incident on the same point, the total displacement at that point is equal to the vector sum of the displacements of the individual waves. If a crest of a wave meets a crest of another wave of the same frequency at the same point, then the magnitude of the displacement is the sum of the individual magnitudes – this is constructive interference. If a crest of one wave meets a trough of another wave then the magnitude of the displacements is equal to the difference in the individual magnitudes – this is known as destructive interference.



Constructive interference occurs when the phase difference between the waves is a multiple of 2π, whereas destructive interference occurs when the difference is π, 3π, 5π, etc. If the difference between the phases is intermediate between these two extremes, then the magnitude of the displacement of the summed waves lies between the minimum and maximum values.

Michelson's interferometer



University of Chicago physicist Albert A. Michelson. This year marks the centennial of Michelson's Nobel Prize in Physics. He was the first American to receive a Nobel Prize in the sciences.

MICHELSON'S INTERFEROMETER

$$\vec{E}_1 = \hat{e}_1 E_1 \exp(i(kL_1 - \omega t))$$
$$\vec{E}_2 = \hat{e}_2 E_2 \exp(i(kL_2 - \omega t))$$

Two plane waves (of the same frequency) are made to interfere each other

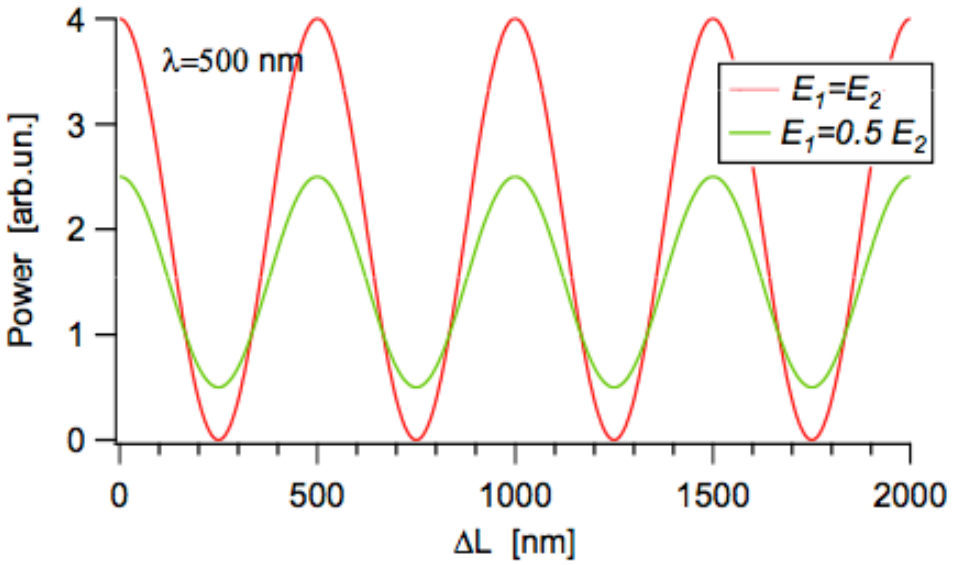
$$\vec{E}_{tot} = \vec{E}_1 + \vec{E}_2 = \exp(i(kL_1 - \omega t))(\hat{e}_1 E_1 + \hat{e}_2 E_2 \exp(ik\Delta L))$$
$$\text{con}: \Delta L = L_2 - L_1$$

Superposition at the detector site

Note: there is no interference if the polarization are mutually orthogonal

$$|\vec{E}_{tot}|^2 = |E_1|^2 + |E_2|^2 + (\hat{e}_1 \cdot \hat{e}_2) E_1 E_2 (\exp(ik\Delta L) + \exp(-ik\Delta L)) =$$
$$= |E_1|^2 + |E_2|^2 + (\hat{e}_1 \cdot \hat{e}_2) E_1 E_2 2\cos(k\Delta L)$$

Intensity read by the detector is proportional to the squared module



The signal read by the detector depends on the difference of optical path Δ*L*

Note that Δ*L* can be evaluated "module λ"

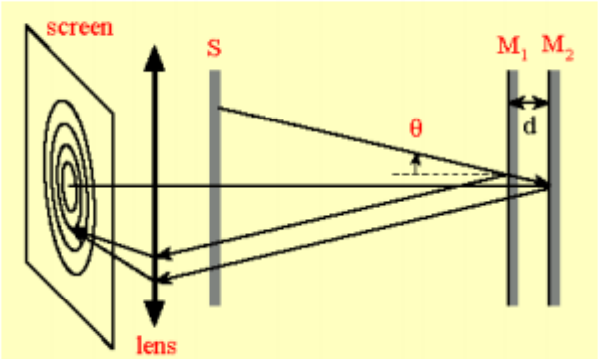
A good sensitivity (tens of nm?) can be easily achieved, depending on the signal-to-noise ratio

The contrast depends on several factors such as, polarization, intensity

INTERFERENCE FRINGES

Actually, Michelson did not measure the intensity with a detector, but he rather used a lens to re-focus the interfering beams and looked at the *interference fringes*

Interference in a Michelson interferometer can be understood in terms of thin-film interference. Imagine that the arms of the interferometer are rotated, such that there is a single optical axis, as shown in the drawing below.



If the two mirrors are precisely aligned such that their planes are exactly perpendicular to one another, thus ensuring that path differences over different regions of the mirrors are constant, the fringe pattern will consist of a series of concentric rings. Each ring will correspond to a different angle of view, measured from the normal to the mirror M_1. These fringes are called fringes of equal inclination. They are analogous to fringes of equal inclination that we observe when we shine light from an extended source on a thin film. When the mirror M_1 is moved so as to approach the condition for zero path difference, the fringe pattern appears to collapse, with all fringes moving towards the center, and disappear.

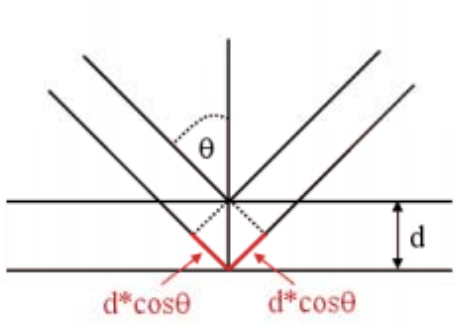
Then reflection from the mirrors M_1 and M_2 is analogous to reflection from two surfaces of an air gap of thickness d. Since the phase shift upon reflection is the same for both mirrors, we find

$$2d\cos\theta = m\lambda$$

for constructive interference.

Fringes are formed because of the difference in optical path

This can be explained by recalling the (already widely encountered) interference “à la Bragg”, i.e., for multiple reflections



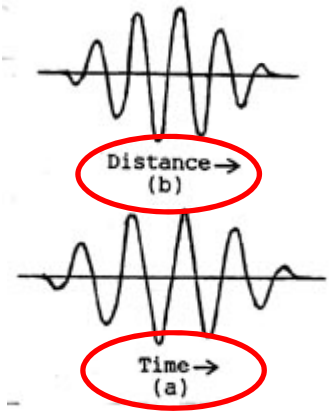
COHERENCE AND INTERFERENCE

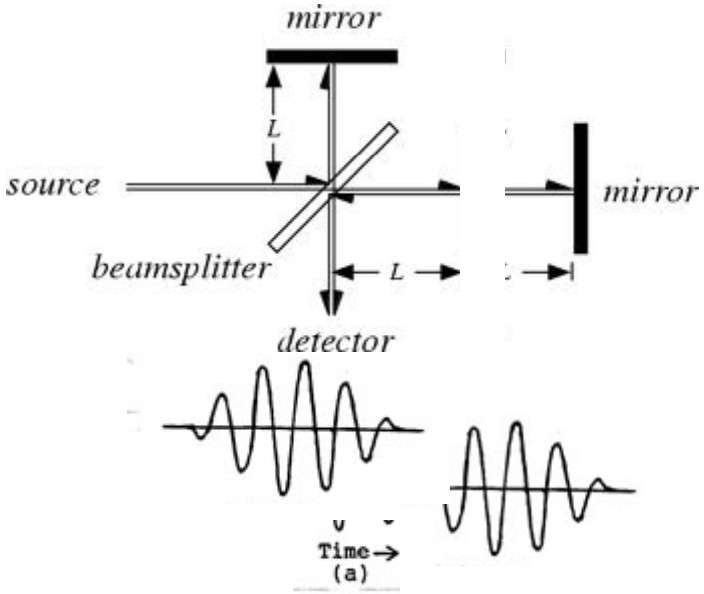
Michelson did use a conventional source (e.g., a vapor lamp) since … no laser was available at that time

How can a laser help?

Remember: indefinite waves cannot exist because of uncertainty principle $\Delta\nu\Delta t \geq 1$
Rather, **wavepackets** must be considered

Since the e.m. wave propagates, wavepackets with a certain (finite) duration will become wavepackets with a certain (finite) length





Moreover: thanks to its monochromaticity laser light allows a reliable "calibration", i.e., relationship between dephasing and difference in optical path
Finally, a collimated beam is needed in order to have intense reflection from a mirror located far away, and here, too, laser is helpful

If the (total) difference in optical path is *too large*, one packet will arrive onto the detector before the other one (or, if you prefer, there will be no superposition in space between the two wavepackets)
--> **No interference appears!**

TEMPORAL AND SPATIAL COHERENCE

2.7.1 Temporal Coherence

Consider a point source PS in the focal plane of a lens forming a parallel light beam which is divided by a beam splitter S into two partial beams (Fig. 2.22). They are superimposed in the plane of observation B after reflection from the mirrors M_1, M_2. This arrangement is called a *Michelson interferometer* (Sect.4.2). The two beams with wavelength λ travel different optical path lengths SM_1SB and SM_2SB, and their path difference in the plane B is

$$\Delta s = 2(SM_1 - SM_2) .$$

The mirror M_2 is mounted on a carriage and can be moved, resulting in a continuous change of Δs. In the plane B, one obtaines maximum intensity when both amplitudes have the same phase, which means $\Delta s = m\lambda$, and minimum intensity if $\Delta s = (2m+1)\lambda/2$. With increasing Δs, the contrast $(I_{max}-I_{min})/(I_{max}+I_{min})$ decreases and vanishes if Δs becomes larger than the coherence length Δs_c (Sect.2.7.4). Experiments show that Δs_c is related to the spectral width $\Delta\omega$ of the incident wave by

$$\Delta s_c \simeq c/\Delta\omega = c/(2\pi\Delta\nu) .$$

Coherence length

This observation may be explained as follows. A wave emitted from a point source with the spectral width $\Delta\omega$ can be regarded as a superposition of many quasi-monochromatic components with frequencies ω_n within the interval $\Delta\omega$. The superposition results in wave trains of finite length $\Delta s_c = c\Delta t = c/\Delta\omega$ because the different components with slightly different frequencies ω_n come out of phase during the time interval Δt and interfere destructively causing the total amplitude to decrease (Sect.3.1). If the path difference Δs in the Michelson interferometer becomes larger than Δs_c, the split wave trains no longer overlap in the plane B. The coherence lengths Δs_c of a light source therefore becomes larger with decreasing spectral width $\Delta\omega$.

Example 2.8

a) A low-pressure mercury spectral lamp with a spectral filter which only transmits the green line $\lambda = 546$ nm has, because of the Doppler width $\Delta\omega_D = 4\cdot10^9$ Hz, a coherence length of $\Delta s_c \simeq 8$ cm.

b) A single-mode HeNe laser with a bandwidth of $\Delta\nu = 1$ MHz has a coherence length of about 50 m.

If the difference in optical path is larger than the coherence length interference will disappear

$$\Delta s_C \approx c/(2\pi\Delta\nu)$$
with $\Delta\nu$ linewidth of the source

Examples:
Vapor lamp (spontaneous emission!) broadened due to Doppler effect:
$\Delta\nu \approx 4$ GHz --> $\Delta s_C \approx 8$ cm

HeNe laser (singlemode operation)
$\Delta\nu \approx 1$ MHz --> $\Delta s_C \approx 50$ m!!

(Temporal/spatial) coherence requires monochromaticity

FIZEAU'S INTERFEROMETER

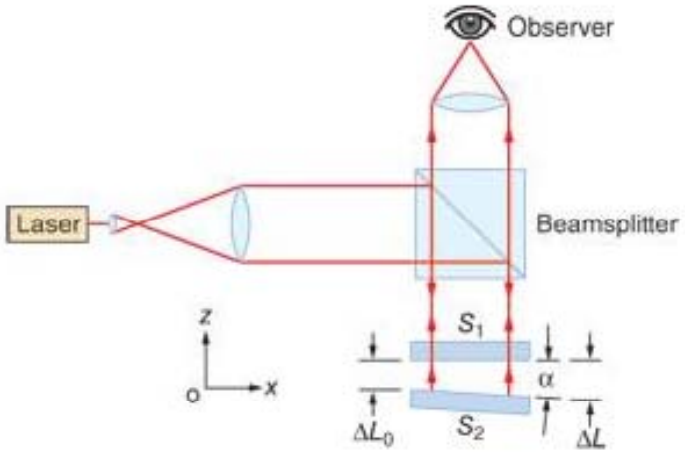


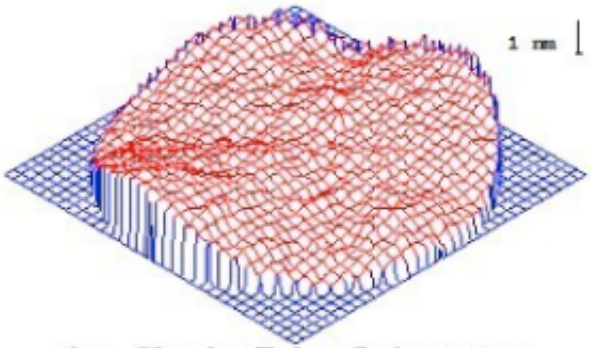
FIGURE 3. In a Fizeau interferometer, the light reflected from the upper side of test mirror S2 interferes with the light reflected from the lower side of reference mirror S1.

