5.4.2 **SCANNING NEAR-FIELD OPTICAL MICROSCOPY**

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Scanning near-field optical microscopy (NSOM or SNOM) is a combination of scanning probe microscopy and optical microscopy. Over the last decade it has become possible for users of classical optics to go beyond the diffraction limit using so-called near-field optics. The strength lies in the potential to combine the best of both worlds. The increasing significance of nanometer-sized structures in electronics, material science, chemistry and biology has made the study of the near-field optical properties of nanostructures highly appropriate and stimulated the development of both instrumentation and theory.

**TYPES OF SCANNING NEAR-FIELD OPTICAL MICROSCOPES**

**Aperture type**

Far-field optical microscopy is based on the imaging of light waves traveling from the substrate through a far-away lens to a detector. However, light coming from a spot on the substrate with sub-wavelength dimensions cannot form a traveling wave. As a consequence, far-away lenses cannot be applied to image nanostructures illuminated with a beam of light. Instead near-field optics is based on some kind of nanometer-sized probe or source that interacts on a sub-wavelength scale with the local sample to be examined. In figure 5.4.4, the
principle of operation for near-field microscopy is explained for the example of an aperture-scanning near-field scanning optical microscope used in the collection mode. In this case the probe is formed by a tapered glass fiber with a nanometer-sized aperture. It collects the light from the underlying nanosize spot on the surface and directs it to a conventional optical detector. The image is constructed by scanning the probe over the surface. The spatial resolution of typically 20 to 100 nm is determined by the size of the probe and is basically independent of the applied wavelength. It may come as a surprise that the light waves—having wavelengths of hundreds of nanometers—can pass through the nanometer-sized tip at all. However, the situation is not much different from the following, more familiar situation. When you listen, you detect sound waves with a wavelength of several meters. Still, these waves have no trouble finding their way through your centimeter-scale ears!

The aperture type is the most widely used configuration. The first near-field optical microscope had a resolution of 20 nm using an aperture at the apex of a sharpened quartz rod. Today, fiber probes are fabricated in efficient and reproducible ways. Figure 5.4.5 shows a typical aperture probe. Of course most of the incoming light is lost. This probe transmits about 10 nanowatts (nW) of light when several milliwatts of laser light is coupled into the fiber. More laser light would burn the aluminum coating.

The probe is the heart of any near-field optical microscope. The probe interacts with the sample and transmits the modulated response to the detector. It therefore determines the contrast mechanism, the spatial resolution and the sensitivity. As such the importance of the probe is comparable to that of the objective lens in far-field optical microscopy. Ideally, the probe is of molecular dimensions. However, it has to be manipulated in a scan pattern over the surface and addressed to collect the near-field interaction information. This is why the probe is always a macroscopic tip ending in a nanometer-sized apex supporting the source or detector. Fabrication of an optical source or detector of nanometer size dimensions is a challenge in itself. Many types of probes have been fabricated. For instance, various experimental designs using luminescent particles or sub-micron-sized light detectors were tried but never fully developed.
The probes are used in various configurations. For instance, the aperture-type near-field scanning optical microscope can also be used in the transmission mode (figure 5.4.6). In this case the probe serves as a source instead of a detector. High-resolution imaging can only be expected while operating the probe at a distance of only a few nanometers from the surface. At this distance— a fraction of the wavelength of the light— the light is confined to the dimension of the aperture. At larger distances the spot size rapidly expands. For many applications it is important that the sample can be viewed with conventional high magnification optics for localization of a specific area of interest. This can be realized using an inverted conventional microscope configuration with sufficient mechanical stability on the nanometer scale (figure 5.4.7). The inverted configuration leaves sufficient space for the mounting of a near-field optical probe in the immediate vicinity of the sample surface.

The next paragraphs describe two other configurations encountered in practical near-field optical microscopy, namely the antenna (or ‘apertureless’) type and the photon-tunneling configuration.
Antenna or 'apertureless' type

The antenna-type probe is simply a passive nanometer-sized metallic tip. The tip is illuminated with a tightly focused laser beam with a submicron spot size. The local interaction with the sample surface is subsequently detected as a modulation in the scattered light (figure 5.4.8). Extreme sensitivity is required to observe this modulation in the presence of the light scattered from the sample (Zenhausern, 1995). Images with a resolution of a few nanometers were obtained in this way. However, this configuration is far from routine. It can only be operated on rather specific samples and requires strong illumination.

![Metallic tip, Fibre type, Micro-lever type](image)

Photon scanning tunneling microscopy

An alternative arrangement is photon scanning tunneling microscopy (PSTM, figure 5.4.9). The operation with uncoated probes makes photon scanning tunneling microscopy experimentally easier than the aperture type. Occasionally, a lateral resolution down to 20 nm is obtained (Moers, 1994a). However, this microscope is very sensitive to far-field scattering. This limits the method to very flat samples and severely limits the application to, for instance, biological samples.

