



SCANNING NEAR-FIELD OPTICAL MICROSCOPY

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ABSTRACT

An average human eye can see details down to 0,07 mm in size. The ability to see smaller details of the matter is correlated with the development of the science and the comprehension of the nature. Today's science needs *eyes* for the nano-world. Examples are easily found in biology and medical sciences. There is a great need to determine shape, size, chemical composition, molecular structure and dynamic properties of nano-structures. To do this, microscopes with high spatial, spectral and temporal resolution are required. Scanning Near-field Optical Microscopy (SNOM) is a new step in the evolution of microscopy. The conventional, lens-based microscopes have their resolution limited by diffraction. SNOM is not subject to this limitation and can offer up to 70 times better resolution.

KEY WORDS: microscopy, optical microscopy, resolution, scanning

INTRODUCTION

The history of microscopy starts with the invention of lenses. Lens is the base element of a conventional microscope. The first written document describing more precisely optical properties of lenses was authored by R. Bacon in 1267. There were several attempts to use lenses in order to make a microscope. It is A. Leeuwenhoeck who is most often named as the inventor of microscope. In the 17th century he made a very simple instrument, based on a single glass lens, which enabled him to discover bacteria, sperm cells and blood cells. Leeuwenhoeck's skill at grinding lenses enabled him to build microscopes that magnified over 200 times. His contemporaries R. Hooke in England and J. Swammerdam in the Netherlands, started building microscopes using two or more lenses. These are very similar to microscopes in use today. Development of conventional microscopes is still underway today: perfection in the shape of lenses, sophisticated combinations of illumination and collection lenses, use of immersion objectives and confocal microscopes are just a few examples of this improvement.

The Resolution Limit of Lens-based Microscopes

The resolution of an optical microscope is defined as the shortest distance between two points in an image of a specimen that can still be distinguished as separate entities. For this purpose, the Rayleigh criterion is generally applied. This criterion states that two points are resolved in an image when the first minimum of the diffraction pattern of one point is aligned with the central maximum of the diffraction pattern of the other point. In the 1870's, German physicist Ernst Abbe worked for Karl Zeiss company. His work is an early example of commercially funded research and development. At that time microscopes were produced in an empirical way and their operation was not truly understood. The aim of Abbe was to establish serious scientific bases for the microscope construction. He demonstrated, closely followed by Lord Rayleigh, that, due to the diffraction, lens-based microscopes are limited in their lateral resolution to (1-4):

$$D = 0.61 \frac{\lambda}{n \sin \theta} \quad (1)$$

In this equation D is the shortest distance between two points on a specimen that can still be distinguished as separate entities, λ is the wavelength of the light producing the image, n is the refractive index of the medium separating the specimen and the objective lens, and θ

is the aperture half-angle of the optical system. In practice, this means that the resolution achievable with the best of the conventional microscopes is around 300 nm. This is a very frustrating limitation for the present-day science that tries to understand the nano-world. This limit, as well as the way to overcome it, can be understood using an analysis based on Fourier optics (5-8).

According to that analysis a plane wave \vec{E} from an object in a plane $U(x, y, 0)$ (see Figure 1) to a plain of forming picture $U(x, y, z)$ can be resolved on components of low and high spatial frequencies. For low spatial frequencies the waves propagate in the z -direction towards the observation plane. These components are the *far-field* components of the angular frequency spectrum. The high spatial frequency components are only present near the sample and decay exponentially in the z direction. The region near the sample containing the high spatial frequency components is called the *near-field* zone. In conventional optical microscopy, lenses with a limited numerical aperture ($NA = n \sin \theta$), are placed in the far-field. Consequently, only waves propagating with their k -vector within the NA will reach the detector, which means that they must fulfill the following condition:

$$\sin(\vec{z}, \vec{k}) < NA. \quad (2)$$

This means that only spatial frequencies which are smaller than NA/λ are detected, corresponding to lateral distances and in the plane $(x, y, 0)$ larger than λ/NA . As a result, the maximum achievable resolution at the image plane is limited to λ/NA ; this corresponds to the diffraction limit defined by Abbe (3). Rayleigh also took into account a minimum contrast needed for a human eye to distinguish two features in