In Fizeau's configuration the interference is measured between the beam retroreflected from a reference mirror and that from a (reflecting, at least, partially) surface under test

The difference in optical path is typically small, but monochromatic light is needed to calibrate the dephasing in terms of length

Moreover, fluctuations in the refractive index of the air are minimized (the distance between test and reference can be made small)

Fizeau interferometers are commonly used for measuring the shape of an optical surface: Typically, a fabricated lens or mirror is compared to a reference piece having the desired shape. In Fig. 1, the Fizeau interferometer is shown as it might be set up to test an optical flat. A precisely figured reference flat is placed on top of the flat being tested, separated by narrow spacers. The reference flat is slightly beveled (only a fraction of a degree of beveling is necessary) to prevent the rear surface of the flat from producing interference fringes. A collimated beam of monochromatic light illuminates the two flats, and a beam splitter allows the fringes to be viewed on-axis.[2][3]



doc. Charles Fabry Laboratory,
Graduate School - Optic Institute

Metrology of (partially) reflecting surfaces can be carried out if a reference flat (a mirror) is available

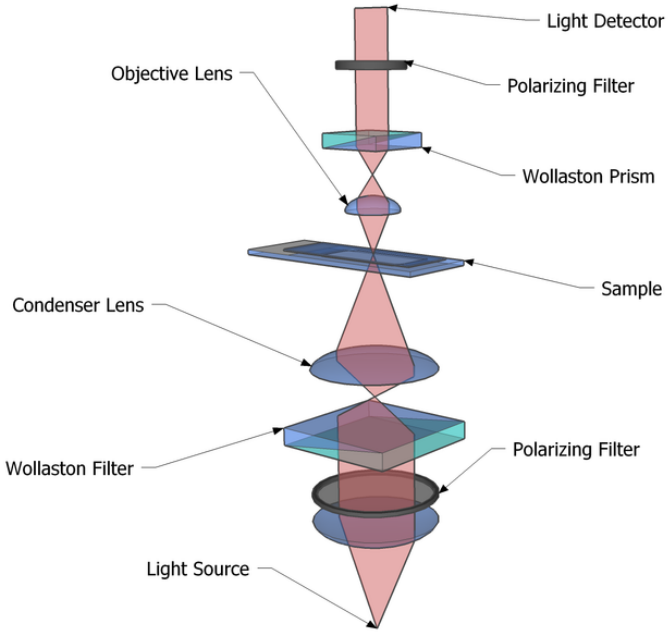
In practice, differences of topography can be measured

DIFFERENTIAL INTERFERENCE CONTRAST

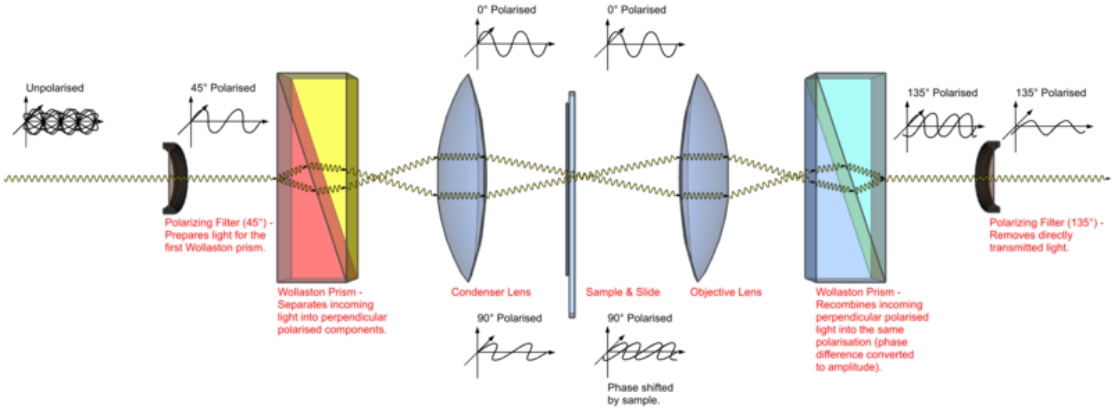
An optical profilometer is a non-contact method for providing much of the same information as a stylus based profilometer. There are many different techniques which are currently being employed, such as laser triangulation (triangulation sensor), confocal microscopy (used for profiling of very small objects), low coherence interferometry and digital holography.

Advantages of optical profilometers

- Speed: For small steps and requirements to do 3D scanning, because the non-contact profilometer does not touch the surface the scan speeds are dictated by the light reflected from the surface and the speed of the acquisition electronics. For doing large steps, a 3D scan on an optical profiler can be much slower than a 2D scan on a stylus profiler.
- Reliability: optical profilometers do not touch the surface and therefore cannot be damaged by surface wear or careless operators. Many non-contact Profilometers are solid-state which tends to reduce the required maintenance significantly.
- Spot size: The spot size, or lateral resolution, of optical methods ranges from a few micrometres down to sub micrometre.



Nomarski's microscope



Differential Interference Contrast relies on the interference between two beams (with mutually orthogonal polarization) sent separately to the sample and then made to recombine (superpose, interfering each other)

Laser light helps in preventing aberration (as in any microscopy) and controlling polarization

"SURFACE" INTERFEROMETRY

Surface interferometry

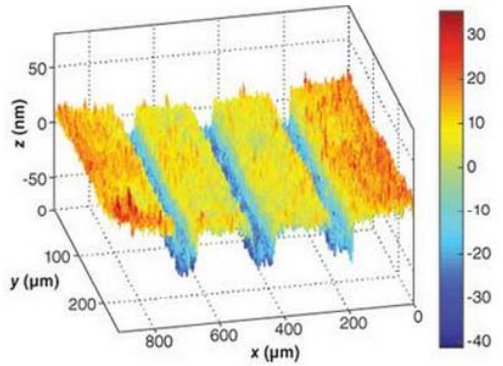
Surface interferometry-which can be carried out in classical Michelson and Fizeau setups-can be considered as a kind of displacement interferometry. Key differences are that an array of photodetectors (like a CCD camera) is used, and the (static) phase difference among several areas on the surface is taken to represent the surface features (see Fig. 3). In this case, the equation on p. 87 is valid for the distance between the reference surface and the surface to be measured as shown in Fig. 2. For an optimum contrast, the intensity is proportional to:

$$I \propto 1 + \cos\frac{2\pi}{\lambda}(\Delta z);$$

$$I(x,y) \propto 1 + \cos[\varphi(x,y)];$$

$$\text{with } \varphi(x,y) = \frac{2\pi z(x,y)}{\lambda}$$

Click here to enlarge image

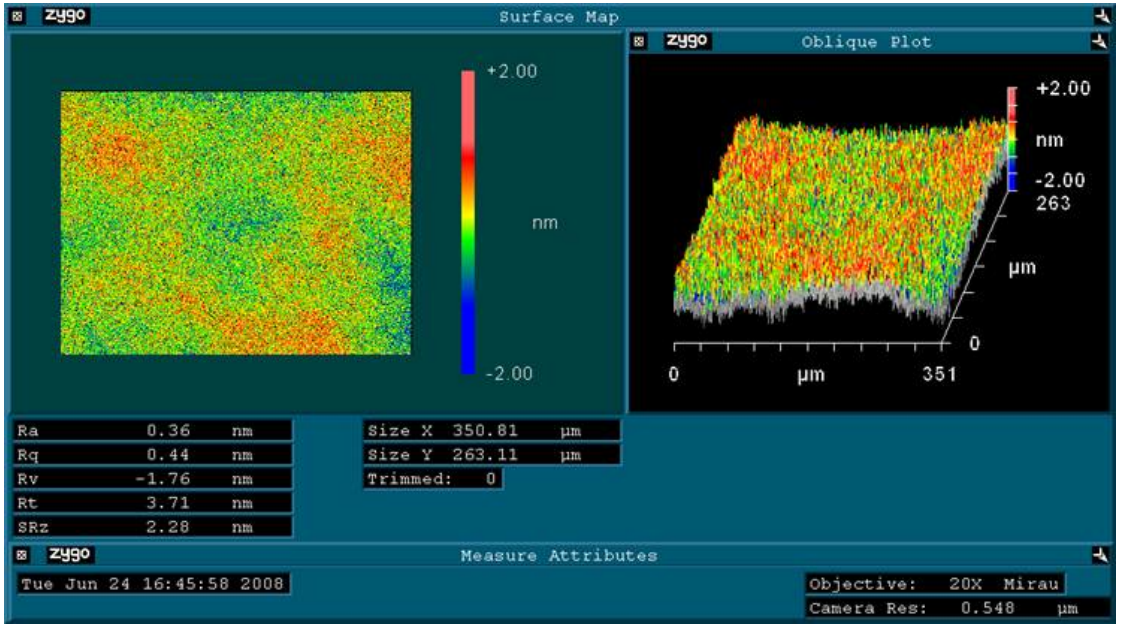


Several methods can be used to determine the surface structure $z(x,y)$. One method is essentially similar to homodyne interferometry, except that CCD cameras are used instead of photodetectors. This method is called instantaneous phase shifting because it takes a very short measuring time and there is no time difference between images taken with a different phase shift.[4]

More commonly used is temporal phase shifting. The reference mirror is displaced over a half wavelength in five (or more) steps, and the phase per pixel is derived from the various intensities.[5] In these cases the interference number N remains undetermined and unimportant. When the phase passes 360°, however, it should continue instead of starting at zero again. The process of removing these discontinuities, a separate subject, is referred to as phase unwrapping.

A third way to determine the surface heights is to consider the whole interference pattern. The reference plane is tilted to guarantee a constant number of fringes, which results in fringes with a determined spatial frequency or carrier frequency. Surface deviations give deviations in this spatial frequency that can be converted to height differences by a Fourier transform and additional manipulations. This method is referred to as the carrier-fringe method, and compared to the other methods it has relatively limited lateral resolution. [6]

OPTICAL PROFILOMETRY



The operation of DIC or other configurations of interferometers, coupled to optical microscopy, enables 3D non-contact profilometry

However: warning!

- Artifacts are possible due to the uneven scattering from nanosized asperities
- Large aspect-ratio machined surfaces are difficult to image
- Calibration is a cumbersome task

HOLOGRAPHY I

Holography is a technique which enables a light field, which is generally the product of a light source scattering off objects, to be recorded and later reconstructed when the original light field is no longer present (due to the absence of the original objects).[17] Holography can be thought of as somewhat similar to sound recording, whereby a sound field created by vibrating matter, like musical instruments or vocal chords, is encoded in such a way that it can be reproduced later without the presence of the original vibrating matter.

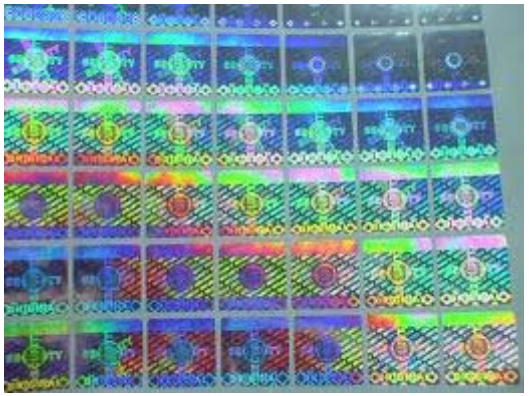
Holograms are recorded using a flash of light that illuminates a scene and then imprints on a recording medium, much in the way a photograph is recorded. A hologram, however, requires a laser as the light source, since lasers can be precisely controlled and have a fixed wavelength, unlike white light, which contains many different wavelengths.

Dennis Gabor
(06/05/1900 – 07/09/1979)
Hungarian scientist (invented the hologram)

A shutter is required when taking a photograph to limit the time in which the film is exposed to light. Holography also requires a specific exposure time, and this can be done using a shutter, or by electronic timing of the laser.

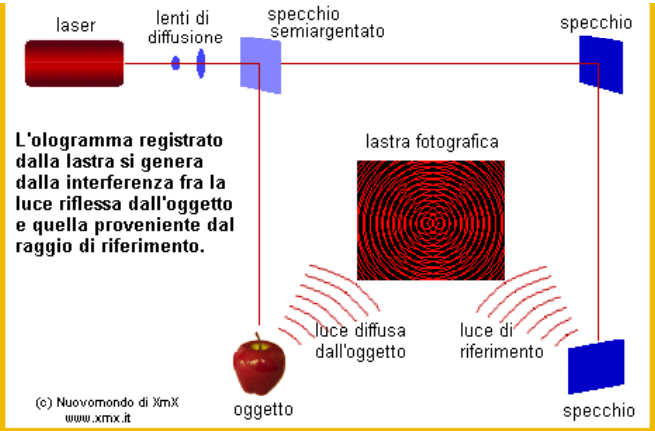
This laser beam is generally aimed through a series of elements that change it in different ways - see Figure 2. The first element is a beam splitter, which divides the beam into two identical beams, each aimed in different directions:

- One beam, known as the illumination or object beam, is spread using lenses and directed onto the scene using mirrors, in order to illuminate it. Some of the light scattered (reflected) from this illumination falls onto the recording medium.
- The second beam, known as the reference beam, is also spread through the use of lenses, but is directed so that it doesn't come in contact with the scene, and instead travels directly onto the recording medium.

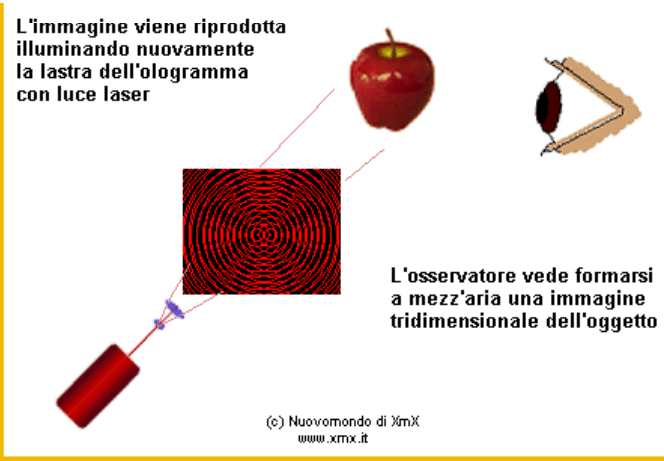


HOLOGRAPHY II

http://www.xmx.it/ologrammi.htm



Qui sopra vediamo come si realizza l'ologramma. Un fascio di luce laser viene sdoppiato: una parte è inviata direttamente sulla lastra, mentre l'altra parte del fascio è diffusa dall'oggetto, prima di cadere sulla lastra. Nel percorrere tragitti diversi, le due componenti del fascio si sfasano l'una rispetto all'altra e, ricongiungendosi, producono una *figura di interferenza* che viene registrata sulla pellicola sotto forma di ologramma. Ad occhio nudo sulla lastra non è visibile alcuna immagine, solo una retinatura di linee sottilissime iridescenti.

