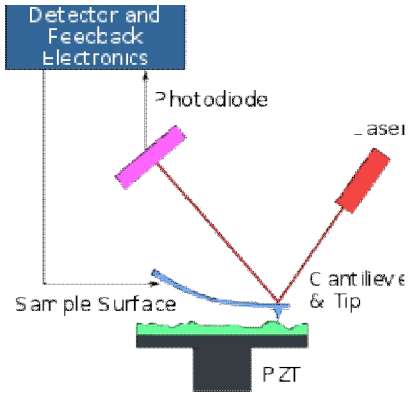
Unit-II Characterization of Nanoscale Materials

Atomic Force Microscopy (AFM)

Atomic force microscopy (AFM) or scanning force microscopy (SFM) is a very-high-resolution type of scanning probe microscopy(SPM), with demonstrated resolution on the order of fractions of a nanometer, more than 1000 times better than the optical diffraction limit.

AFM is a type of scanning probe microscopy (SPM), with demonstrated resolution on the order of fractions of a nanometer, more than 1000 times better than the optical diffraction limit. The information is gathered by "feeling" or "touching" the surface with a mechanical probe. Piezoelectric elements that facilitate tiny but accurate and precise movements on (electronic) command enable precise scanning.



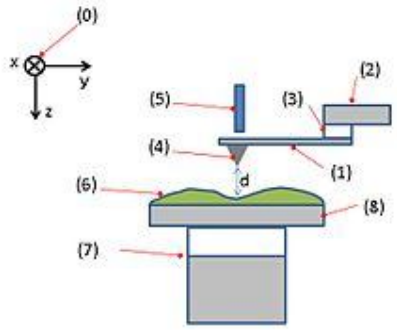
The AFM has three major abilities: force measurement, imaging, and manipulation. In force measurement, AFMs can be used to measure the forces between the probe and the sample as a function of their mutual separation. This can be applied to perform force spectroscopy, to measure the mechanical properties of the sample, such as the sample's Young's modulus, a measure of stiffness.

For imaging, the reaction of the probe to the forces that the sample imposes on it can be used to form an image of the three-dimensional shape (topography) of a sample surface at a high resolution. This is achieved by raster scanning the position of the sample with respect to the tip and recording the height of the probe that corresponds to a constant probe-sample interaction (see section topographic imaging in AFM for more details). The surface topography is commonly displayed as a pseudocolor plot.

In manipulation, the forces between tip and sample can also be used to change the properties of the sample in a controlled way. Examples of this include atomic manipulation, scanning probe lithography and local stimulation of cells.

Simultaneous with the acquisition of topographical images, other properties of the sample can be measured locally and displayed as an image, often with similarly high resolution. Examples of such properties are mechanical properties like stiffness or adhesion strength and electrical properties such as conductivity or surface potential. In fact, the majority of SPM techniques are extensions of AFM that use this modality.

Figure shows an AFM, which typically consists of the following features. Numbers in parentheses correspond to numbered features in Fig. 3. Coordinate directions are defined by the coordinate system (0).



Typical configuration of an AFM.

(1): Cantilever, (2): Support for cantilever, (3): Piezoelectric element(to oscillate cantilever at its eigen frequency.), (4): Tip (Fixed to open end of a cantilever, acts as the probe), (5): Detector of deflection and motion of the cantilever, (6): Sample to be measured by AFM, (7): xyz drive, (moves sample (6) and stage (8) in x, y, and z directions with respect to a tip apex (4)), and (8): Stage.

The small spring-like cantilever (1) is carried by the support (2). Optionally, a piezoelectric element (typically made of a ceramic material) (3) oscillates the cantilever (1). The sharp tip (4) is fixed to the free end of the cantilever (1). The detector (5) records the deflection and motion of the cantilever (1). The sample (6) is mounted on the sample stage (8). An xyz drive (7) permits to displace the sample (6) and the sample stage (8) in x, y, and z directions with respect to the tip apex (4). Although Fig. 3 shows the drive attached to the sample, the drive can also be attached to the tip, or independent drives can be attached to both, since it is the relative displacement of the sample and tip that needs to be controlled. Controllers and plotter are not shown in Fig.

According to the configuration described above, the interaction between tip and sample, which can be an atomic scale phenomenon, is transduced into changes of the motion of cantilever which is a macro scale phenomenon. Several different aspects of the cantilever motion can be used to quantify the interaction between the tip and sample, most commonly the value of the deflection, the amplitude of an imposed oscillation of the cantilever, or the shift in resonance frequency of the cantilever.

Detector

The detector (5) of AFM measures the deflection of the cantilever and converts it into an electrical signal. The intensity of this signal will be proportional to the displacement of the cantilever. Various methods of detection can be used, e.g. interferometry, optical levers, the piezoresistive method, the piezoelectric method, and STM-based detectors. The AFM signals, such as sample height or cantilever deflection, are recorded on a computer during the x-y scan. They are plotted in a pseudocolor image, in which each pixel represents an x-y position on the sample, and the color represents the recorded signal.

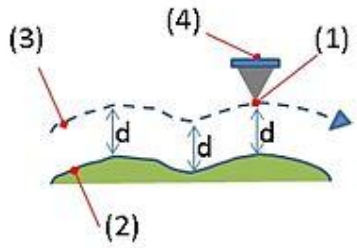


Fig: Topographic image forming by AFM. **(1)**: Tip apex, **(2)**: Sample surface, **(3)**: Z-orbit of Tip apex, **(4)**: Cantilever.