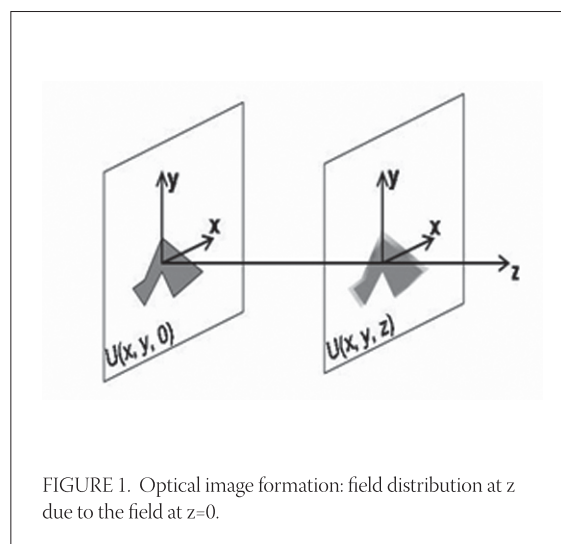


FIGURE 1. Optical image formation: field distribution at z due to the field at $z=0$.

an image. He defined the resolution limit as the distance between two points for which the intensity maximum of the diffraction pattern of the first point coincides with the first minimum in intensity of the diffraction pattern of the second point. The Rayleigh criterion separation distance corresponds to a contrast value of 26,4%. This led to the generally quoted resolution limit of lens based microscopes given by the Equation 1. Waves containing the high spatial frequency information of the object do not propagate but decay exponentially with the distance from the object. SNOM allows detection of these non-propagating evanescent waves in the near-field zone, thus giving the high spatial frequency information about the object. To do this, a probe is brought into the near-field zone, close to the sample, to either detect the near-field light directly, or to convert the evanescent waves into propagating waves and detect these in the far-field. The probe is either a nanometer-size scatter source or a waveguide with sub-wavelength size aperture.

New Microscopy Concepts and Techniques

Several new microscopy techniques were developed in the last century to overcome the resolution limit of the conventional microscopes. The most successful ones are electron microscopy (Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM)) and Scanning Probe Microscopy (SPM) family, which includes SNOM, Scanning Tunneling Microscope (STM) and Atomic Force Microscope (AFM). There is an important conceptual difference between the electron microscopy and the SPM: Electron microscopes work in a manner very similar to that of conventional microscopes. In electron microscopes electrons play the same role photons play in conventional microscopy. They are focused and/or collected by magnetic or electrostatic lenses. Magnetic and electrostatic lenses have the same focusing effect with electrons as the glass lenses have with photons. Since they use lenses, electron microscopes are also subject to diffraction limit: Their resolution is defined by the same equation (Equation 1) as the resolution of conventional microscopes. The high resolution they achieve is due to short relativistic wavelengths of electrons. SPM represents a real conceptual revolution in the way of observing an object: Instead of putting a detector far from the sample and using the propagation of the physical quantity (photons, electrons, ions, waves) to transfer information from the sample to the detector, the detector is set very close to the sample, in a so called near-

field zone. Here the notion of the propagation becomes meaningless. Conventional microscopes use lenses to form magnified images of samples. SPM instruments do not use lenses. SPM is based on a very sharp probe that scans the sample, collecting signal point per point. The signal from each of these points becomes one pixel of the final image. There are three principal members of the SPM family: STM, AFM and SNOM.