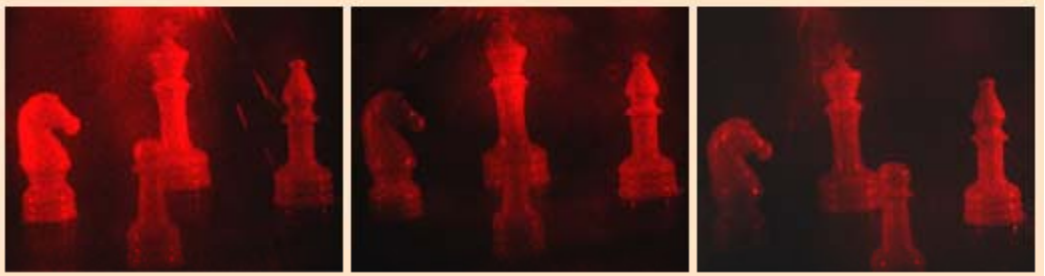


...qui sopra, per riprodurre l'ologramma lo osserviamo con la luce laser, proiettandone un fascio sulla lastra. Apparentemente a mezz'aria l'osservatore vede formarsi l'immagine tridimensionale, attorno alla quale si può anche girare per osservarla da tutti i punti di vista,

Holography is a kind of "lensless" (3D) photography based on the occurrence of interference

Laser illumination is mandatory since difference in optical path can be relevant (and a long coherence length is required)

Moreover laser can be defocused (enlarged) without losing coherence (the same way it can be focused)



These three images of the same hologram were taken by positioning the camera at three positions, moving from left to right. Note that the pawn appears on the left side of the king in the left photo, but transitions to the right of the king as you sweep your eye across the hologram. This is real parallax, which tells you that the image is truly 3-dimensional. Each perspective corresponds to looking through the hologram at a particular point.

HOLOGRAPHY III

Multi-Wavelength digital holographic metrology

Carl C. Aleksoff
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The concepts of holography find applications in various metrology methods useful for instance for real-time evaluation of deformation, stress, strains

Very complicated…

One of the most useful outgrowths of Emmett Leith's holographic work is its use in metrology[1]. The measurement of parts via holographic techniques started early at the Willow Run Labs of the University of Michigan with object deformation interferometry[2, 3], shape contouring[4] , and vibration analysis[5, 6]. In this paper we will consider a more modern outgrowth of using multi-wavelength digital holography to generate 3D (three dimensional) computer-based precision imagery of manufactured parts. These digital holographic techniques have been incorporated into the Coherix Shapix™ systems*, for which Emmett Leith was a consultant.

The output digital image is a 2D array of numbers, where each number represents a height *H* from a reference plane for a pixel. This data can be processed to generate various views on a monitor or mined for features such a deviation from specified shape or surface roughness.

The basic multi-wavelength measurement concept can be described from the standpoint of synthetic-aperture laser-radars[7, 8] or via interferometers[9]. In this paper will develop the basics of the process from an interferometric perspective because we are describing an image plane holographic system where each acquired image pixel can be described as part of a simple interferometer.

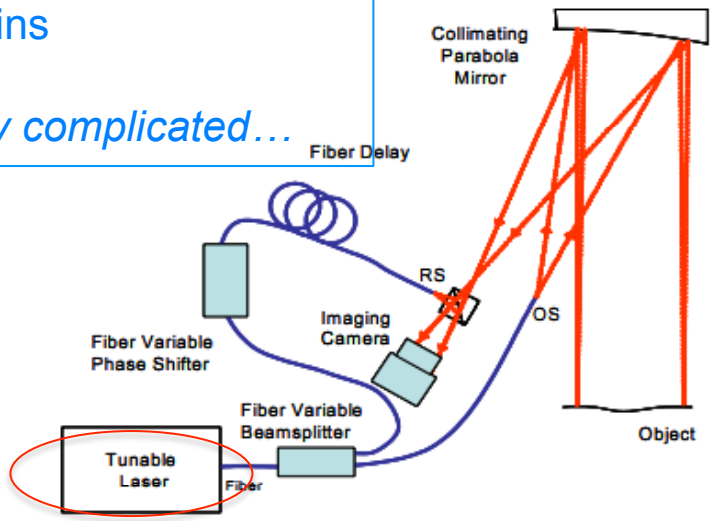


Fig. 1 The optical arrangement is shown for measuring the object height as a function lateral position illumination source (OS) is the end of one fiber and the reference source (RS) is at the other end reference source is at the same apparent position and distance from the camera as the focus of light v parabola from the object. The camera images the object as well as accepting a coaxial reference wav interference via the cube beamsplitter.

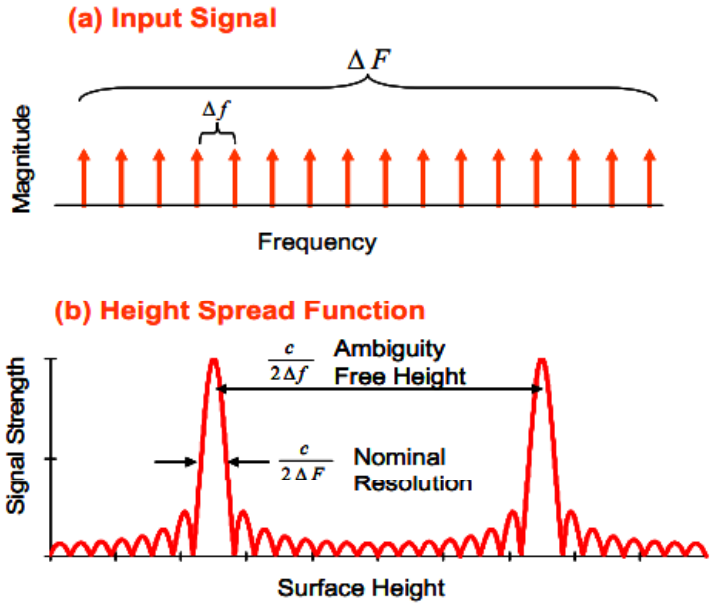


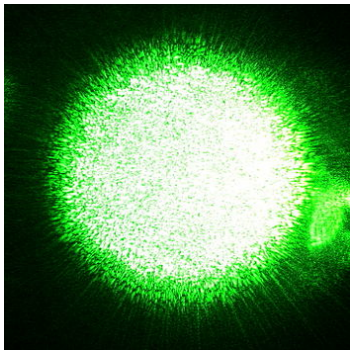
Fig. 3. These diagrams show the basic relationships the input and output data. The input data is a set of delta functions with the frequency spacing of the measurement frequencies (assumed equal spacing) and measured phases. The output is the magnitude of the Fourier Transform of the input data. The calculated surface height is at the first peak of the output.

SPECKLE PATTERN INTERFEROMETRY I

A **speckle pattern** is an intensity pattern produced by the mutual interference of a set of wavefronts.[1] This phenomenon has been investigated by scientists since the time of Newton, but speckles have come into prominence since the invention of the laser and have now found a variety of applications.

Speckles (brilliant spots) appear when coherent, i.e., laser light is scattered by a non-flat surface

They are due to constructive/destructive interference (in fact, they cannot be "focused" by the eye!)



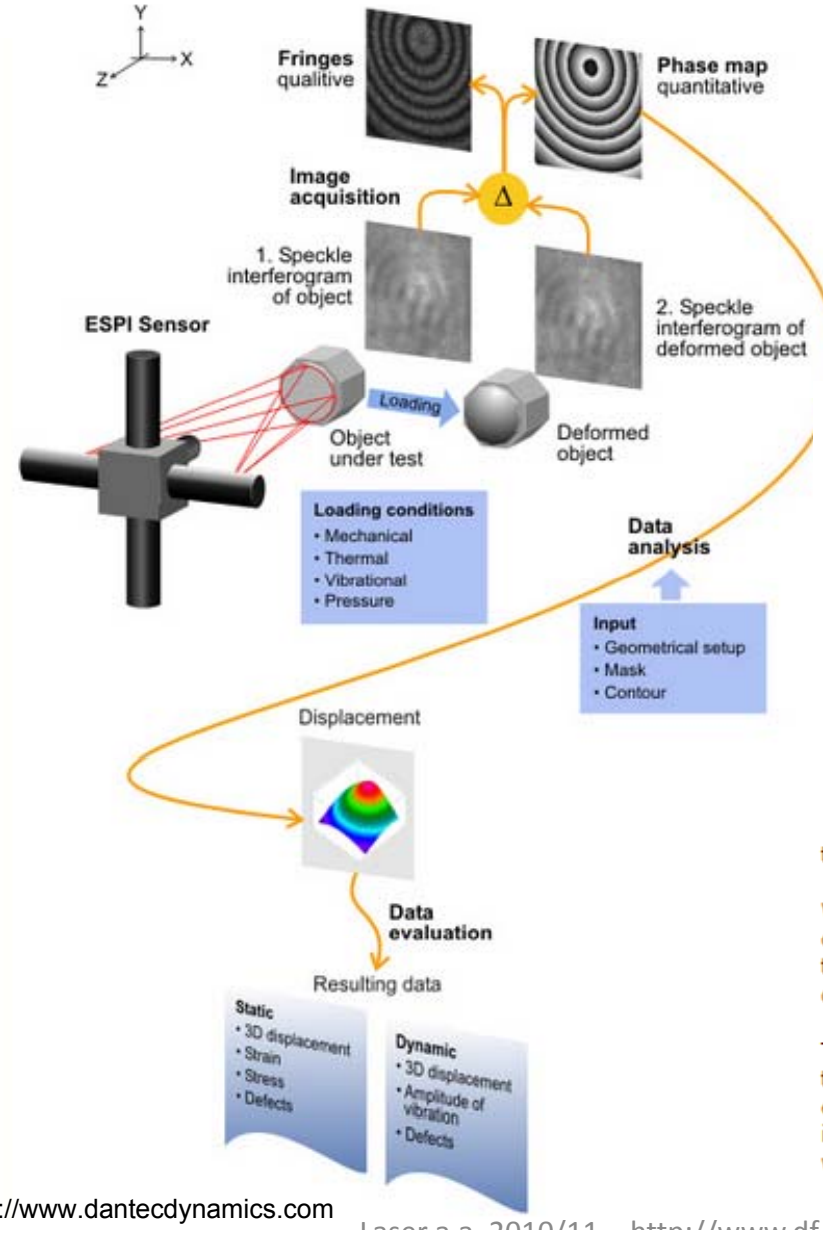
Picture of a green laser pointer sent onto a rough surface

Electronic Speckle Pattern Interferometry (ESPI)[1], also known as TV Holography, is a technique which uses laser light, together with video detection, recording and processing to visualise static and dynamic displacements of components with optically rough surfaces. The visualisation is in the form of fringes on the image where each fringe normally represents a displacement of half a wavelength of the light used (i.e quarter of a micrometre or so).

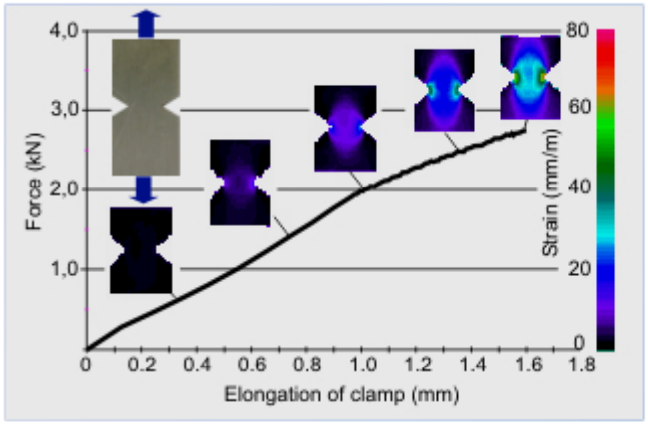
ESPI can be used for stress and strain measurement, vibration mode analysis and nondestructive testing.

ESPI is similar to holographic interferometry in many ways, but there are also significant differences between the two techniques.

SPECKLE PATTERN INTERFEROMETRY II



Comparison between the speckle pattern of a undeformed and of a deformed object can provide with *quantitative* information of the stress localization (through the use of non-trivial algorithms operated in real-time)



Principles
The illumination of a rough surface with coherent laser light and subsequent imaging using a CCD camera generates statistical interference patterns, the so-called speckles.

Like a fingerprint, these speckles are inherent to the investigated surface. Superimposing a reference light, which is split out of the same laser source, on these speckles results in an interferogram.

When the object under test is loaded, e.g. by mecha-nical means, and the surface is deformed, the speckle interferogram also changes. Comparing an interfero-gram of the surface before and after loading will result in a fringe pattern, which reveals the displacement of the surface during loading as contour lines of deformation.

These qualitative fringe images are of low contrast and noisy due to the presence of the speckles. A procedure called phase shifting takes a series of speckle images for each surface state and calculates a quantitative phase map. In contrast to the fringe images this phase map furthermore contains quantitative and directional information which can directly be transformed into a displacement value.

http://www.dantecdynamics.com

SPECKLE PATTERN INTERFEROMETRY III

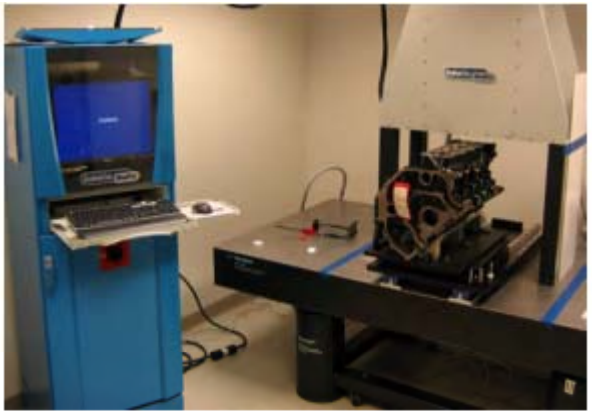


Fig.4. The Shapix system with an engine block inserted for measurement.

Fig. 5. A picture of the engine showing the cylinder deck face to be measured for flatness

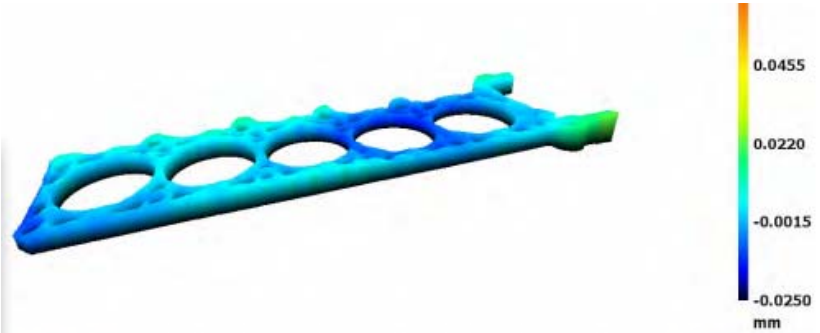


Fig. 6a A Shapix measured engine cylinder deck is shown as a 3D perspective image. This deck was cut with a new tool bit.