Applications

The AFM has been applied to problems in a wide range of disciplines of the natural sciences, including solid-state physics, semiconductor science and technology, molecular engineering, polymer chemistry and physics, surface chemistry, molecular biology, cell biology, and medicine. Applications in the field of solid state physics include (a) the identification of atoms at a surface, (b) the evaluation of interactions between a specific atom and its neighboring atoms, and (c) the study of changes in physical properties arising from changes in an atomic arrangement through atomic manipulation.

In molecular biology, AFM can be used to study the structure and mechanical properties of protein complexes and assemblies. For example, AFM has been used to image microtubules and measure their stiffness. In cellular biology, AFM can be used to attempt to distinguish cancer cells and normal cells based on a hardness of cells, and to evaluate interactions between a specific cell and its neighboring cells in a competitive culture system. AFM can also be used to indent cells, to study how they regulate the stiffness or shape of the cell membrane or wall. In some variations, electric potentials can also be scanned using conducting cantilevers.

Biological applications and other

Force spectroscopy is used in biophysics to measure the mechanical properties. of living material or detect structures of different stiffness buried into the bulk of the sample using the stiffness tomography. Another application was to measure the interaction forces between from one hand a material stuck on the tip of the cantilever, and from another hand the surface of particles either free or occupied by the same material. From the adhesion force distribution curve, a mean value of the forces has been derived. It allowed to make a cartography of the surface of the particles, covered or not by the material.

Identification of individual surface atoms

The AFM can be used to image and manipulate atoms and structures on a variety of surfaces. The atom at the apex of the tip "senses" individual atoms on the underlying surface when it forms incipient chemical bonds with each atom

Advantages and disadvantages

Advantages

Provides a two-dimensional projection or a two-dimensional image of a sample, the AFM provides a three-dimensional surface profile. AFM modes can work perfectly well in ambient air or even a liquid environment. AFM can provide higher resolution than SEM.

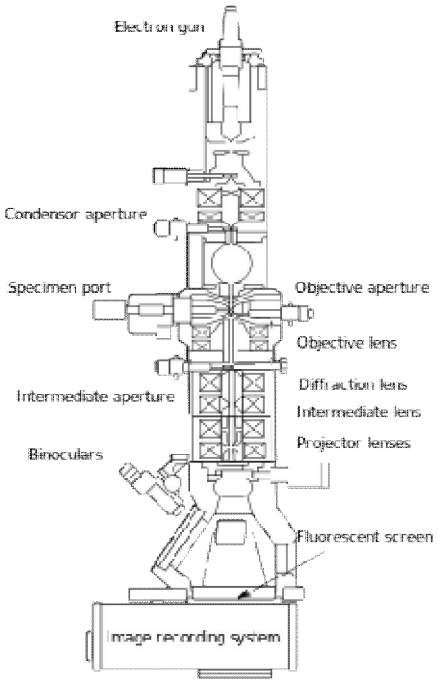
Disadvantages

The AFM can only image a maximum scanning area of about 150×150 micrometers and a maximum height on the order of 10-20 micrometers. The scanning speed of an AFM is also a limitation. AFM images can also be affected by nonlinearity, hysteresis, and creep of the piezoelectric material and cross-talk between the *x*, *y*, *z*axes that may require software enhancement and filtering. Due to the nature of AFM probes, they cannot normally measure steep walls or overhangs.

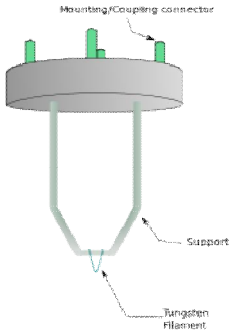
Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM, also sometimes conventional transmission electron microscopy or CTEM) is a microscopytechnique in which a beam of electrons is transmitted through a specimen to form an image. The specimen is most often an ultrathin section less than 100 nm thick or a suspension on a grid. An image is formed from the interaction of the electrons with the sample as the beam is transmitted through the specimen. The image is then magnified and focused onto an imaging device, such as a fluorescent screen, a layer of photographic film, or a sensor such as a charge-coupled device.

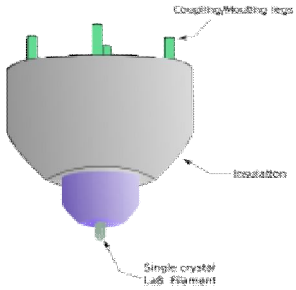
Transmission electron microscopes are capable of imaging at a significantly higher resolution than light microscopes, owing to the smaller de Broglie wavelength of electrons. Electrons are usually generated in an electron microscope by a process known as thermionic emission from a filament, usually tungsten, in the same manner as a light bulb, or alternatively by field electron emission.