SNOM versus Other Microscopy Techniques

There is a great need to determine shape, size, chemical composition, molecular structure and dynamic properties of nano-structures. It requires microscopes with high spatial, spectral and temporal resolution. There are several microscopy techniques that achieved technological maturity, are commercially available, widely used and well understood in larger scientific community. Conventional lens-based microscopy, SEM, TEM, AFM and STM are the best examples. The classical, lens-based optical microscope has a very good spectral and temporal resolution, but its spatial resolution is limited to more than the half of the illumination wavelength, i.e. from 0,3 to 0,5 micrometers for visible light. Electron microscopes, STM and AFM easily achieve 10 nm spatial resolution and beyond, but they are poor performers with respect to spectral and dynamic properties. Moreover, SEM, TEM and STM have to be operated in vacuum and often require a heavy sample preparation, which limits their application in life sciences. Most importantly, SNOM is an optical microscopy technique. None of the other microscopy techniques is capable of gathering optical information: AFM, for example, allows sensing the shape of the sample, not seeing it. SNOM gives images with resolution well beyond the diffraction limit. It makes possible spectroscopic studies with resolution reaching well into the sub-100 nm regime. It is a totally non destructive, relatively inexpensive technique, which does not require any particular sample preparation. SNOM offers a considerable advantage over other microscopy techniques because it is an *optical* technique with a resolution of a scanning probe microscopy. "Optical" means that SNOM can exploit the same contrast mechanisms that are used by our eye. It also allows the use of spectroscopic methods. All the imaging techniques that were extensively developed for conventional microscopy studies, such as fluorescence labeling, can also be used with SNOM. SNOM is no longer an exotic method: It has matured into a valuable tool, ready to be applied to a large variety of problems in physics, chemistry and biology.

Historical Development of SNOM

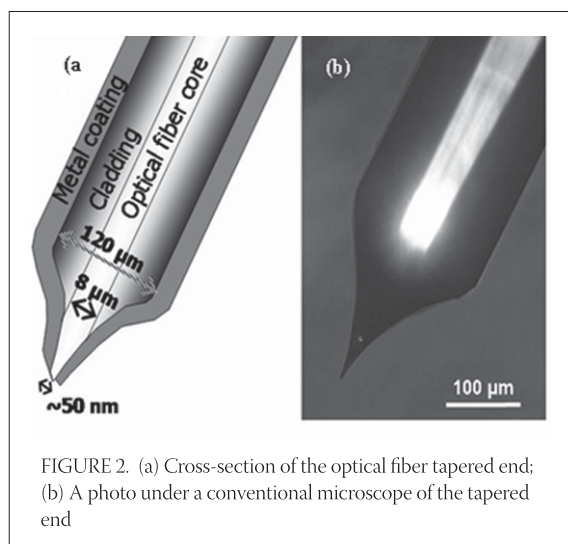
The first to propose a SNOM concept was an Irish scientist E. Syngé in 1928 (9). He described an experimental scheme that would allow optical resolution to extend into the nanometer regime without the use of lenses. He proposed to use a strong light source behind a thin, opaque, metal film with a 100 nm diameter hole in it as a very small light source. He imposed the condition that the aperture in the metal film be no further away from the sample than the aperture diameter, i.e., less than 100 nm. Images were to be recorded point by point, detecting the light transmitted through the sample by a sensitive photo-detector. Signal recorded at each point would be just one pixel of the final image. In 1932 he even proposed a way to move with great precision a small aperture in a metal plane in the last few nanometers from the surface using piezo-electric actuators (10). Syngé's proposition for moving the detector is the main method used today in all scanning probe microscopy instruments. Unfortunately, his work was completely forgotten, until uncovered by D. McMullan in 1990s (11), well after the first SNOM instrument appeared. In 1956 J. O'Keefe described an experimental similar approach. In a short letter he presented a general idea on how to extend the optical microscopy resolution (12). He did not propose any practical solutions on how to make such an instrument. In this sense Syngé's work is much more advanced. Yet, since Syngé's ideas were forgotten O'Keefe was often cited as the inventor of the SNOM concept. The first experimental demonstration of the validity of the SNOM concept had to wait until the seventies. In 1972 Ash and Nichols described a microwave experiment that yielded scans widely cited as the first demonstration of near-field sub-wavelength imaging (13). They imaged 0,5 mm wide lines of aluminum deposited on glass slides using electromagnetic radiation with a 3 cm wavelength. They achieved the resolution of $\lambda/60$ using microwaves and thus demonstrated that the SNOM concept is valid. Another important step in the development of a practical SNOM instrument was the invention of a scanning tunneling microscope (STM) in 1981. by two groups independently: D. W. Pohl and his colleagues in IBM labs in Zurich, Switzerland (14) and A. Lewis and co-workers at Cornell University (15). Pohl and Lewis teams made the first SNOM instruments working with visible light. This established a straight-forward link between SNOM and conventional microscopy. The light waves containing the high spatial frequency information do not propagate and their intensity de-