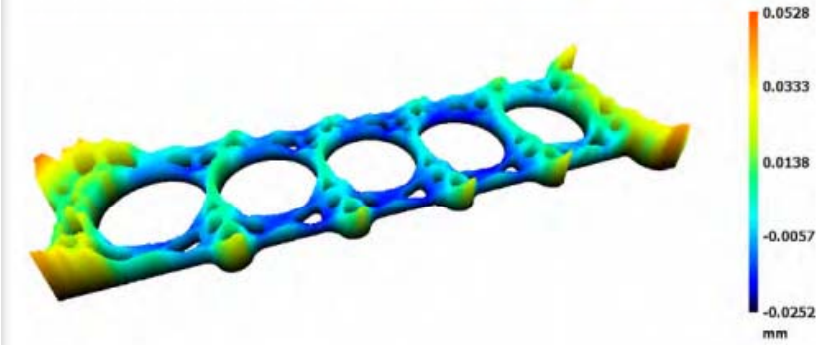


Fig. 6b. A Shapix measured engine cylinder deck is shown as a 3D perspective image. This deck was the 1800 unit cut with the tool bit.

Coherix Inc. Announces New Product Line - The ShaPix® 1500 Series

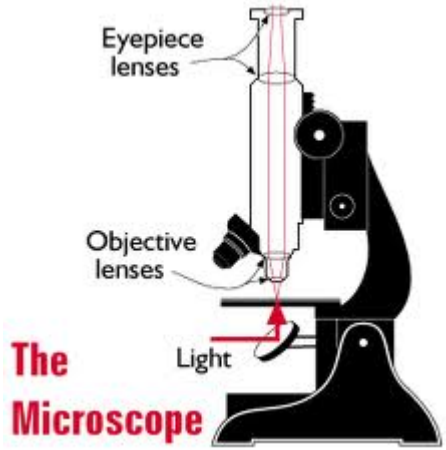
High vertical resolution combined with very low in-plane resolution (needed to inspect large size samples!)

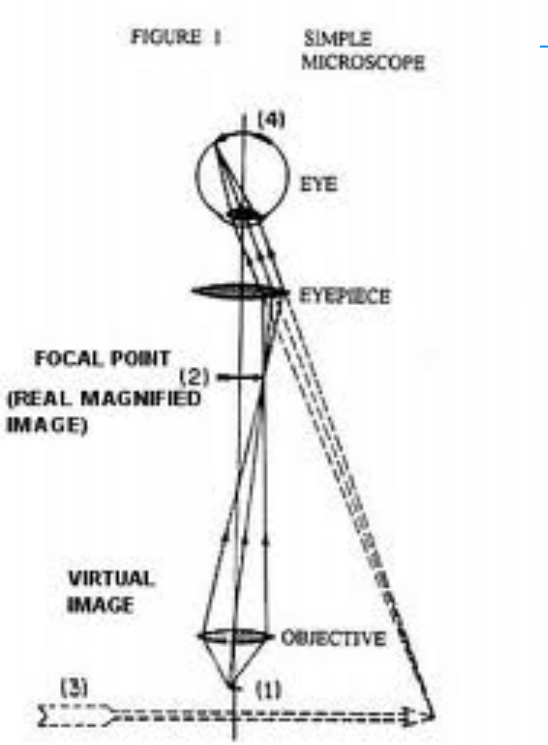
APPLICATIONS TO MICROSCOPY

In principle, laser metrology (based on interference) is sensitive to “one direction” (i.e., the direction of beam propagation) --> *profilometry (i.e., topographical variations of a surface)*

It can obviously be implemented in microscopes to obtain images, but *the in-plane resolution is typically limited*

How can laser improve optical microscopy?
(Note: this entails also achieving non-metrological information, in principle all enabled by conventional microscopy!)





HISTORICAL REMARKS

During the 1st century AD (year 100), glass had been invented and the Romans were looking through the glass and testing it. They experimented with different shapes of clear glass and one of their samples was thick in the middle and thin on the edges. They discovered that if you held one of these "lenses" over an object, the object would look larger.

Someone also discovered that you can focus the rays of the sun with one of these special "glasses" and start a fire. These early lenses were called magnifiers or burning glasses. The word lens by the way, is derived from the latin word lentil, as they were named because they resembled the shape of a lentil bean (look up lens in a dictionary).

These lenses were not used much until the end of the 13th century when spectacle makers were producing lenses to be worn as glasses.

The early simple "microscopes" which were really only magnifying glasses had one power, usually about 6X - 10X . One thing that was very common and interesting to look at was fleas and other tiny insects. These early magnifiers were hence called "flea glasses".

Sometime about the year 1590, two Dutch spectacle makers, Zaccharias Janssen and his father Hans started experimenting with these lenses. They put several lenses in a tube and made a very important discovery. The object near the end of the tube appeared to be greatly enlarged, much larger than any simple magnifying glass could achieve by itself! They had just invented the compound microscope (which is a microscope that uses two or more lenses).

Galileo heard of their experiments and started experimenting on his own. He described the principles of lenses and light rays and improved both the microscope and telescope. He added a focusing device to his microscope and of course went on to explore the heavens with his telescopes.

Anthony Leeuwenhoek of Holland became very interested in lenses while working with magnifying glasses in a dry goods store. He used the magnifying glass to count threads in woven cloth. He became so interested that he learned how to make lenses. By grinding and polishing, he was able to make small lenses with great curvatures. These rounder lenses produced greater magnification, and his microscopes were able to magnify up to 270X!

Anthony Leeuwenhoek became more involved in science and with his new improved microscope was able to see things that no man had ever seen before. He saw bacteria, yeast, blood cells and many tiny animals swimming about in a drop of water. From his great contributions, many discoveries and research papers, Anthony Leeuwenhoek (1632-1723) has since been called the "Father of Microscopy".

http://www.microscope-microscope.org

Optical microscope is one of the most common scientific instrumentation (since a long, long time….)

MAGNIFICATION AND CONTRAST

Magnification is found (from geometrical optics) to be simply dependent on the inverse of the objective focal length f_O

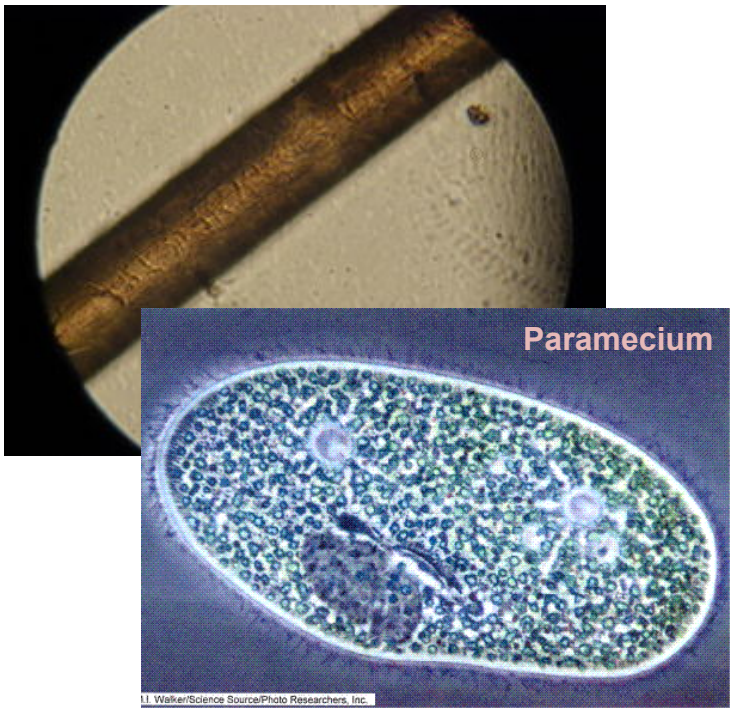
Microscope: The angular magnification is given by

$$\mathrm{MA} = M_o \times M_e$$

where M_o is the magnification of the objective and M_e the magnification of the eyepiece. The magnification of the objective depends on its focal length f_o and on the distance d between objective back focal plane and the focal plane of the eyepiece (called the tube length):

$$M_o = \frac{d}{f_o}.$$

The magnification of the eyepiece depends upon its focal length f_e and calculated by the same equation as that of a magnifying glass (above).



Main contrast mechanisms:
- Opacity/reflectance → morphology
- Color (often after staining) → selection of individual (functional) components

No scan is needed and the eye acts as a (very sophisticated) detector

DIFFRACTION FROM A CIRCULAR APERTURE

The far-field diffraction of a plane wave incident on a circular aperture is often referred to as the Airy Disk. The variation in intensity with angle is given by

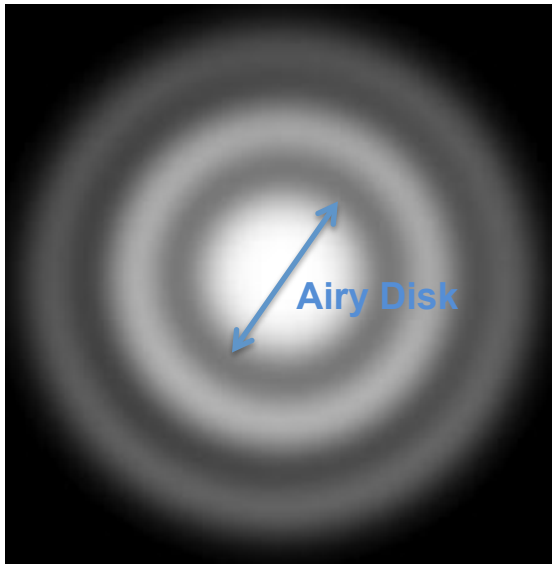
$$I(\theta) = I_0 \left(\frac{2J_1(ka \sin\theta)}{ka \sin\theta} \right)^2,$$

where a is the radius of the circular aperture, k is equal to $2\pi/\lambda$ and J_1 is a Bessel function. The smaller the aperture, the larger the spot size at a given distance, and the greater the divergence of the diffracted beams.

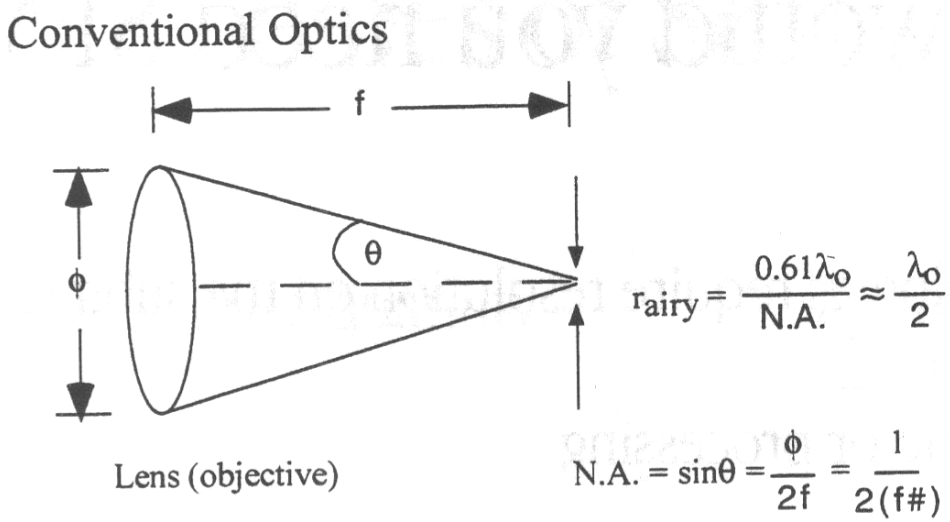
$J_1 = 0$ for $ka\sin\theta \approx 3.8$ --> $\sin\vartheta \approx 0.6\,\lambda/a$

Microscopy suffers from diffraction since **a finite aperture** objective is used to collect light!

Magnification can be ideally increased without any limit, but **spatial resolution** remains finite



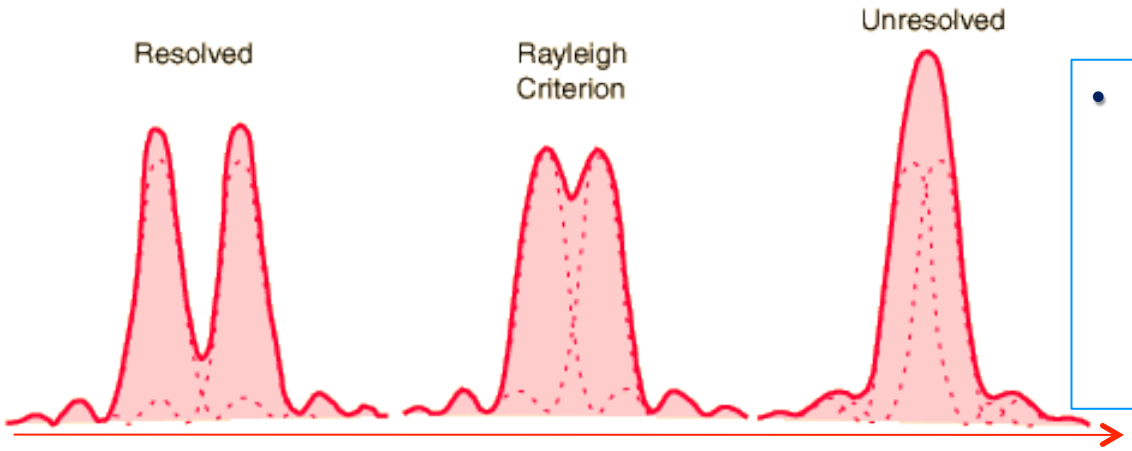
RAYLEIGH CRITERION AND ABBE'S LIMIT



RAYLEIGH $\Delta x_{min} = \frac{1.22\lambda}{2n\sin\theta}$

ABBE $P_{min} = \frac{\lambda}{2n\sin\theta}$

Numerical Aperture (N.A.) plays a role in defining the resolution: $n\sin\theta$ = N.A. objective

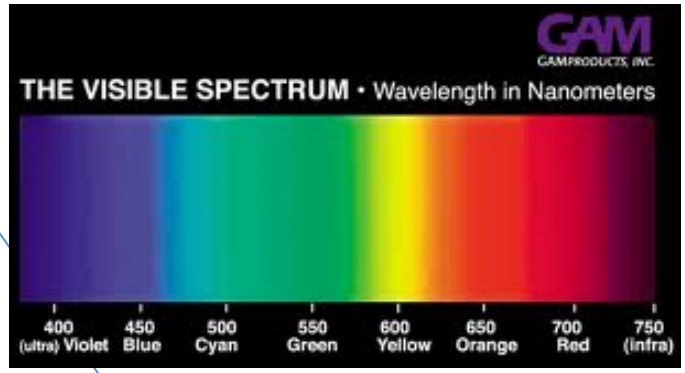


- **Rayleigh criterion**
  - Resolving two equal intensity Airy spots
  - Separation ~ 0.61 λ / N.A. (circular aperture)

HOW TO IMPROVE RESOLUTION (IN PRINCIPLE…)

Rayleigh criterion/Abbe limit

$$\Delta x_{\min} = \frac{0.61\lambda}{n \sin\theta}$$



In the visible, the maximum resolving power is ~λ/2, i.e., 200-300 nm

Increase *n* by using a high-*n* transparent oil

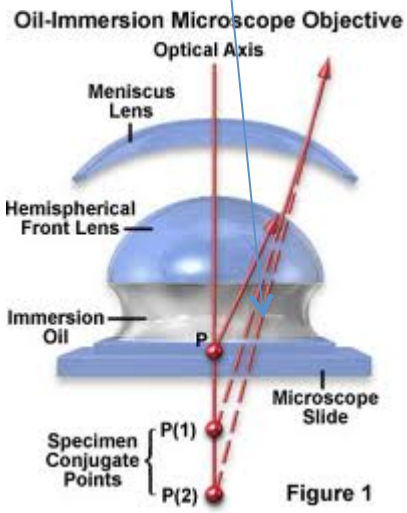


Figure 1

Decrease λ, e.g., by using particle beams

Remember: $p \approx \sqrt{2mE} = \hbar k$ --> λ ≈ 1-10 nm

HOW TO TAKE ADVANTAGE OF LASERS

Electron microscopy is extremely powerful and effective in providing with very good resolution

But: it has drawbacks such as, cost, need of sample preparation (very cumbersome if you aim at high resolution!), sample damage (hardly compatible with soft/organic matter), absence of “quantitative” contrast mechanisms

Moreover, quite often sensitivity to *optical properties* (e.g., reflectivity, refractive index, scattering, luminescence, optical activity, etc.) is strictly required

Optical microscopy is still the subject of important innovation!