Layout of optical components in a basic TEM



Hairpin style tungsten filament



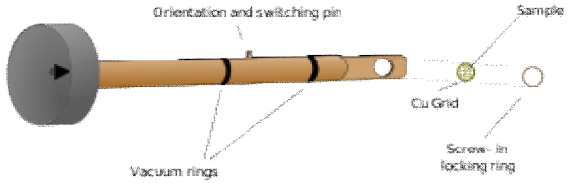
Single crystal LaB_6filament

From the top down, the TEM consists of an emission source, which may be a tungsten filament or needle, or a lanthanum hexaboride (LaB_6) single crystal source. The gun is connected to a high voltage source (typically ~100–300 kV) and, given sufficient current, the gun will begin to emit electrons either by thermionic or field electron emission into the vacuum. The electron source is typically mounted in a Wehnelt cylinder to provide preliminary focus of the emitted electrons into a beam. The upper lenses of the TEM then further focus the electron beam to the desired size and location.

Manipulation of the electron beam is performed using two physical effects. The interaction of electrons with a magnetic field will cause electrons to move according to the left hand rule, thus allowing for electromagnets to manipulate the electron beam. The use of magnetic fields allows for the formation of a magnetic lens of variable focusing power, the lens shape originating due to the distribution of magnetic flux. Additionally, electrostatic fields can cause the electrons to be deflected through a constant angle. Coupling of two deflections in opposing directions with a small intermediate gap allows for the formation of a shift in the beam path, allowing for beam shifting in TEM, which is important for STEM.

Typically a TEM consists of three stages of lensing. The stages are the condenser lenses, the objective lenses, and the projector lenses. Imaging systems in a TEM consist of a phosphor screen, which may be made of fine (10–100 μm) particulate zinc sulfide, for direct observation by the operator, and, optionally, an image recording system such as film based or doped YAG screen coupled CCDs. A TEM is composed of several components, which include a vacuum system in which the electrons travel, an electron emission source for generation of the electron stream, a series of electromagnetic lenses, as well as electrostatic plates.

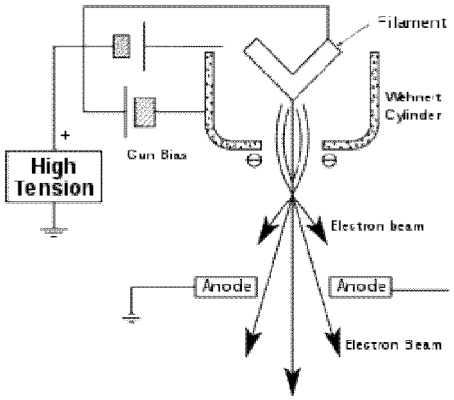
TEM specimen stage designs include airlocks to allow for insertion of the specimen holder into the vacuum with minimal loss of vacuum in other areas of the microscope. The specimen holders hold a standard size of sample grid or self-supporting specimen. Standard TEM grid sizes are 3.05 mm diameter, with a thickness and mesh size ranging from a few to 100 µm. Two main designs for stages in a TEM exist, the side-entry and top entry version. Each design must accommodate the matching holder to allow for specimen insertion without either damaging delicate TEM optics or allowing gas into TEM systems under vacuum.



A diagram of a single axis tilt sample holder for insertion into a TEM goniometer. Titling of the holder is achieved by rotation of the entire goniometer

Insertion procedures for side-entry TEM holders typically involve the rotation of the sample to trigger micro switches that initiate evacuation of the airlock before the sample is inserted into the TEM column.

Electron gun



The electron gun is formed from several components: the filament, a biasing circuit, a Wehnelt cap, and an extraction anode. By connecting the filament to the negative component power supply, electrons can be "pumped" from the electron gun to the anode plate, and the TEM column, thus completing the circuit.

Electron lens

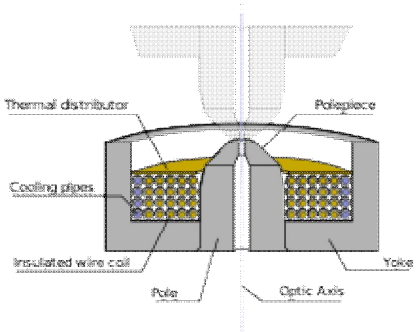


Diagram of a TEM split pole piece design lens

Electron lenses are designed to act in a manner emulating that of an optical lens, by focusing parallel electrons at some constant focal distance. Electron lenses may operate electrostatically or magnetically. The majority of electron lenses for TEM use electromagnetic coils to generate a convex lens.

Amplitude–contrast is obtained due to removal of some electrons before the image plane. During their interaction with the specimen some of electrons will be lost due to absorption, or due to scattering at very high angles beyond the physical limitation of microscope or are blocked by the objective aperture.

Sample preparation

Sample preparation in TEM can be a complex procedure. TEM specimens should be less than 100 nanometers thick for a conventional TEM. Unlike neutron or X-Ray radiation the electrons in the beam interact readily with the sample, an effect that increases roughly with atomic number squared (Z^2). High quality samples will have a thickness that is comparable to the mean free path of the electrons that travel through the samples, which may be only a few tens of nanometers. Preparation of TEM specimens is specific to the material under analysis and the type of information to be obtained from the specimen.

Tissue sectioning

Biological tissue is often embedded in a resin block then thinned to less than 100 nm on an ultramicrotome. Inorganic samples, such as aluminium, may also be embedded in resins and ultrathin sectioned in this way, using either coated glass, sapphire or larger angle diamond knives. To prevent charge build-up at the sample surface when viewing in the TEM, tissue samples need to be coated with a thin layer of conducting material, such as carbon.