cays exponentially with the distance from the sample surface. These waves remain in the near-field are called non-propagating or evanescent waves. There are two methods that allow detecting these waves, which resulted in two distinct SNOM instrument designs: apertureless SNOM and aperture SNOM. In apertureless SNOM (16, 17), the probe is a nanometer-size metal tip which is used as a scatter source: It is brought close to the sample (0-10nm from its surface) where it converts the evanescent waves into propagating waves by scattering. These propagating waves originating in the near-field are detected in the far-field. In aperture SNOM the probe is a waveguide with one end tapered and ending with a very small, sub-wavelength size aperture. The aperture is brought in the near-field zone (0-10 nm from the surface of the sample) where it collects the near-field light, and guides it through the waveguide to the detector. Resolution-wise, the best reported results were obtained with apertureless technique. Ideal apertureless SNOM uses an atomically sharp metallic probe (a probe tapered so sharply that its tip ends with a single atom), very similar to the STM or AFM probe. The probe scans the sample. During the scanning, the distance between the probe tip and the sample surface has a constant, very small (0-10 nm) value. The sample is illuminated from the far-field, for example by focusing a laser spot on the scanned zone around the probe's tip. The metallic probe further enhances the illumination field in an antenna-like manner or by surface plasmon field effects, and thus concentrates and amplifies the field at its tip. The enhanced field interacts with the sample and is scattered from the near-field zone by the tip. The detector is positioned in the far-field. One of the main challenges in apertureless SNOM is to distinguish and take into account the light coming from under the tip only, since there is a relatively big surface of the sample around the tip which is also illuminated, giving rise to a strong background signal. One way to overcome this problem is to introduce a modulation of the tip/sample distance and to take into account only the signal that is modulated with the same frequency. Today, a typical resolution of an apertureless SNOM is somewhere between 5 and 30 nm, while the one of an aperture-SNOM is between 50 and 150 nm. The limited resolution of the aperture-SNOM is due to the fact that reducing the aperture diameter decreases the number of photons that can pass through the aperture. This in turn lowers the signal levels at the detector and decreases the signal-to-noise ratio, which imposes a conflicting limit on the resolution improvement that can

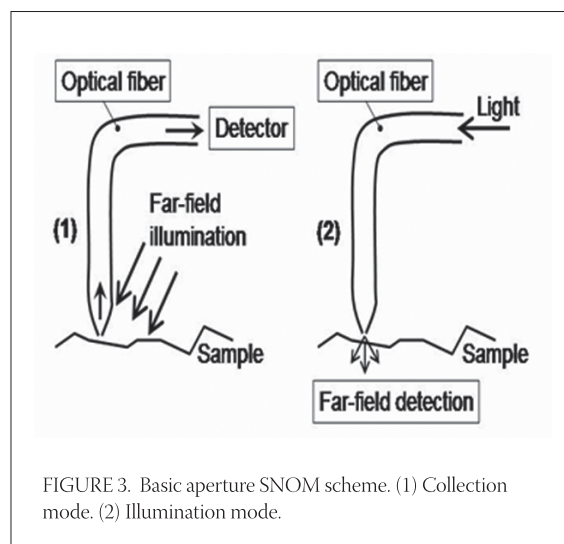
be achieved by further reduction of aperture diameter. Despite this, aperture-SNOM is presently the most widely used and developed near-field optical technique. All the commercial instruments currently available use aperture probes. One of the reasons for this lies in the fact that the aperture-SNOM was the first to be developed in the mid 80's and the first apertureless-SNOM experiments were presented later, in the mid 90's (16, 17). There is another important advantage of aperture setup. Aperture is a very confined light source without any background. This is in contrast to apertureless SNOM, where a rather large, intensive laser spot is focused on a metallic nanometer sized probe apex. In fluorescence SNOM applications this leads to photo-bleaching of the entire illuminated zone around the tip, even before it could be scanned. In fluorescence experiments a localized illumination is extremely important. Application of the SNOM technique to biological problems is essential and fluorescence labeling and imaging is presently one of the principal methods in bio-research.