Three examples (among many) where the use of laser is able to improve spatial resolution and sensitivity in optical microscopy:

- Confocal microscopy
- STimulated Emission Depletion microscopy (STED)
- Scanning Near-field Optical Microscopy (SNOM

A FEW WORDS ON FOURIER OPTICS

Fourier analysis useful to transfer the problem:
Time domain --> frequency domain
but also Real space domain --> reciprocal (wavevector) domain

A surface pattern f(x,y) (i.e., an object to be imaged!) can be always described in terms of a (2D) Fourier superposition

Fourier components are function of the reciprocal space k_X k_Y :

$f(x,y) = \int f'(k_X, k_Y) \exp(-ik_X x) \exp(-ik_Y y)\, dk_X\, dk_Y$

Large k_X (and k_Y) values imply large angular displacement

⇩

A faithful pattern reproduction (i.e., an accurate image) implies collecting the largest number of k_X and k_Y

⇩

Large numerical aperture (*NA=nSinθ*) needed for high resolution

but

In non point-like illumination schemes (conventional) rays emitted from different points of the surface can be "spuriously" collected

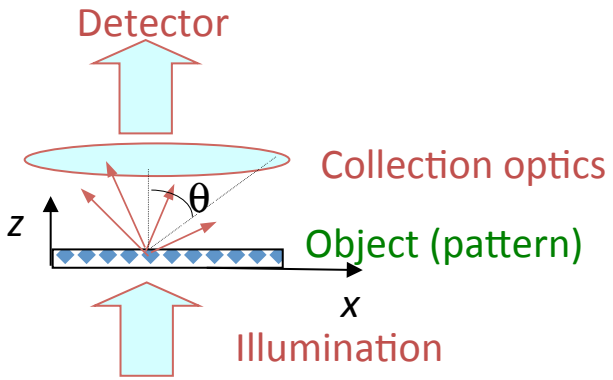
⇩

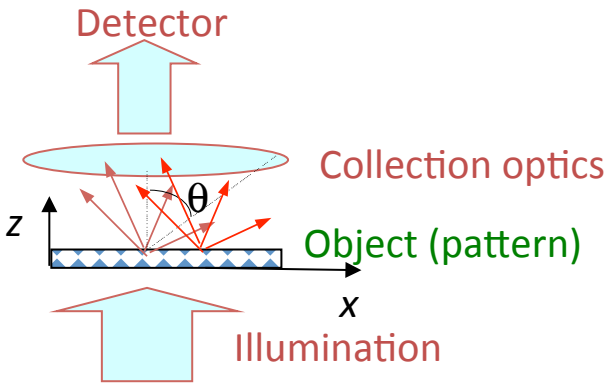
Large numerical aperture leads to sensitivity to "stray (unwanted) light"

⇩

Contrast falls down and high space resolution is hampered

e.g.: transmission microscopy



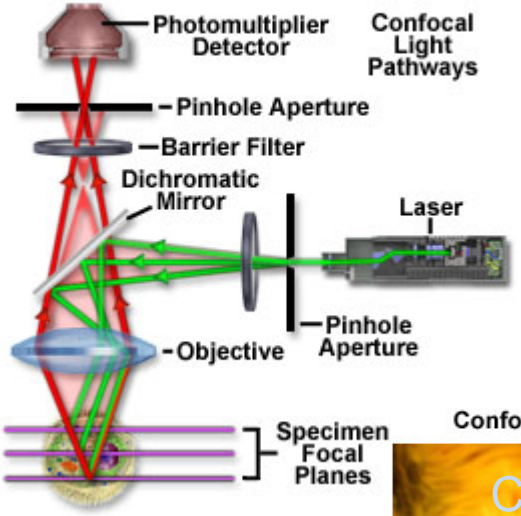


CONFOCAL MICROSCOPY I

Theory of Confocal Microscopy

Unsurpassed Optics OLYMPUS

Laser scanning confocal microscopy represents one of the most significant advances in optical microscopy ever developed, primarily because the technique enables visualization deep within both living and fixed cells and tissues and affords the ability to collect sharply defined optical sections from which three-dimensional renderings can be created. The principles and techniques of confocal microscopy are becoming increasingly available to individual researchers as new single-laboratory microscopes are introduced. Development of modern confocal microscopes has been accelerated by new advances in computer and storage technology, laser systems, detectors, interference filters, and fluorophores for highly specific targets.



Illumination is point-like thanks to the ability of focusing laser radiation
Collection is from the same point
--> Stray light prevented

Confocal and Widefield Fluorescence Microscopy

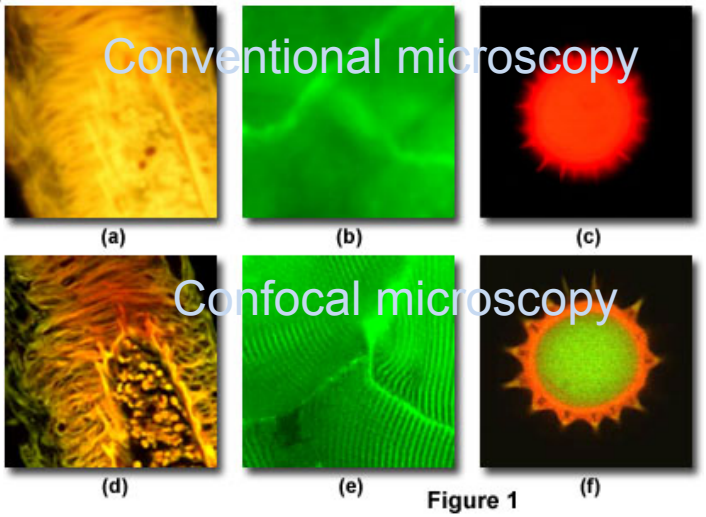
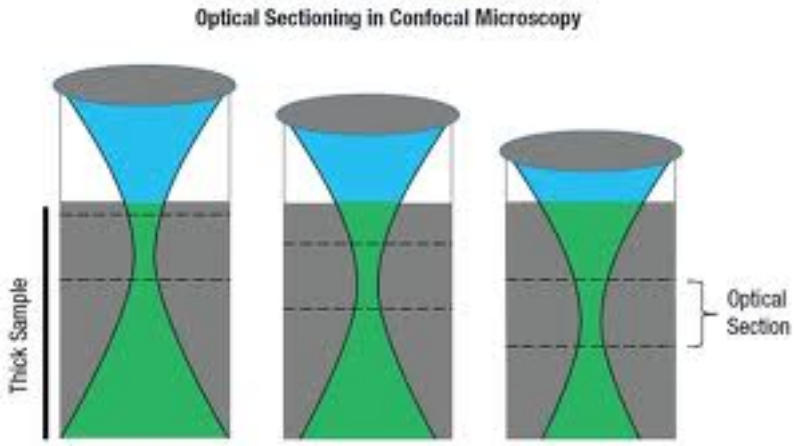


Figure 1

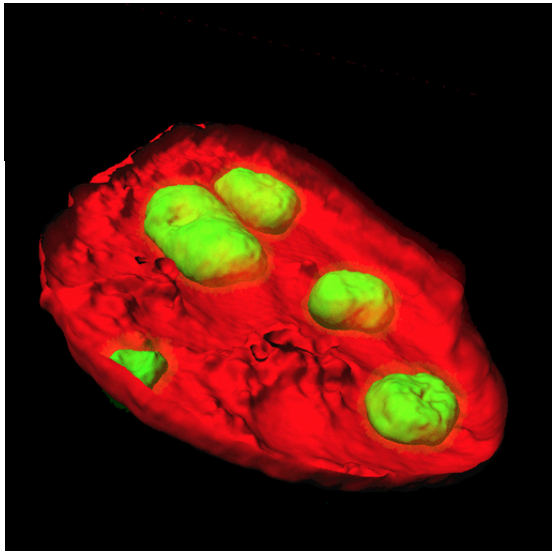
Presented in Figure 1 are a series of images that compare selected viewfields in traditional widefield and laser scanning confocal fluorescence microscopy. A thick section of fluorescently stained human medulla in widefield fluorescence exhibits a large amount of glare from fluorescent structures above and below the focal plane (Figure 1(a)). When imaged with a laser scanning confocal microscope (Figure 1(d)), the medulla thick section reveals a significant degree of structural detail. Likewise, widefield fluorescence imaging of whole rabbit muscle fibers stained with fluorescein produce blurred images (Figure 1(b)) lacking in detail, while the same specimen field (Figure 1(e)) reveals a highly striated topography in confocal microscopy. Autofluorescence in a sunflower pollen grain produces an indistinct outline of the basic external morphology (Figure 1(c)), but yields no indication of the internal structure. In contrast, a thin optical section of the same grain (Figure 1(f)) acquired with confocal techniques displays a dramatic difference between the particle core and the surrounding envelope.

CONFOCAL MICROSCOPY II

Besides increase of spatial resolution ***down to the diffraction limit (i.e., 200-300 nm!)***, the tight focusing of the illumination /collection enables sectioning (tomography-like) of the images to get 3D information



Confocal microscopy is widely used in biology to investigate stained (typically, with fluorescent dyes) living cells or biological tissues



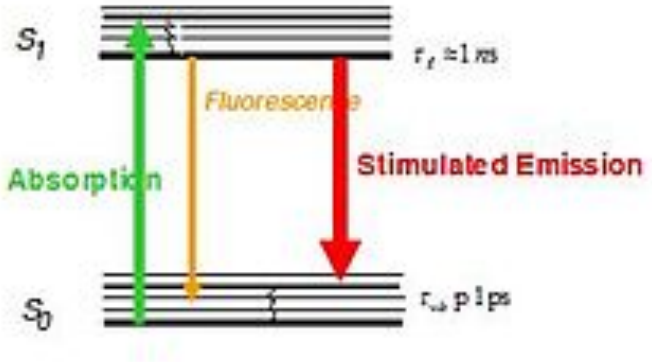
Variant (made possible by pulsed lasers): two-photon

The concept of two-photon excitation is based on the idea that two photons of comparably lower energy than needed for one photon excitation can also excite a fluorophore in one quantum event. Each photon carries approximately half the energy necessary to excite the molecule. An excitation results in the subsequent emission of a fluorescence photon, typically at a higher energy than either of the two excitatory photons. The probability of the near-simultaneous absorption of two photons is extremely low. Therefore a high flux of excitation photons is typically required, usually a femtosecond laser. The purpose of employing the two-photon effect is that the axial spread of the point-spread-function is substantially lower than for single-photon excitation. As a result, the resolution along the z dimension is improved, allowing for thin optical sections to be cut. Two-photon microscopes are less damaging to the sample than a single-photon confocal microscope.

STED MICROSCOPY I

Stimulated emission depletion (STED) microscopy is a process that provides super resolution by selectively deactivating fluorophores to enhance the imaging in that area.[1] It was developed by Stefan W. Hell in 1994, and was first experimentally shown in 1999. This is one of several types of super resolution microscopy techniques that have recently been developed. Super resolution microscopy is a set of techniques to bypass the diffraction limit of microscopy to achieve better resolution. Both Photoactivated Localization Microscopy (PALM) and Stochastic Optical Reconstruction Microscopy (STORM) are also super resolution microscopy techniques, although they use a different process than STED to achieve this resolution.

Because STED selectively deactivates the fluorescence, it can achieve resolution better than the traditional confocal microscopy. Normal fluorescence occurs by exciting an electron from the ground state into an excited electronic state which, after relaxing back to the ground state, emits a photon. STED interrupts this process before the photon is released. The excited electron is forced to relax into a higher vibration state than the fluorescence transition would enter, causing the photon to be released to be red shifted as shown in the image below.[2] Because the electron is going to a higher vibrational state, the energy difference of the two states is lower than the normal fluorescence difference. This lowering of energy raises the wavelength, and causes the photon to be shifted farther into the red end of the spectrum. This shift differentiates the two types of photons, and allows the stimulated photon to be ignored.



STED MICROSCOPY II

In the STED the fluorescence is “killed” (depleted) by exciting the molecule *before spontaneous emission occurs*

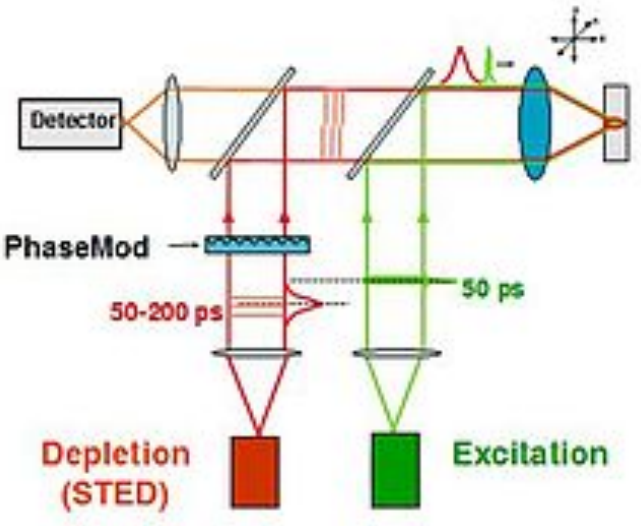
→ *Short-pulsed tunable (narrow band) lasers are needed, typically Ti:Sa*

→ *High laser intensity is needed (but not desired…)*

To force this alternative emission to occur, an incident photon must strike the fluorophore. This need to be struck by an incident photon has two implications for STED. First, the number of incident photons directly impacts the efficiency of this emission, and, secondly, with sufficiently large numbers of photons fluorescence can be completely suppressed.[3] To achieve the large number of incident photons needed to suppress fluorescence, the laser used to generate the photons must be of a high intensity. Unfortunately, this high intensity laser can lead to the issue of photobleaching the fluorophore. Photobleaching is the name for the destruction of fluorophores by high intensity light.