Sample staining

Compounds of heavy metals such as osmium, lead, uranium or gold may be used prior to TEM observation to selectively deposit electron dense atoms in or on the sample in desired cellular or protein region. This process requires an understanding of how heavy metals bind to specific biological tissues and cellular structures.

Mechanical milling

A diamond, or cubic boron nitride polishing compound may be used in the final stages of polishing to remove any scratches that may cause contrast fluctuations due to varying sample thickness. Even after careful mechanical milling, additional fine methods such as ion etching may be required to perform final stage thinning.

Chemical etching

Certain samples may be prepared by chemical etching, particularly metallic specimens. These samples are thinned using a chemical etchant, such as an acid, to prepare the sample for TEM observation. Devices to control the thinning process may allow the operator to control either the voltage or current passing through the specimen, and may include systems to detect when the sample has been thinned to a sufficient level of optical transparency.

Scanning TEM

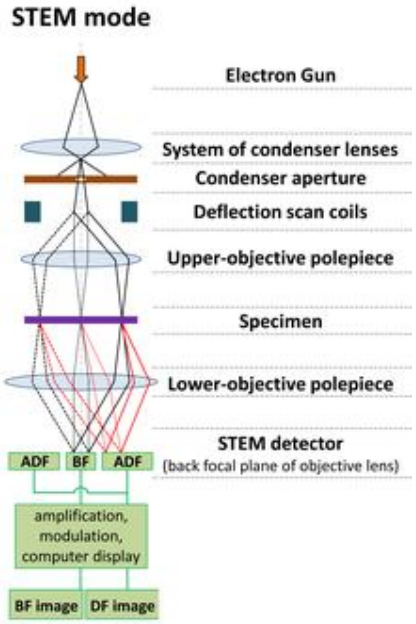
Scanning coils are used to deflect the beam, such as by an electrostatic shift of the beam, where the beam is then collected using a current detector such as a Faraday cup, which

acts as a direct electron counter. By correlating the electron count to the position of the scanning beam, the transmitted component of the beam may be measured. The non-transmitted components may be obtained either by beam tilting or by the use of annular dark field detectors.

Limitations

There are a number of drawbacks to the TEM technique. Many materials require extensive sample preparation to produce a sample thin enough to be electron transparent, which makes TEM analysis a relatively time consuming process with a low throughput of samples. The structure of the sample may also be changed during the preparation process. Also the field of view is relatively small, raising the possibility that the region analyzed may not be characteristic of the whole sample. There is potential that the sample may be damaged by the electron beam, particularly in the case of biological materials.

Scanning Transmission Electron Microscopy (STEM)



A scanning transmission electron microscope (STEM) is a type of transmission electron microscope (TEM). As with a conventional transmission electron microscope (CTEM), images are formed by electrons passing through a sufficiently thin specimen. However, unlike CTEM, in STEM the electron beam is focused to a fine spot (with the typical spot size 0.05 – 0.2 nm) which is then scanned over the sample in a raster illumination system constructed in a way that at each point sample illuminated with the beam parallel to the optical axis.

The addition of an aberration corrector to STEMs enables electron probes to be focused to sub-angstrom diameters, allowing images with sub-angstrom resolution to be acquired. This has made it possible to identify individual atomic columns with unprecedented clarity. Aberration-corrected STEM was demonstrated with 1.9 Å resolution in 1997 and soon after in 2000 with roughly 1.36 Å resolution. Advanced aberration-corrected STEMs have since been developed with sub-50 pm resolution. Aberration-corrected STEM provides

the added resolution and beam current critical to the implementation of atomic resolution chemical and elemental spectroscopic mapping.

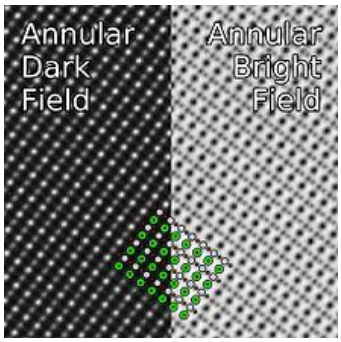
Applications

Scanning transmission electron microscopes are used to characterize the nanoscale, and atomic scale structure of specimens, providing important insights into the properties and behaviour of materials and biological cells. Scanning transmission electron microscopy has been applied to characterize the structure of a wide range of material specimens, including solar cells, semiconductor devices, complex oxides, batteries, fuel cells, catalysts, and 2D materials.

The first application of STEM to the imaging of biological molecules was demonstrated in 1971. The advantage of STEM imaging of biological samples is the high contrast of annular dark-field images, which can allow imaging of biological samples without the need for staining. STEM has been widely used to solve a number of structural problems in molecular biology.

STEM detectors and imaging modes

Annular dark-field



Atomic resolution imaging of $SrTiO_3$, using annular dark field (ADF) and annular bright field (ABF) detectors. Overlay: strontium (green), titanium (grey) and oxygen (red).

In annular dark-field mode, images are formed by fore-scattered electrons incident on an annular detector, which lies outside of the path of the directly transmitted beam. By using a high-angle ADF detector, it is possible to form atomic resolution images where the contrast of an atomic column is directly related to the atomic number (Z-contrast image). Directly interpretable Z-contrast imaging makes STEM imaging with a high-angle detector an appealing technique in contrast to conventional high resolution electron microscopy, in which phase-contrast effects mean that atomic resolution images must be compared to simulations to aid interpretation.