Solid immersion lens (SIL)

Optical microscopy with a solid immersion lens (SIL) is a way to push the diffraction limit for imaging by working in near-field operation in the vertical direction. In contrast to other ('real') near-field methods, it works in far-field operation for the lateral directions, just like confocal microscopy. However, the solid-immersion-lens method is of considerable industrial importance because it allows for high-density recording (section 1.2). It pushes the current technology for optical recording by reducing the distance between the focusing lens and the storage medium below the wavelength of the light. Under these circumstances, the evanescent field overlap allows the lens to act like a magnifying glass lying on a newspaper. The addition of a solid immersion lens will reduce the spot size by a factor of \( n^2 \), with \( n \) the refractive index of the lens. One factor of \( n \) arises from the wavelength reduction inside the lens and one factor from the increase in the maximum angle of incidence due to refraction at the lens surface (Mamlin, 1995).
Using a glass lens with a refractive index of 1.9, this allows for a reduction of the spot diameter by a factor of 3.5, to about 125 nm. This means an increase in storage density by a factor of 10 compared to commercially available optical data storage media and tough competition for the current magnetic hard disks. The advantages of near-field data storage using a solid immersion lens are non-contact, high-density, high-speed (up to at least several MHz) operation. The main limitation is that the spot size is still diffraction-limited due to the far-field operation in the lateral direction, and cannot be reduced any further. Using near-field operation in all three directions could push the spot size beyond the diffraction limit down to 20 to 80 nm, which would add an extra factor of 10 to the optical data density, though with loss of light and an ultrashort working distance as complicating factors. Densities as high as 170 Gb/in$^2$ (60-nm bits) have already been reported [Hosaka, 1997].

**Shear force microscopy**

The use of a fiber probe in close proximity to the sample surface requires a highly sensitive distance sensing and regulating mechanism to keep the fiber from crashing into the surface. Fortunately, with the development of scanning tunneling and atomic force microscopy, this problem has been solved through the use of piezo-electric manipulators responding to the measured probe-sample interaction (sections 5.2 and 5.3). Force microscopy with tapered fibers was first demonstrated in 1992 [e.g., Toledo-Crow, 1992]. The fiber probe is attached to a piezo-electric element. This element brings the fiber into a rapid forced oscillation parallel to the surface over a distance of about 25 nm, thereby only slightly decreasing the lateral resolution. The oscillation amplitude decreases on approaching the sample surface due to 'shear' forces between probe and sample. This change is detected by the electronic actuators of the piezo-element and converted into distance information. Using an electronic feedback system based on this shear force detection, the distance between probe and surface can be adjusted between 1 and 15 nm with a vertical sensitivity of about 0.1 nm. Simultaneously a topographic 'shear' force image of the surface, similar to regular atomic force microscopy operation, is obtained 'for free'.

**Applications**

The feasibility of near-field optics has been explored experimentally since 1982 [Pohl, 1984] only one year after the demonstration of scanning tunneling microscope in 1981, and four years before atomic force microscopy. Though among the first in the expanding variety of scanning probe methods, near-field optics has been struggling with probe fabrication for almost a decade, while tunneling and especially force microscopy have developed into widely used surface analysis tools with nanometer resolution. Yet, despite the outstanding vertical and lateral sensitivity of scanning tunneling and atomic force microscopy, optical
microscopy has remained essential. It is convenient, non-invasive, non-destructive, it can operate in a native environment (in vivo). It also has a high contrast, is fast and in particular highly chemically specific due to the obtained spectroscopic information. In comparison, the chemical specificity of force sensing is only beginning to emerge [Dammer, 1995]. The first applications of near-field optical microscopy to biological and chemical samples used the relatively efficient aperture probes [Moers, 1994b]. Near-field optics has only recently started to show its latent promises of optical contrast at nanometer dimensions with improving efficiency and versatility [Paesler, 1996]. The steadily growing number of applications in material science, optoelectronics, chemistry and biology is indicative of its future importance in nanotechnology [van Hulst, 1996].

**Figure 5.4.10**

A Langmuir-Blodgett monolayer of polymers on a glass substrate. 
(a) Shear force image with a lateral resolution of about 50 nm, showing the topography of several uniform 8-nm thick polymer monolayer domains, including some surface roughness and a few non-fluorescent structures. 
(b, c) Simultaneously recorded near-field fluorescence images with about 200-nm lateral resolution for excitation with mutually perpendicular directions of linearly polarized light (indicated by the arrows). The domains that emit fluorescent light in one image are dark in the other. This clearly shows the high anisotropy of the film and indicates the orientation of the polymer chain, which is uniform over each domain due to the crystallinity of the film. Reprinted with permission from [Moers, 1994b]. Copyright 1994 American Chemical Society.

**Monolayers**

A Langmuir-Blodgett film is a highly organized and oriented one-molecule-thick layer. Generally these films serve as model systems for molecular organization in (bio)chemical membranes. For near-field optical microscopy, Langmuir-Blodgett films are ideal objects of study, because of their homogeneous, ultra-flat surface and their well-defined molecular orientation. An example clearly demonstrating the advantage of near-field optics in combination with force microscopy is given in figure 5.4.10.