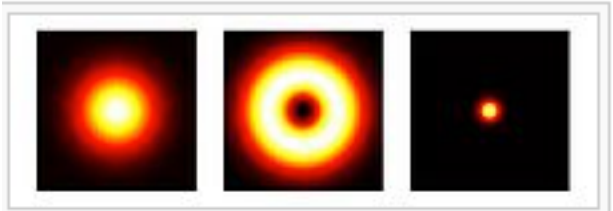
Sub-diffraction spatial resolution is obtained by properly **shaping** the depleting laser beam in order to “kill” the fluorescence in a small region only

Careful optical engineering required!



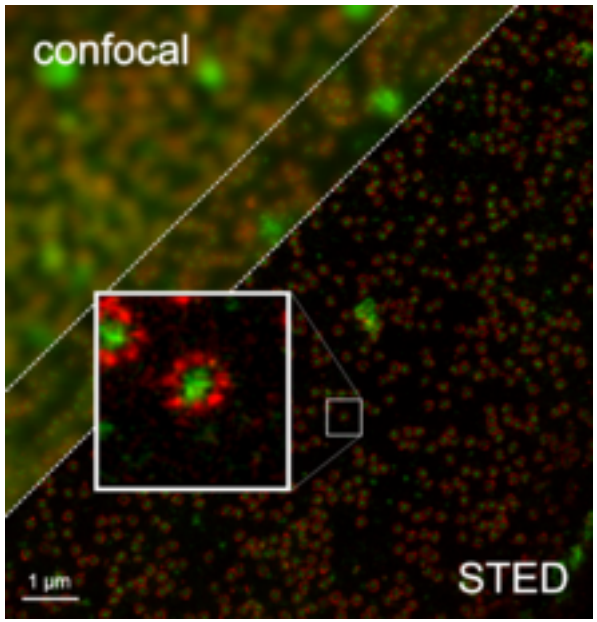
STED MICROSCOPY III

STED functions by depleting specific regions of the sample while leaving a center focal spot active to emit fluorescence. This focal area can be engineered by altering the properties of the pupil plane of the objective lens.[4][5][6] The most common early example of this diffractive optical elements, or DOEs, is a doughnut shape used in two dimensional lateral confinement shown below. The red zone is depleted, while the green spot is left active. This DOE is generated by a circular polarization of the depletion laser, combined with a helical phase ramp. The lateral resolution of this DOE is typically between 30 and 80 nm. However, values down to 2.4 nm have been reported.[7] Using different DOEs, axial resolution on the order of 100 nm have been demonstrated.[8] A modified Abbe's equation describes this sub diffraction resolution as: $D=\lambda/(2n\sin\alpha*\sqrt{(1+I/Isat)})$. To optimize the effectiveness of STED, the destructive interference in the center of the focal spot needs to be as close to perfect as possible. That imposes certain constraints on the optics that can be used.



Excitation spot (2D, left), doughnut-shape de-excitation spot (center) and remaining area allowing fluorescence (right).

Both excitation and depletion spots are separately diffraction-limited, but their "difference" is sub-diffraction sized



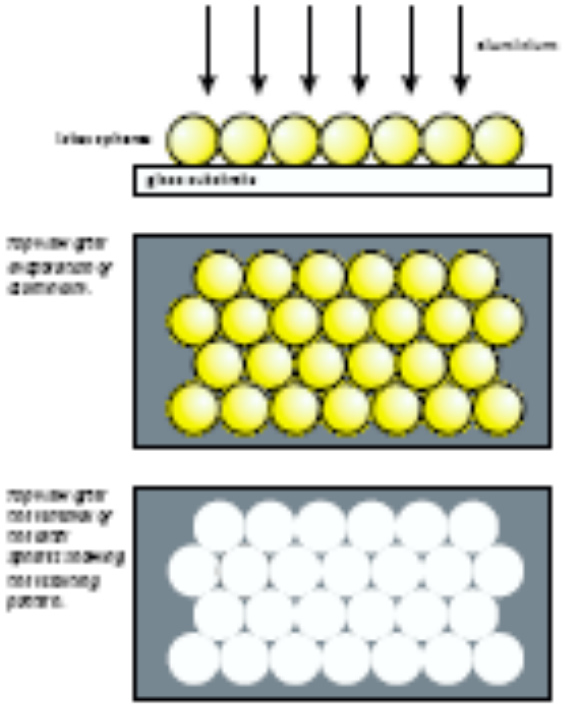
Note:
the STED works on dye molecules (with suitable energy levels!) hence cannot be applied thoroughly at any material! Promised major exploitation is in the biological area (stained materials)

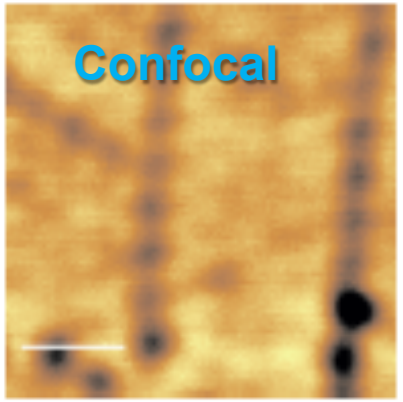
NEAR-FIELD AND ITS PROMISES

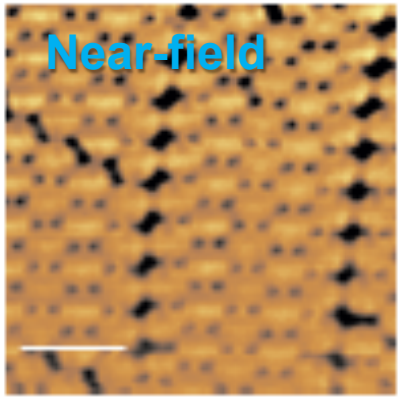
Comparison of optical resolution between Confocal Microscopy (CM, top image) and Scanning Near-field Optical Microscopy (SNOM, lower image). The scale bar is 1 micron.

The sample is a latex projection pattern. In the confocal image only the dislocation lines are visible, whereas the SNOM image also shows the Al islands clearly (same sample area).

Latex projection pattern are produced by evaporating aluminium onto a glass substrate covered with latex spheres. These latex spheres have a very uniform diameter. After the evaporation process, the latex spheres are removed.

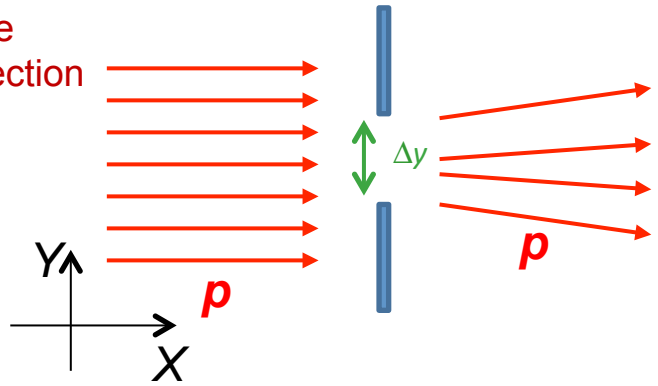






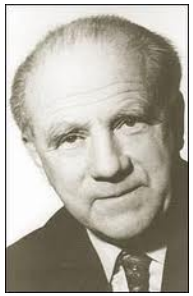
DIFFRACTION FROM ANOTHER POINT OF VIEW

Photons in a perfectly collimated plane wave have an impulse along the *X*-direction



When passing through the slit, photons are "localized" along *Y*, hence Δy gets a finite value

The impulse gets a non-zero component along *Y* in order for the uncertainty principle (Heisenberg) to hold valid



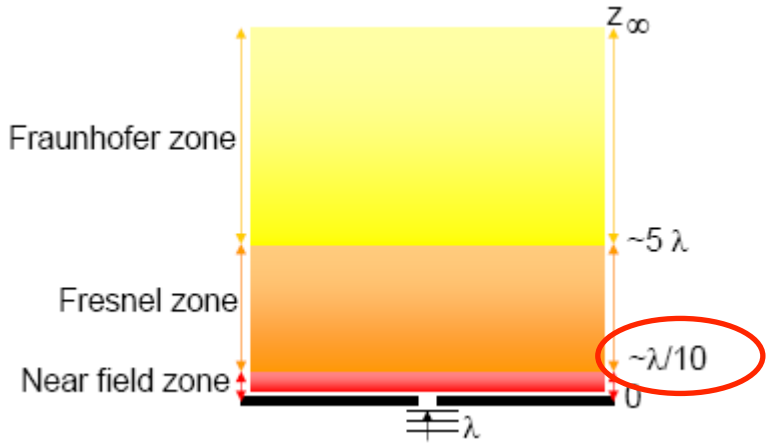
$$\Delta y \Delta p_Y \geq \frac{\hbar}{2}$$

$$\vec{p} = \hbar \vec{k}$$

Diffraction can be seen also in a particle-like picture

Diffraction means acquisition of ***in-plane components*** of the wavevectors

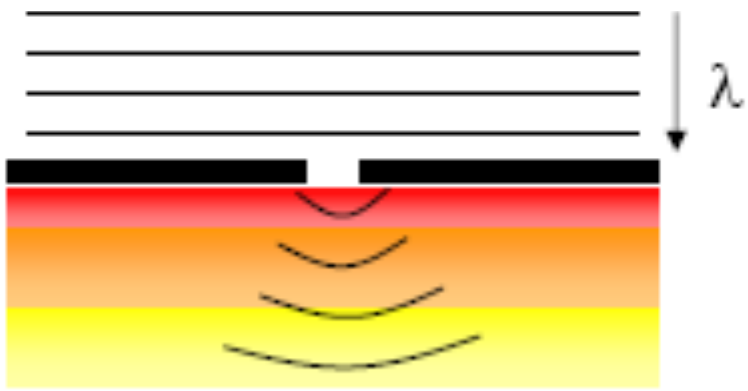
THE BASIC IDEA OF NEAR-FIELD MICROSCOPY



Basic idea:

- Stay (excite/observe) ***extremely* close** to the surface
- Use non-propagating e.m. fields extinguishing in ***extremely small*** scales

Extremely means (practically) (well) **below λ /10**



Near-fields
(e.g., produced by very small apertures)

HISTORY OF THE IDEA

1928/1932

E.H. Synge proposes the idea of using a small aperture to image a surface with sub-wavelength resolution using optical light. For the small opening, he suggests using either a pinhole in a metal plate or a quartz cone that is coated with a metal except for at the tip. He discusses his theories with A. Einstein, who helps him develop his ideas. [E.H. Synge, "A suggested method for extending the microscopic resolution into the ultramicroscopic region" Phil. Mag. 6, 356 (1928); E.H. Synge, "An application of piezoelectricity to microscopy", Phil. Mag., 13, 297 (1932)].

Basic idea (Synge) from diffraction theory

1956

J.A. O'Keefe, a mathematician, proposes the concept of Near-Field Microscopy without knowing about Synge's earlier papers. However, he recognizes the practical difficulties of near field microscopy and writes the following about his proposal: "The realization of this proposal is rather remote, because of the difficulty providing for relative motion between the pinhole and the object, when the object must be brought so close to the pinhole." [J.A. O'Keefe, "Resolving power of visible light", J. of the Opt. Soc. of America, 46, 359 (1956)].

In the same year, Baez performs an experiment that acoustically demonstrates the principle of near field imaging. At a frequency of 2.4 kHz (ë = 14 cm), he shows that an object (his finger) smaller than the wavelength of the sound can be resolved.

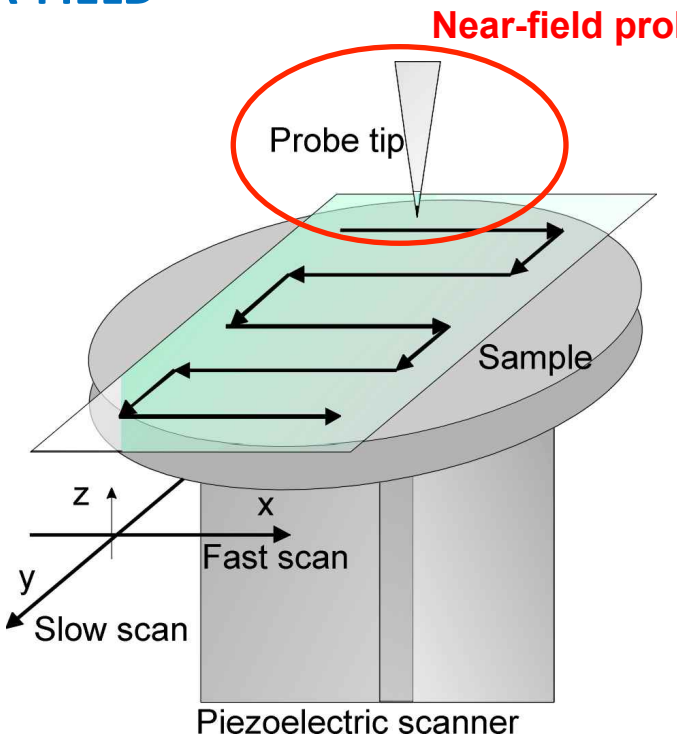
Confirmation through theoretical modelling

1972

E.A. Ash and G. Nichols demonstrate ë / 60 resolution in a scanning near field microwave microscope using 3 cm radiation. [E.A. Ash and G. Nichols, "Super-resolution aperture scanning microscope", Nature 237, 510 (1972)].

Experimental demonstration ***in the MW***

SCANNING PROBE NEAR-FIELD

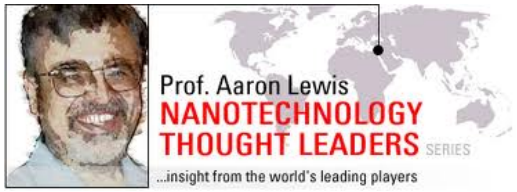


1984

The first papers on the application of NSOM appear. These papers are the first to show that NSOM is a practical possibiltity, spurring the growth of this new scientific field. [A. Lewis, M. Isaacson, A. Harootunian and A. Murray, Ultramicroscopy 13, 227 (1984); D.W. Pohl, W. Denk and M. Lanz, APL 44, 651 (1984)].

Optical near-field microscopy made possible thanks to advancements of SPM technologies

Dr. Dieter Pohl, Zurich, Switzerland, proved that it is possible to build a light microscope which uses no lenses, but which transports light to the specimen through a fine probe. In this way, the resolution limit of the microscope which was considered as insurmountable for more than 100 years has been lowered by at least one order of magnitude: These near-field microscopes today operate with a typical resolution of 100 nm, with 10 nm already being possible and even 1 nm within reach.