Aperture SNOM Basics

In the first SNOM experiment in 1984, Pohl et al. (14) used a metal coated quartz tip which was pressed against a surface to create an aperture. To create an image, the probe was put in contact with the sample at each pixel, and retracted during the movement to the next pixel in order to avoid the tip damage. Since then, both the near-field probe design and the scanning process significantly improved. In 1991, Betzig et al. (18) introduced SNOM probe made from a single-mode optical fiber; one end of the fiber is tapered to a tip size of approximately 50 nm; the taper is coated with aluminum; a sub-wavelength aperture is created in the aluminum coating at the tip of the taper (Figure 2). To



keep the fiber within the near-field region of the sample a distance regulation mechanism was implemented: the shear-force control (19, 20); this mechanism enables scanning the aperture always at a constant distance of a few nanometers from the sample surface. The size of the aperture approximates the maximum resolution of the aperture SNOM. There are two methods that make possible a routine production of 50-100nm diameter apertures with optical fibers: chemical etching and melting-pulling method. The left side of the figure 2 shows a cross section of the fiber tip and indicates most common dimensions of its core, cladding and aperture. The right side of the figure is a photo of the tapered end of the optical fiber used in the infrared SNOM experiments. The aperture has to be brought into the near-field zone, very close to the sample, to collect the light waves containing the high spatial frequency sample information. Because of the exponential decay of the near-field waves the probe has to be kept at a constant distance from the sample. Otherwise, the intensity changes due to different probe-sample distances would shadow the desired contrast mechanisms (such as the absorption and reflection properties of the sample). The aperture is raster scanned over the surface of the sample, while it either illuminates, or collects the light from the sample surface (Figure 3). During the scanning, the fiber tip has to be maintained at a constant and a very small distance (of few nanometers) from the sample: In this way, the light is collected from only a very small surface of the sample which is under the aperture, and which has approximately the same size as the aperture. As the aperture is scanned over the sample, the light signal is recorded point per point, using a suitable photo-detector. The total light intensity recorded at a certain point during scanning corresponds to only one pixel of the final image.



There are three main SNOM operation modes, two of which are shown in the Figure 3: (1) Collection mode: The sample is illuminated from the far-field; this illumination gives rise to the evanescent light at the sample surface; the evanescent light is converted by the aperture into propagating waves inside the fiber, and conducted through the fiber to a suitable detector; (2) Illumination mode: Light is coupled into the fiber at its flat end, and conducted through it to the tapered end, where it is squeezed towards the aperture; the sub-wavelength aperture converts the propagating light into the evanescent field (21, 22); the evanescent field interacts with the fine structure of the sample; this interaction converts the evanescent field into a propagating light wave which is detected in the far-field. The third operation mode of an aperture SNOM is a combination of the two modes just mentioned; it is usually called "illumination-collection" mode: aperture is used both as the light source and as the collector; this operation mode requires a beam-splitter to separate the light directed to the sample from the light coming back from the sample and which is directed to the detector. In each of these modes we can perform measurements both in transmission and in reflection.

Scanning

The most important element of a SNOM instrument, besides the probe, is a scanning system. To get a nanometer resolution with SNOM one first must have a nanometer resolution scanner. SNOM, STM and AFM use the same scanners. These scanners are made of piezo-electric materials, most usually a PZT ceramic (Pb-Zr-Ti-O).