**Chromosomes**

Human chromosome structure has been investigated with all microscopic techniques available. Chromosomes can be chemically labeled with small molecules with a technique called in-situ hybridization. By using label molecules that emit fluorescent light upon illumination with, for instance, a laser, the labels can be detected using a microscope. Fluorescence in-situ hybridization (FISH) has become one of the major methods for the analysis of human cellular genetic material. It enables direct visualization of topological or positional information of gene sequences in a conventional fluorescence microscope, allowing rapid localization of DNA fragments in morphologically preserved chromosomes. By using labels emitting light with different colors, multiple processes can be examined simultaneously through multicolor fluorescence detection. However, in conventional fluorescence microscopy, the position of the fluorescence labels
can only be determined with a resolution of about 0.3 μm due to the fundamental diffraction limit. Localization of the numerous closely linked genes requires mapping at higher resolution. This can be achieved using electron or force microscopy. Using electron microscopy, some DNA samples can be imaged with nanometer resolution. Atomic force microscopy can discriminate morphological labels of 75 to 100 nm diameter [Putman, 1993]. However, these methods lack the multiplicity of fluorescence detection. Near-field fluorescence microscopy has the potential to combine the best of both: optical resolution beyond the diffraction limit and multiplicity with sensitivity down to the single molecular level. Figure 5.4.11 shows a fluorescence near-field optical microscopy scan combined with simultaneous shear force detection of human chromosomes.

![Figure 5.4.11](image)

**A 7x7 μm² scan of a human chromosome.** The chromosome was labeled with fluorescent molecules by in-situ hybridization (FISH). (a) Shear force image showing the chromosome topography with some substructure; (b) corresponding near-field image of the green fluorescence light emitted upon laser illumination, displaying about ten labeling molecules in the central area of the chromosome. Reprinted with permission from [Moers, 1996]. Copyright 1998 Royal Microscopical Society, Oxford, UK.

**Single fluorescent molecules**

The observation of individual molecules is an application of near-field optical microscopy as a scientific tool. By tracking the exact position and orientation of a molecule, many dynamic biological processes can be studied, such as protein and molecular conformational changes. Moreover, the single molecular response provides a sensitive tool to study the local environment of a single molecule at biologically relevant conditions. For instance, the accuracy of a measurement of the fluorescence energy transfer between a donor and acceptor pair depends critically on their relative distance and molecular dipole orientation. In addition, selective excitation of molecules with a particular orientation can be exploited as a tool to trigger or inhibit specific biological reactions. To date, light microscopy of single molecules at ambient conditions with high spatial and temporal resolution is readily achievable by ultrasensitive fluorescence detection [Xie, 1996]. Also, single molecules can be imaged using the near-field method. They have been localized within a few nanometers and their orientation in three dimensions could be determined [Betzig, 1993]. This achievement was directly followed by single molecular spectroscopy [Trautman, 1994] and single molecular fluorescence lifetime detection [Xie, 1994].

A typical example of the dynamics of single molecules as observed in near-field fluorescence is shown in figure 5.4.12 on page 327. A sample consisting of carbocyanine fluorescent molecules embedded in a thin PMMA (polymethyl methacrylate) layer was prepared by spin-coating (section 3.7). This resulted in
a 5 to 10 nm layer with a surface coverage of typically a few dye molecules per square micrometer. Figure 5.4.12 (on page 327) shows a series of 'snapshot' images displaying single carboxylic molecules. Note that molecule 7 remains fixed during one hour in images (c) to (h), while molecule 4 appears to gradually rotate from the red to the green direction between images (b) to (d) and back again to the red direction between images (e) to (h). Fast rotational activity is observed for molecule 6 with sudden fluctuations from one scan line to the other within the same image. Sudden appearances and disappearances of molecules are also observed. For instance, molecules 8 and 9 are only present from image (f) on, while molecules 3 and 6 have disappeared in image (h). Molecule 1 emits light in image (e), (f) and (h), but not in (g). This behavior is probably caused by a quantum-mechanical transition to a non-emitting molecular state. Finally, figure 5.4.13 (also on page 327) shows the time-resolved sliding of a repair-enzyme over a DNA chain.

Conclusions
Near-field scanning optical microscopy allows fluorescence, absorption and polarization contrast with nanometer lateral resolution, unlimited by diffraction. In particular, near-field fluorescence microscopy gives a clear high-resolution contrast and induces virtually no bleaching as opposed to conventional fluorescence microscopy. Shear-force feedback is essential for reliable operation of the aperture type based on fibers, especially while scanning over the soft surfaces of cells and chromosomes. The aperture-type near-field fluorescent microscope gives chemical specificity and orientational information in addition to the simultaneously acquired topographical image using force feedback. For bio-nanoscience, the technique offers a large potential for the structural and chemical analysis of cellular material and for the detection of molecular organization on membranes (van Hulst, 1997a). Applications of photon tunneling microscopy are less numerous because this technique is limited to non-scattering surfaces. For further information on recent advances in the development and application of near-field optical microscopy the reader is referred to recent conference proceedings (Paesler, 1995; van Hulst, 1997b).