HOW TO GET RID OF HEISENBERG

Propagating waves:
k_i are real and $|k_Y| \le k = 2\pi/\lambda$

$\Downarrow$

Heisenberg's principle: $k_Y \ge 2\pi / \Delta y$

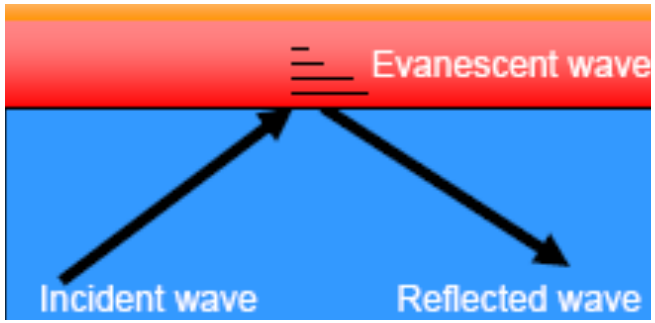
$\Delta y \ge ..\lambda$
(actual parameters give the Abbe's limit)

… but …

In **non-propagating (e.g., evanescent)** waves:
k_i can be imaginary, and, e.g.: $|k_Y| \ge k = 2\pi/\lambda$

The Heisenberg's principle is no longer ruling the ultimate resolution!

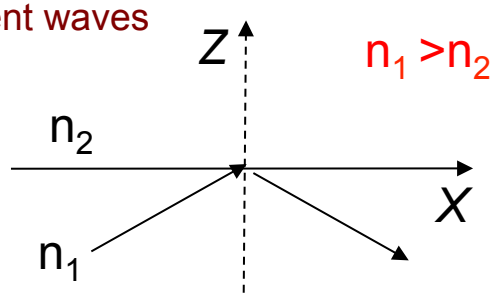
Example of waves with imaginary components of the wavevector:



Evanescent waves (e.g., by TIR)

REMINDERS OF EVANESCENT WAVES

A vivid example of non-propagating waves are the so-called evanescent waves



From Maxwell's equations, we know that the component of the electric field tangent to the interface of two dielectrics must be continuous across that interface. For a plane wave moving from one dielectric to another,

$$k_i \, Sin[\theta_i] = k_t \, Sin[\theta_t]$$

where k is the wave vector, theta is the angle between the wave propagation and the interface normal, and subscripts i and t stand for incident and transmitted wave fronts, respectively.

The frequency of the wave is identical on either side of the interface, so we have Snell's law:

$$n_i \, Sin[\theta_i] = n_t \, Sin[\theta_t]$$

with n the index of refraction of each of the media.

At incoming angles equal to and above the critical angle

$$\theta_c = ArcSin\left[\frac{n_t}{n_i}\right]$$

The reflectance is 1, the transmittance is 0, and all energy is reflected back to the incoming side of the interface. However, as electric fields impinge on our uncharged dielectric interface, the boundary conditions due to Maxwell's equations and the conservation of momentum demand that there be a matching field on the far side. The component of k_t parallel to the interface is still equal to the component of k_i to obey the boundary conditions.

We have an incoming plane wave

$$E_i[x, t] = \mathcal{E}_i \, e^{(i\,(k_{ix}\,x + k_{iz}\,z - \omega\,t))}$$

With amplitude $\mathcal{E}i$, wave vector k and frequency w. The z direction is normal to the plan of interface of two dielectrics, and the x direction is chosen so that the wave vector lies entirely in the xz plane.

Tom Hunt http://www.physics.harvard.edu/~tomhunt/pubs/evanescent.pdf

$E_t[\mathbf{r},t] = E_{0t}\exp(i(\mathbf{k}_t\cdot\mathbf{r}-\omega t))$

$\mathbf{k}_t\cdot\mathbf{r}=k_{tx}x + k_{tz}z$

$k_{tx}=k_t\sin\theta_t$; $k_{tz}=k_t\cos\theta_t$

With Snell's law:

$k_t\sin\theta_t = n_1 k_t \sin\theta_i/n_2$

$k_t\cos\theta_t = \pm k_t\,(1-n_1^2\sin^2\theta_i/n_2^2)^{1/2}$

$= \pm i\, k_t\,(n_1^2\sin^2\theta_i/n_2^2-1)^{1/2} = \pm i\,\beta$

Hence:

$E_t[\mathbf{r},t] = E_{0t}\exp(-\beta z)\exp(i(k_x x-\omega t))$

- The k_z component of the wave-vector is imaginary
- The field intensity along *Z* is exponentially decaying
- **The field is non-propagating**

HOW TO EXPLAIN RESOLUTION ACCORDING TO FOURIER

Fourier optics:
every field diffracted by an aperture (or an object) described through Fourier superposition of plane waves

$$E(x,y,z) = \iint \tilde{E}(u,v,z)\exp[i2\pi(ux+vy)]dudv$$

u, v: *spatial frequencies*

Fourier components:

$$\tilde{E}(u,v,z) = Ao(u,v)\exp[ik_z z]$$
$$= Ao(u,v)\exp\left[i\frac{2\pi}{\lambda}z\sqrt{1-\lambda^2u^2-\lambda^2v^2}\right]$$

$k^2 = (k_z^2+u^2+v^2) = (2\pi/\lambda)^2$

Hole (or object) size > λ

- low spatial frequencies
- root > 0
- k_z real
- progressive wave along *z*

$$\tilde{E}(u,v,z) = Ao(u,v)\exp[-jk_z z]$$

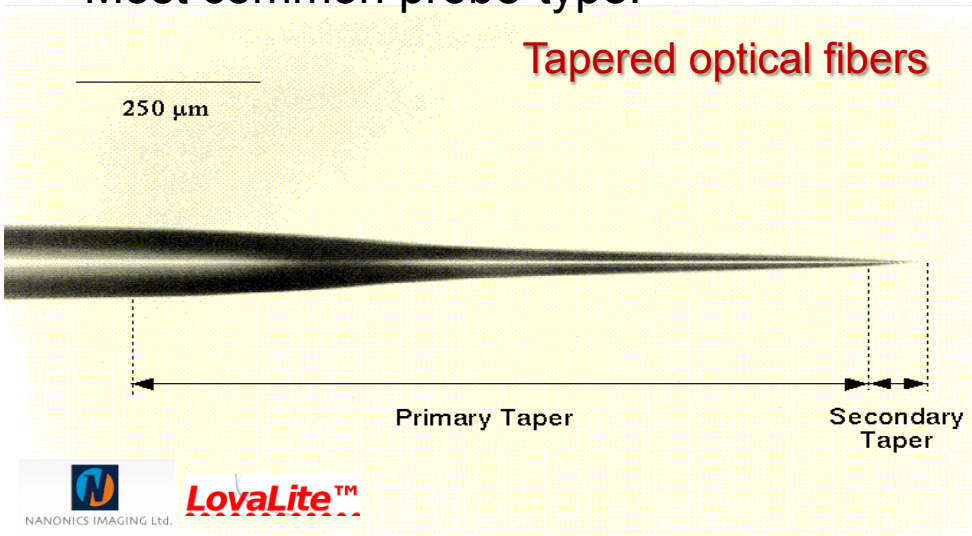
Hole (or object) size < λ

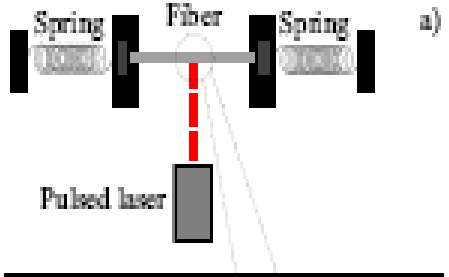
- high spatial frequencies
- root < 0
- k_z imaginary
- evanescent wave along *z*

$$\tilde{E}(u,v,z) = Ao(u,v)\exp[-\alpha z]$$

HOW TO PRACTICALLY REALIZE NEAR-FIELD EMITTERS

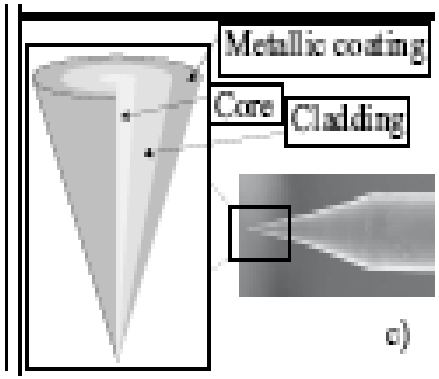
Most common probe-type:



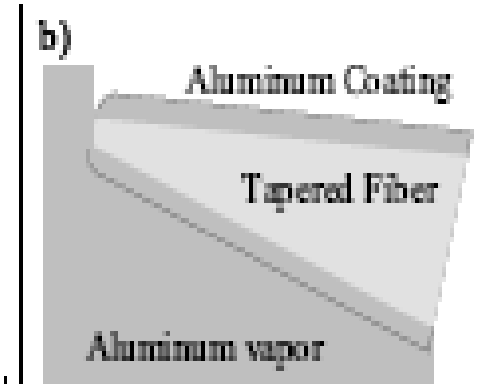


1) An optical fiber is heated and pulled

2) A tapered end is achieved



4) An "aperture" is left in the coating layer at the taper apex

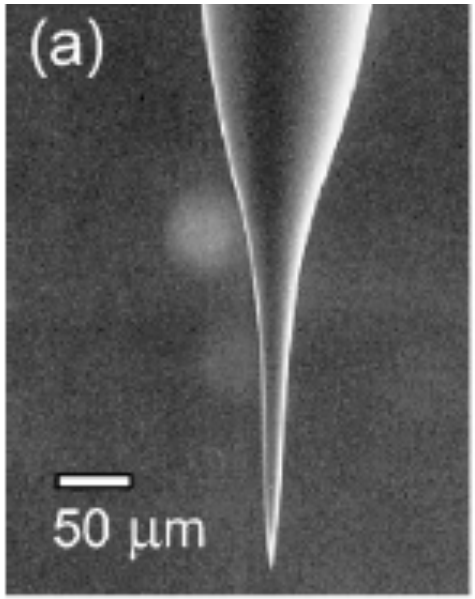


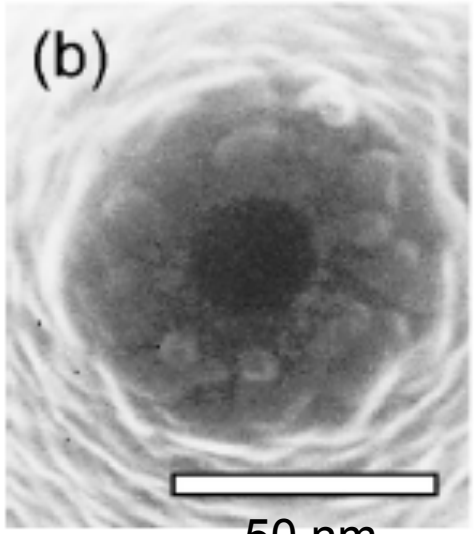
3) The taper cone is metal coated

Typically, single-mode optical fibers are used

Typically, 50-100 nm aperture size are obtained

OPTICAL FIBER NEAR-FIELD PROBES





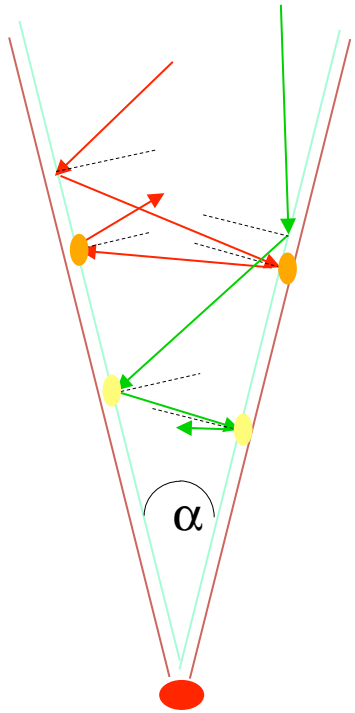
Advantages:

- great flexibility
- easy to use
- relatively inexpensive
- available in different fashions
- relatively small aperture size (50 nm, typ)

Disadvantages:

- small throughput (~10^{-4} -10^{-6})
- limitations in the power
- wear

Multiple reflections at the (metal-coated) walls leads to back-reflection (and damages due to absorption of the laser power)



$$\theta_{i,n} = \theta_{i,n-1} - \alpha$$

Use of lasers is mandatory in order to properly and efficiently couple radiation into the probe!

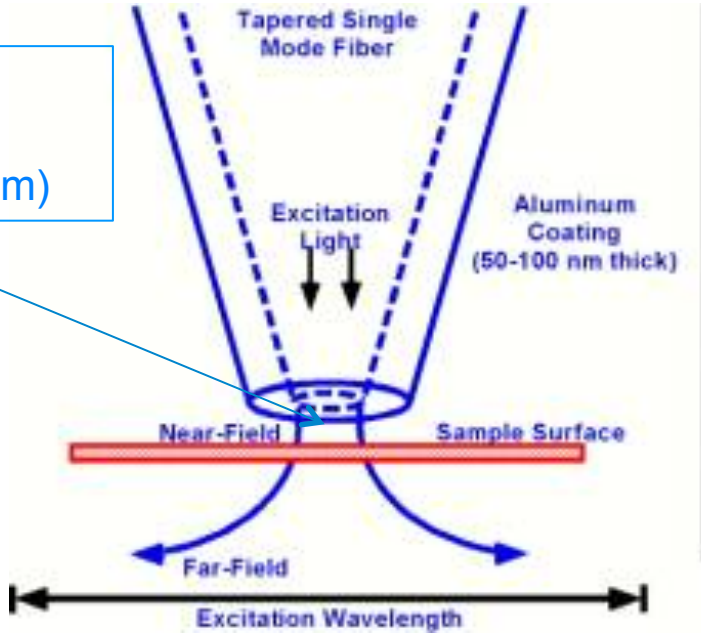
THE NEED FOR APPROACH

The evanescent near-field extinguishes in a very short range

$$I(0,0,z) \cong \left(\frac{8ka}{3\pi}\right)^2 e^{\frac{-3\pi z}{4a}}$$

(ideal case, after Bethe)

Typically, the spatial resolution is ruled by the aperture size (i.e., ~ 50 nm)



The tip must be kept very close (typ < 10 nm) to the surface for the sample to be concerned by the near-field

Constant-gap operation needed!!