STEM tomography

STEM tomography allows the complete three-dimensional internal and external structure of a specimen to be reconstructed from a tilt-series of 2D projection images of the specimen acquired at incremental tilts. High angle ADF STEM is a particularly useful imaging mode for electron tomography because the intensity of high angle ADF-STEM images varies only with the projected mass-thickness of the sample, and the atomic number of atoms in the sample. This yields highly interpretable three dimensional reconstructions.

Cryo-STEM

Cryo-electron microscopy in STEM (Cryo-STEM) allows specimens to be held in the microscope at liquid nitrogen or liquid helium temperatures. This is useful for imaging specimens that would be volatile in high vacuum at room temperature. Cryo-STEM has been used to study vitrified biological samples, vitrified solid-liquid interfaces in material specimens and specimens containing elemental sulfur, which is prone to sublimation in electron microscopes at room temperature.

In situ/environmental STEM

In order to study the reactions of particles in gaseous environments, a STEM may be modified with a differentially pumped sample chamber to allow gas flow around the sample, whilst a specialized holder is used to control the reaction temperature. Alternatively a holder mounted with an enclosed gas flow cell may be used. Nanoparticles and biological cells have been studied in liquid environments using liquid-phase electron microscopy in STEM, accomplished by mounting a microfluidic enclosure in the specimen holder.

Scanning Tunneling Microscope (STM)

A scanning tunneling microscope (STM) is an instrument for imaging surfaces at the atomic level. STM is based on the concept of quantum tunneling. When a conducting tip is brought very near to the surface to be examined, a bias (voltage difference) applied between the two can allow electrons to tunnel through the vacuum between them. The resulting *tunneling current* is a function of tip position, applied voltage, and the local density of states (LDOS) of the sample. Information is acquired by monitoring the current as the tip's position scans across the surface, and is usually displayed in image form. STM can be a challenging technique, as it requires extremely clean and stable surfaces, sharp tips, excellent vibration control, and sophisticated electronics, but nonetheless many hobbyists have built their own.

Procedure

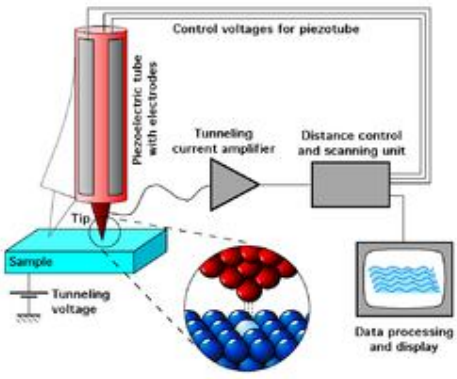
First, a voltage bias is applied and the tip is brought close to the sample by coarse sample-to-tip control, which is turned off when the tip and sample are sufficiently close. At close range, fine control of the tip in all three dimensions when near the sample is typically piezoelectric, maintaining tip-sample separation W typically in the 4-7 Å (0.4-0.7 nm) range, which is the equilibrium position between attractive ($3<W<10$Å) and repulsive ($W<3$Å) interactions. In this situation, the voltage bias will cause electrons to tunnel between the tip and sample, creating a current that can be measured. Once tunneling is established, the tip's bias and position with respect to the sample can be varied (with the details of this variation depending on the experiment) and data are obtained from the resulting changes in current.

If the tip is moved across the sample in the x-y plane, the changes in surface height and density of states causes changes in current. These changes are mapped in images. This change in current with respect to position can be measured itself, or the height, z, of the tip corresponding to a constant current can be measured. These two modes are called constant height mode and constant current mode, respectively. In constant current mode, feedback electronics adjust the height by a voltage to the piezoelectric height control mechanism. This leads to a height variation and thus the image comes from the tip topography across the sample and gives a constant charge density surface; this means contrast on the image is due to variations in charge density. In constant height mode, the voltage and height are both held

constant while the current changes to keep the voltage from changing; this leads to an image made of current changes over the surface, which can be related to charge density. The benefit to using a constant height mode is that it is faster, as the piezoelectric movements require more time to register the height change in constant current mode than the current change in constant height mode. All images produced by STM are grayscale, with color optionally added in post-processing in order to visually emphasize important features.

In addition to scanning across the sample, information on the electronic structure at a given location in the sample can be obtained by sweeping voltage and measuring current at a specific location. This type of measurement is called scanning tunneling spectroscopy(STS) and typically results in a plot of the local density of states as a function of energy within the sample. The advantage of STM over other measurements of the density of states lies in its ability to make extremely local measurements: for example, the density of states at an impurity site can be compared to the density of states far from impurities. Framerates of at least 25 Hz enable so called video-rate STM. Framerates up to 80 Hz are possible with fully working feedback that adjusts the height of the tip. Due to the line-by-line scanning motion, a proper comparison on the speed requires not only the framerate, but also the number of pixels in an image: with a framerate of 10 Hz and 100x100 pixels the tip moves with a line frequency of 1 kHz, whereas it moves with only with 500 Hz, when measuring with a faster framerate of 50 Hz but only 10x10 pixels. Video-rate STM can be used to scan surface diffusion.