Piezo ceramics are characterized by the fact that their dimensions change as a function of voltage applied to them. Typically, several volts applied to a piezo ceramic scanner induce a few nanometers variation of its size. When the voltage is no longer applied, the ceramic goes back to its initial size. The size change is proportional to the value of the voltage applied. Thus, piezo ceramics are a perfect material for high precision actuators and scanners. Depending on the piezoelectric type and the scanner size and design, the largest area that the scanner can cover is usually several tens of microns. The scanner usually contains three piezo blocs, moving the SNOM tip in x, y and z direction. The most usual SNOM feedback mechanism controlling the tip-to-sample distance is based on the use of the so-called shear force (23). Shear force is a short range force and its intensity is significant only a few nanometers from the sample. The shear force feedback mechanism works in the following way: Resonant lateral (x-y plane) oscillation of the fiber tip is induced by applying an AC voltage to the dither piezo (Figure 4). The tapered end of the optical fiber is approached towards the sample. When the tip of the fiber is at a few nanometers from the sample, the amplitude of the oscillation starts to be damped by shear forces. The fiber tip is fixed to the bimorph piezo (Figure 4). Oscillation and bending of the fiber bends the bimorph piezo too. The bending of the piezo induces an AC voltage in it. The amplitude of the AC voltage is equivalent to the amplitude of the tip oscillation. Consequently, the amplitude of the oscillation of the tip can be monitored by monitoring the AC voltage from the bimorph

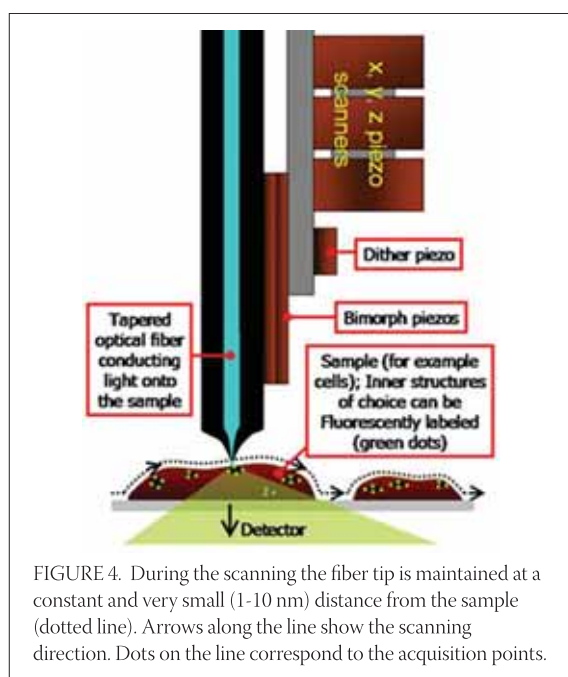


FIGURE 4. During the scanning the fiber tip is maintained at a constant and very small (1-10 nm) distance from the sample (dotted line). Arrows along the line show the scanning direction. Dots on the line correspond to the acquisition points.

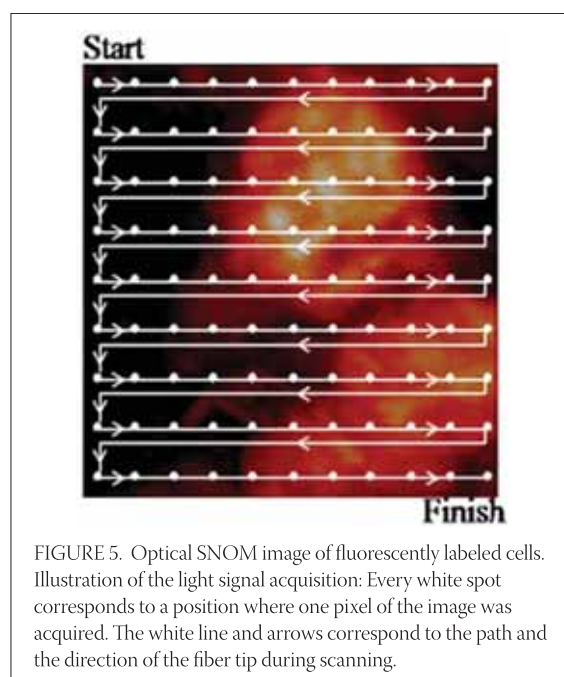


FIGURE 5. Optical SNOM image of fluorescently labeled cells. Illustration of the light signal acquisition: Every white spot corresponds to a position where one pixel of the image was acquired. The white line and arrows correspond to the path and the direction of the fiber tip during scanning.