THE SHEAR-FORCE METHOD

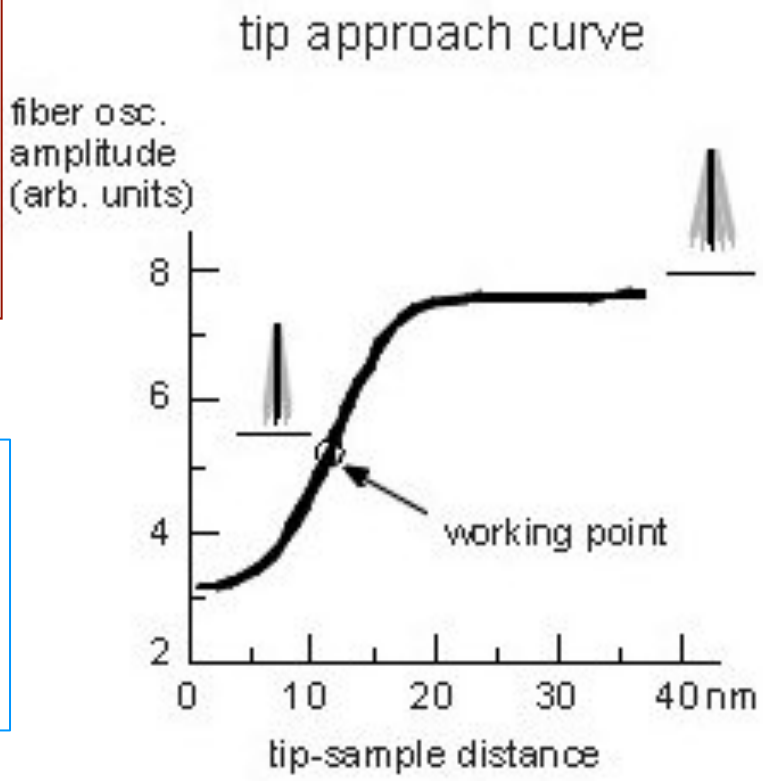
- Probe kept in oscillation parallel to the surface
- Oscillation amplitude monitored by a tuning-fork
- Shear-forces (mostly associated with friction of the air layers between tip and surface) efficiently damp the system oscillations at distances < 10 nm

Notes:

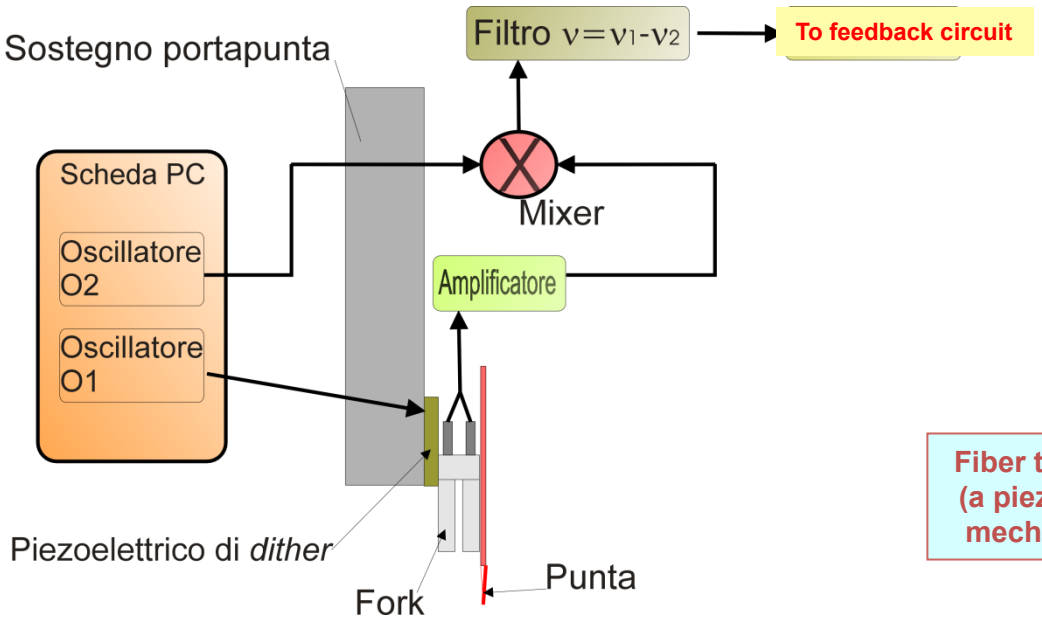
- Oscillation amplitude must be small (typ ~ 1 nm) to prevent resolution loss
- Oscillation at resonance frequency required

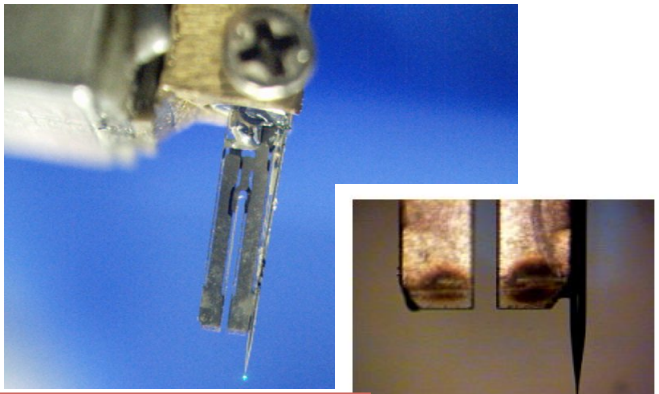
Feedback is applied, as in any SPM to keep constant the oscillation amplitude

The distance feedback provides a topography image simultaneously with every SNOM scan



A CUSTOM-MADE SOLUTION

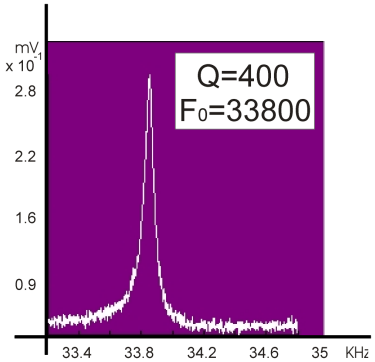




Fiber tip is glued onto a *tuning fork* (a piezoelectric device sensing the mechanical oscillation amplitude)

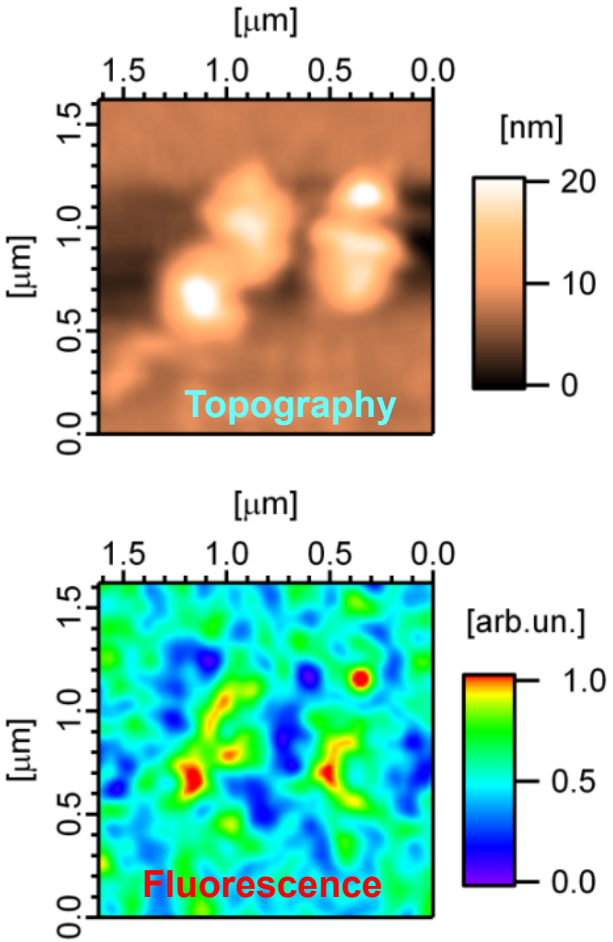
- A dithering piezoelectric transducer keeps the probe tip in oscillation along a direction **parallel** to the surface
- Oscillation amplitude is monitored by a *tuning fork*
- When the distance gets smaller (typ., below 10 nm), the oscillation is **damped** (and phase is changed) due to **shear-forces**
- Similar to AFM in tapping mode, but for the oscillation direction, the relevant distance and the involved mechanisms

Mech resonance spectrum (undamped oscillation)

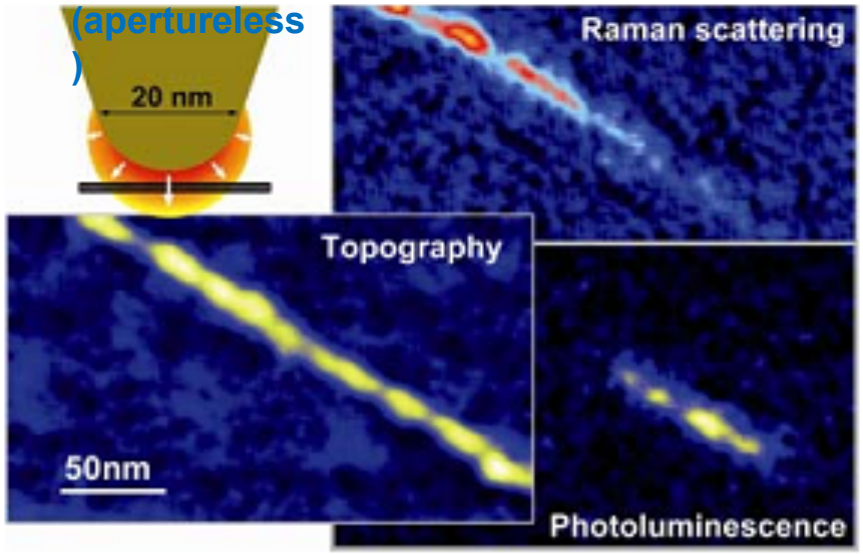


SNOM EXAMPLES I

Photoluminescence of NV_0 color centers in isolated diamond



Tip-enhanced (aperturele ss)



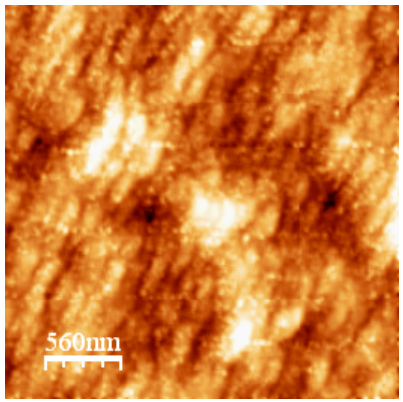
Photoluminescence and tip-enhanced Raman nanoscopy of individual single-wall Carbon Nanotubes

H. Harutyunyan *et al.*, Nano Lett. **9**, 2010 (2009)

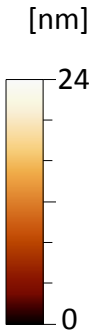
Single, isolated nanostructures, nanoparticles, or emitting molecules can be analyzed in spectral terms

SNOM EXAMPLES II

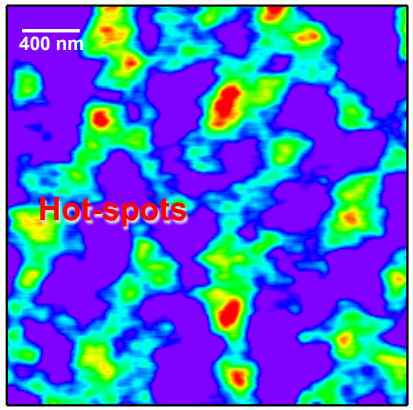
Topography



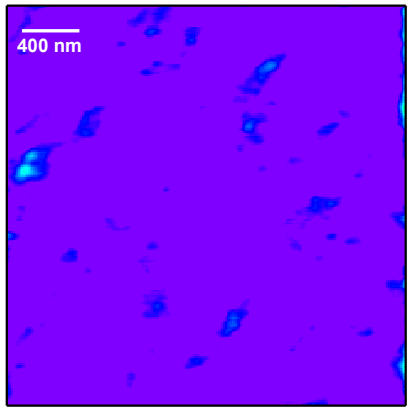
Gold nanowire sample

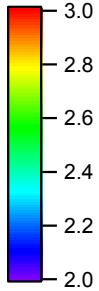


Map of the collected near-field



Resonant excitation 690nm

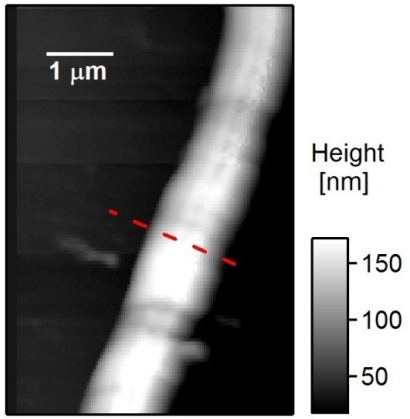




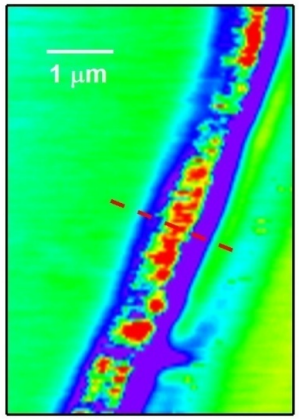
Non-resonant excitation 532nm

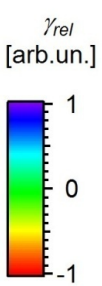
Maps of e.m. localization in a plasmonic substrate

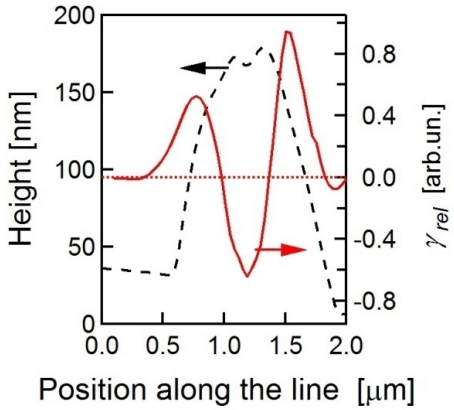
Topography



Map of material dichroism







Map of optical activity in a polymer electrospun nanofiber

SNOM extends the **measurement** ability of microscopy to sub-diffraction spatial resolution

CONCLUSIONS

- ✓Among a wide (and still growing) array of applications, the advent of lasers has enabled remarkable improvements in optical technologies such as interferometry (and related metrology) and microscopy
- ✓Optical profilometry, holograms, speckle interference methods have taken great advantage from lasers
- ✓Lasers are also the protagonist of a revival in interest for optical microscopy
- ✓Substantial progress in spatial resolution towards nanotechnology applications has been achieved in STED and SNOM, both made possible thanks to the availability and diffusion of laser sources

CREDITS

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R. Waser (ed.), *Nanoelectronics and information technology* (Wiley-VCH, 2003)

F. Fuso, Fisica delle Nanotecnologie, trasparenze lezioni 2007/08, e referenze citate

Other, quoted on the slides