Instrumentation



The components of an STM include scanning tip, piezoelectric controlled height and x,y scanner, coarse sample-to-tip control, vibration isolation system, and computer.

The resolution of an image is limited by the radius of curvature of the scanning tip of the STM.

Principle of operation

Tunneling is a functioning concept that arises from quantum mechanics. Classically, an object hitting an impenetrable barrier will not pass through. In contrast, objects with a very small mass, such as the electron, have wavelike characteristics which permit such an event, referred to as tunneling. Electrons behave as beams of energy, and in the presence of a potential $U(z)$, assuming 1-dimensional case, the energy levels $\psi_n(z)$ of the electrons are given by solutions to Schrödinger's equation,

Near-Field Scanning Optical Microscope (NSOM)

Near-field scanning optical microscopy (NSOM/SNOM) is a microscopy technique for nanostructure investigation that breaks the far field resolution limit by exploiting the properties of evanescent waves. In SNOM, the excitation laser light is focused through an aperture with a diameter smaller than the excitation wavelength, resulting in an evanescent field (or near-field) on the far side of the aperture. When the sample is scanned at a small distance below the aperture, the optical resolution of transmitted or reflected light is limited only by the diameter of the aperture. In particular, lateral resolution of 20 nm and vertical resolution of 2–5 nm have been demonstrated.

As in optical microscopy, the contrast mechanism can be easily adapted to study different properties, such as refractive index, chemical structure and local stress. Dynamic properties can also be studied at a sub-wavelength scale using this technique. NSOM/SNOM is a form of scanning probe microscopy.

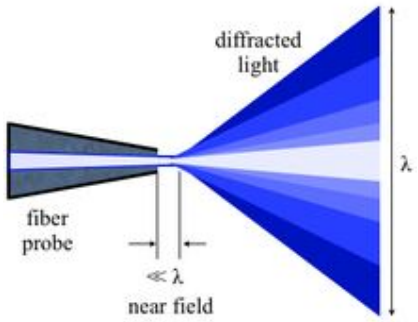
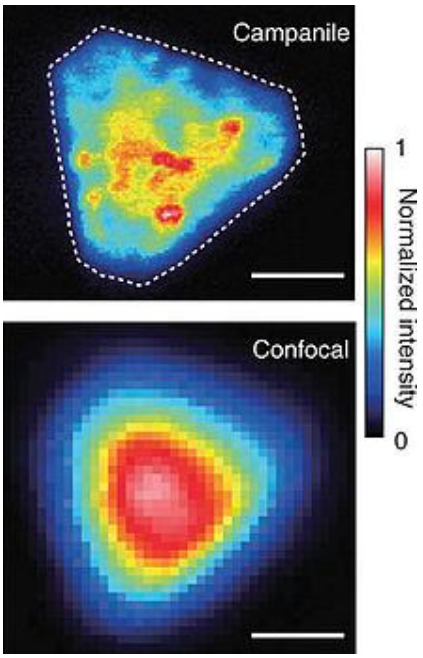


Diagram illustrating near-field optics, with the diffraction of light coming from NSOM fiber probe, showing wavelength of light and the near-field.



Comparison of photoluminescence maps recorded from a molybdenum disulfide flake using NSOM with a campanile probe (top) and conventional confocal microscopy (bottom). Scale bars: 1 μm.

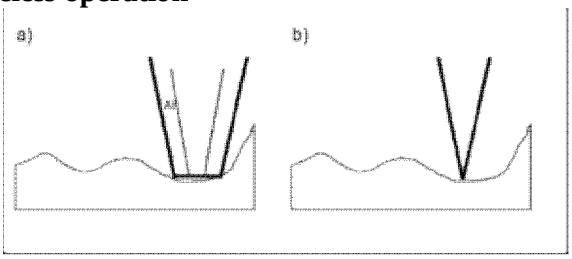
Theory

According to Abbe's theory of image formation, developed in 1873, the resolving capability of an optical component is ultimately limited by the spreading out of each image point due to diffraction. Unless the aperture of the optical component is large enough to collect all the diffracted light, the finer aspects of the image will not correspond exactly to the object.

This treatment only assumes the light diffracted into the far-field that propagates without any restrictions. NSOM makes use of evanescent or non propagating fields that exist only near the surface of the object. These fields carry the high frequency spatial information about the object and have intensities that drop off exponentially with distance from the object. Because of this, the detector must be placed very close to the sample in the near field zone, typically a few nanometers. As a result, near field microscopy remains primarily a surface inspection technique. The detector is then rastered across the sample using a piezoelectric stage. The scanning can either be done at a constant height or with regulated height by using a feedback mechanism.