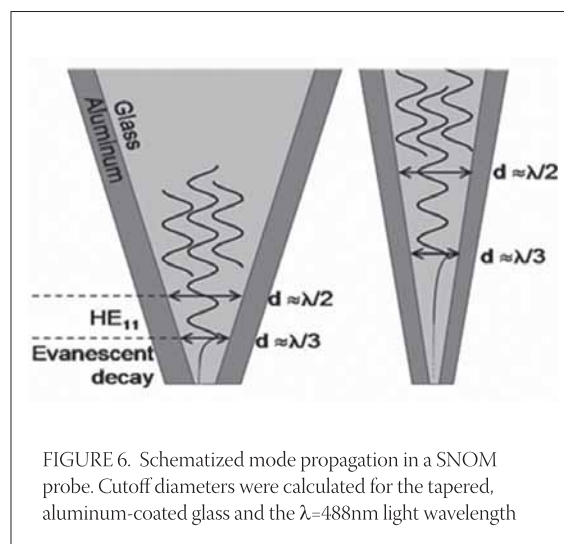
piezo. This voltage is fed into a feedback loop, which then moves the tip in the z direction (towards and from the sample) in order to have a constant damped amplitude of the oscillation during scanning. By maintaining constant the damped amplitude of the oscillation, we actually maintain constant the distance between the fiber tip and the sample. The aperture is maintained at a very small and constant distance from the sample surface during an entire scan. The fiber tip is being scanned laterally by x-y piezos, and the z piezo simultaneously moves it up and down in a way to maintain constant the distance between the aperture and the sample.

The origin of the shear force is a controversial subject: first it was hypothesized that it is due to Van der Waals forces; today, some experiments demonstrate that it is actually a viscous damping in a thin water layer confined between the tip and the sample (24) (water originating from the ambient humidity), and some indicate that the damping is caused by an intermittent contact (25) (where the tip actually touches the sample once per oscillation). In SNOM applications, the topographic resolution is usually higher than the optical one, and seems much more limited by the size of the fiber probe than by the shear-force scanning mechanism. In rare cases, when sample contains physically very different regions (for example if one part of the sample is hydrophobic and the other hydrophilic) one must take into account possible shear-force scanning artifacts. Since the evanescent field decreases exponentially away from the sample surface, very small variation of the aperture-sample distance induces strong change in the optical signal in SNOM. Thus, topographic artifacts induce also the optical ones. The Figure 5 shows a typical scanning path of a SNOM probe. The probe scans the sample line by line, point by point. It stops at an acquisition point (x-y movement), stabilizes its z position, the signal is recorded, and it moves to the next point. A user can choose several parameters: the size of the zone to scan, the density of the acquisition points (the distance between two acquisition point in a scan line), the scan velocity (the time that the fiber will take to move from one point to another) are just some of the most usual parameters that can be modified by a user. Shear-force scanning is a time consuming technique: ideally, the fiber tip should make hundreds of oscillations when moving from one point to the other in order to detect very small shifts in the oscillation amplitude caused by topographic variations of the sample, and adapt the tip position correspondingly. That is why a compromise must be made between the scan size, the density of the acquisition

points and the time for a single scan to be completed. If possible, one would choose a maximum scan size (hundreds of microns), with the biggest number of points (thousands). Unfortunately, this would take hours with shear-force high resolution scanning. Consequently, it is much more common to see 10 by 10 micron images with acquisition points every 25 to 50 nanometers.

Light Transmission through a Sub-wavelength Size Aperture

Typical light transmission of aperture SNOM probe is very limited: only 10^{-3} - 10^{-6} of the light power coupled into the fiber is emitted by the aperture. The rest of the light is either back-reflected or absorbed by the coating in the taper region. The light transmission is determined by two factors: one is the taper cone angle and the other is the size of the aperture. Influence of the taper angle on the light throughput can be extrapolated from calculations presented in (26): The efficiency of guiding light to the aperture is determined by the distribution of propagating modes in the tapered waveguide (in single mode fibers, when the guiding core gets thinner in the taper, the guided mode spreads); the mode structure in a metallic waveguide is a function of the core (glass) diameter; this model shows that one mode after the other runs into cutoff when the core diameter gradually decreases; when the diameter of the dielectric (core) becomes smaller than $\lambda/2$, only the HE_{11} mode still propagates (Figure 6); another cutoff occurs when diameter becomes smaller than $\lambda/3$. Above this limit the HE_{11} mode becomes an evanescent wave; this means that the mode field decays exponentially (26, 27); the amount of light that reaches the probe aperture depends on the distance separating the aperture and the $\lambda/3$ diameter section of the taper.



This distance is smaller in fibers tapered with larger cone angles than in those with smaller cone angles. The transmission coefficient of a sub-wavelength aperture in an infinitely thin perfectly conducting screen has been calculated rigorously in (21) and (22). This calculation predicts that the transmission coefficient is proportional to d_4/λ_4 , where d is the diameter of the aperture and λ the wavelength of the illumination light:

$$T \propto d_4/\lambda_4. \quad (3)$$

Transmission data were calculated for a realistic tip structure. For a full taper cone angle of about 30° and a 100 nm aperture diameter, a transmission around 10^{-5} is expected. For a taper angle of about 42° the transmission values are around 10^{-3} (28). These values are in reasonable agreement with experimental results (29). Working with really small apertures (< 40 nm diameter) is often impossible because the transmission coefficient decrease dramatically with decreasing aperture size, leading to a signal to noise ratio which is insufficient to get reliable results. This cannot be by-passed by increasing the input light power because of a low damage threshold of the coating (≈ 10 mW). Large taper cone angles improve greatly the transmission, but this kind of probes is suitable only for very flat samples (Figure 7). The field distribution at the SNOM aperture depends strongly on the ratio d/λ (8, 27). Roughly, when the diameter of the aperture becomes smaller than the wavelength of the incident light, one part of the field transmitted through the aperture still propagates into the far-field, but the rest of the transmitted field remains strongly bound to the aperture and exponentially decreases away from it. When the aperture is much smaller than the wavelength, the transmitted field is dominated by the evanescent components, and the far-field power emitted by the aperture strongly decreases.

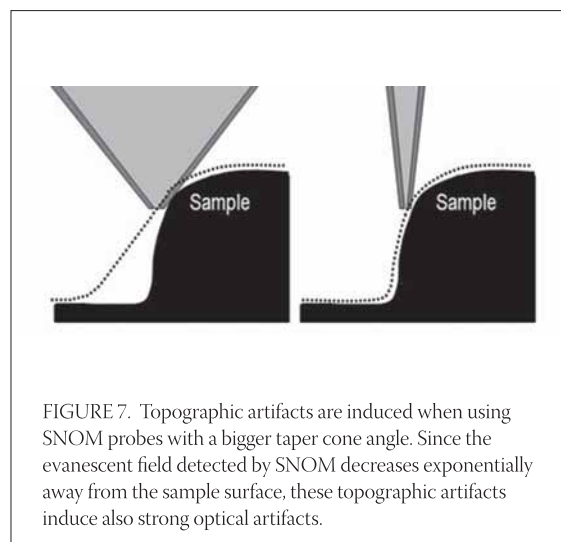


FIGURE 7. Topographic artifacts are induced when using SNOM probes with a bigger taper cone angle. Since the evanescent field detected by SNOM decreases exponentially away from the sample surface, these topographic artifacts induce also strong optical artifacts.

Consider an aperture in the x - y plane and a propagating field arriving at the aperture in the positive z direction. The evanescent field E_z on the exit side of the aperture obeys the following equation:

$$E_z = C e^{-\frac{z}{\lambda}}, \quad (4)$$

where C is a constant depending on the refractive index of the medium before (usually a glass taper) and after the aperture (8). In the common aperture SNOM experiments this means that the light is strongly localized at the aperture. This strong localization of the field at the aperture is responsible for the resolution that can be achieved with SNOM (30). SNOM is a surface selective technique: it is not the best instrument for studies of structures that are deeply imbedded below the sample surface. Experimental studies performed on highly transparent samples (cells) indicate that structures more than 150 nm below the sample surface have negligible contributions to the observed signal (31).

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