Modes of operation

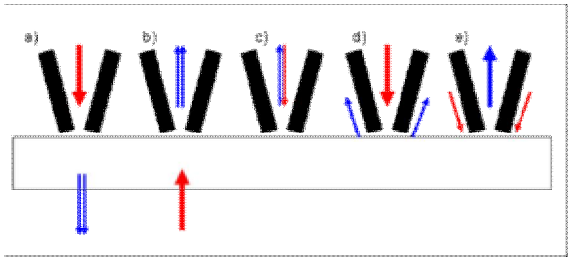
Aperture and apertureless operation



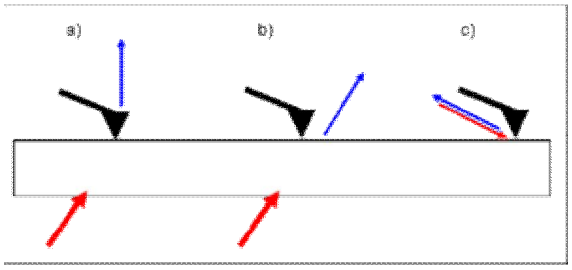
Sketch of a) typical metal-coated tip, and b) sharp uncoated tip.

There exist NSOM which can be operated in so-called aperture mode and NSOM for operation in a non-aperture mode. As illustrated, the tips used in the aperture less mode are very sharp and do not have a metal coating.

Though there are many issues associated with the aperture tips (heating, artifacts, contrast, sensitivity, topology and interference amongst others), aperture mode remains more popular. This is primarily because aperture less mode is even more complex to set up and operate, and is not understood as well. There are five primary modes of aperture NSOM operation and four primary modes of aperture less NSOM operation. The major ones are illustrated in the next figure.



Aperture modes of operation: a) illumination, b) collection, c) illumination collection, d) reflection and e) reflection collection.



Aperture less modes of operation: a) photon tunneling (PSTM) by a sharp transparent tip, b) PSTM by sharp opaque tip on smooth surface, and c) scanning inter ferometric aperture less microscopy with double modulation.

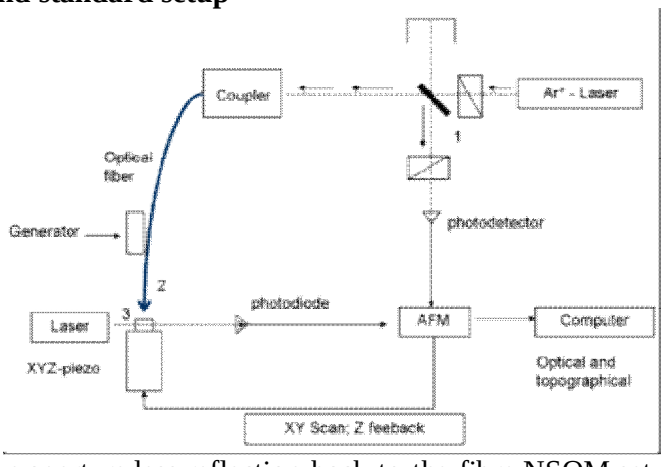
Some types of NSOM operation utilize a campanile probe, which has a square pyramid shape with two facets coated with a metal. Such a probe has a high signal collection efficiency (>90%) and no frequency cutoff. Another alternative is “active tip" schemes, where the tip is functionalized with active light sources such as a fluorescent dye or even a light emitting diode that enables fluorescence excitation.

Feedback mechanisms

Feedback mechanisms are usually used to achieve high resolution and artifact free images since the tip must be positioned within a few nanometers of the surfaces. Some of these mechanisms are:

- Constant force feedback: This mode is very similar to the feedback mechanism used in atomic force microscopy (AFM). Experiments can be performed in contact, intermittent contact, and non-contact modes.
- Shear force feedback: In this mode, a tuning fork is mounted alongside the tip and made to oscillate at its resonance frequency. The amplitude is closely related to the tip-surface distance, and thus used as a feedback mechanism.

Instrumentation and standard setup



Block diagram of an aperture less reflection-back-to-the-fibre NSOM setup with shear-force distance control and cross-polarization; 1: beam splitter and crossed polarisers; 2: shear-force arrangement; 3: sample mount on a piezo stage.

The primary components of an NSOM setup are the light source, feedback mechanism, the scanning tip, the detector and the piezoelectric sample stage. The light source is usually a laser focused into an optical fibre through a polarizer, a beam splitter and a couple. The polarizer and the beam splitter would serve to remove stray light from the

returning reflected light. The scanning tip, depending upon the operation mode, is usually a pulled or stretched optical fibre coated with metal except at the tip or just a standard AFM cantilever with a hole in the centre of the pyramidal tip. Standard optical detectors, such as avalanche photodiode, photomultiplier tube (PMT) or CCD, can be used. Highly specialized NSOM techniques, Raman NSOM for example, have much more stringent detector requirements.

Near-field spectroscopy

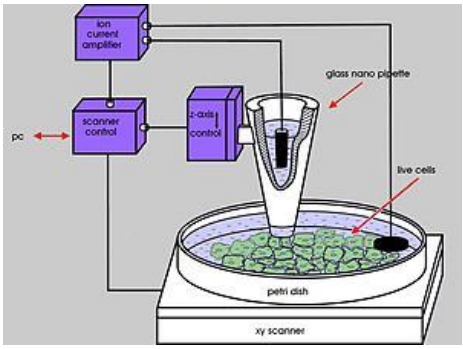
As the name implies, information is collected by spectroscopic means instead of imaging in the near field regime. Through Near Field Spectroscopy (NFS), one can probe spectroscopically with subwavelength resolution. Raman SNOM and fluorescence SNOM are two of the most popular NFS techniques as they allow for the identification of nanosized features with chemical contrast. Some of the common near-field spectroscopic techniques are:

- *Direct local Raman NSOM:* Aperture Raman NSOM is limited by very hot and blunt tips, and by long collection times. However, apertureless NSOM can be used to achieve high Raman scattering efficiency factors (around 40). Topological artifacts make it hard to implement this technique for rough surfaces.
- *Tip-enhanced Raman spectroscopy (TERS) is an offshoot of Surface enhanced Raman spectroscopy (SERS).* This technique can be used in an apertureless shear-force NSOM setup, or by using an AFM tip coated with gold or silver. The Raman signal is found to be significantly enhanced under the AFM tip. This technique has been used to give local variations in the Raman spectra under a single-walled nanotube. A highly sensitive optoacoustic spectrometer must be used for the detection of the Raman signal.
- *Fluorescence NSOM:* This highly popular and sensitive technique makes use of the fluorescence for near field imaging, and is especially suited for biological applications. The technique of choice here is the apertureless back to the fiber emission in constant shear force mode. This technique uses merocyanine based dyes embedded in an appropriate resin. Edge filters are used for removal of all primary laser light. Resolution as low as 10 nm can be achieved using this technique.
- *Near field infrared spectrometry and near field dielectric microscopy*:[18] near-field probes may be used to combine sub-micron microscopy with localized IR spectroscopy.
- *nano-FTIR*: is a broadband nanoscale spectroscopy that uses broadband illumination and FTIR detection to obtain a complete infrared spectrum at every spatial location. Sensitivity to a single molecular complex and nanoscale resolution up to 10 nm has been demonstrated with nano-FTIR.

Limitations

- Very low working distance and extremely shallow depth of field.
- Normally limited to surface studies, however can be applied for subsurface investigations within the corresponding depth of field.
- In shear force mode and other contact operation is not conducive for studying soft materials.
- Long scan times for large sample areas for high resolution imaging.

Scanning Ion-Conductance Microscopy (SICM)



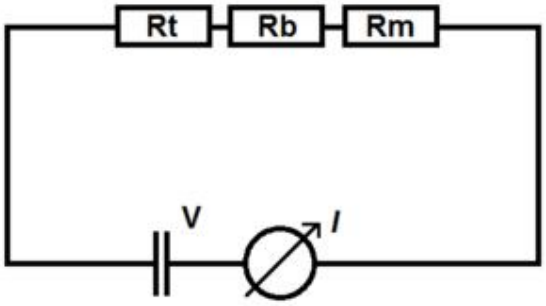
Scanning ion-conductance microscopy diagram.

Scanning ion-conductance microscopy (SICM) is a scanning probe microscopy technique that uses an electrode as the probe tip. SICM allows for the determination of the surface topography of micrometer and even nanometer-range structures in aqueous media conducting electrolytes. The samples can be hard or soft, are generally non-conducting, and the non-destructive nature of the measurement allows for the observation of living tissues and cells, and biological samples in general. It is able to detect steep profile changes in samples and can be used to map a living cell's stiffness in tandem with its detailed topography, or to determine the mobility of cells during their migrations.

Working principle

Scanning ion conductance microscopy is a technique using the increase of access resistance in a micro-pipette in an electrolyte-containing aqueous medium when it approaches a poorly conducting surface. It monitors the ionic current flowing in and out of the micro/nano-pipette, which is hindered if the tip is very close to the sample surface since the gap through which ions can flow is reduced in size.

The SICM setup is generally as follows: A voltage is applied between the two Ag/AgCl electrodes, one of which is in the glass micro-pipette, and the other in the bulk solution. The voltage will generate an ionic current between the two electrodes, flowing in and out of the micro-pipette. The conductance between the two electrodes is measured, and depends on the flux of ions. Movements of the pipette are regulated through piezoelectrics.



Equivalent electrical circuit of a SICM setup.

The total resistance of the setup (Rtot) is the sum of the three resistances: Rb, Rm, and Rt. Rb the resistance of the electrolyte solution between the tip of the micro-pipette and the electrode in the bulk of the solution. Rm is the resistance of the electrolyte solution between

the electrode in the micro-pipette and the tip. Rt is the resistance of the current flowing through the tip. Rb and Rm depend on the electrolyte conductivity, and the position and shape of the Ag/AgCl electrodes. Rt depends on the size and shape of the aperture, and on the distance between the tip and the sample. All the parameters except the distance between tip and sample are constant within a given

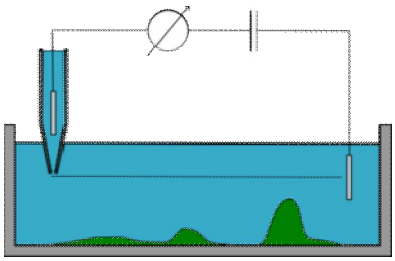
Comparison with other scanning probe microscopy techniques

SICM has a worse resolution than AFM or STM, which can routinely reach resolutions of about 0.1 nm. The resolution of SICM measurement is limited to 1.5 times the diameter of the tip opening in theory, but measurements taken with a 13 nm opening-diameter managed a resolution of around 3–6 nm. SICM can be used to image poorly or non-conducting surfaces, which is impossible with STM.

In SICM measurements, the tip of the micro-pipette does not touch the surface of the sample; which allows the imaging of soft samples (cells, biological samples, cell villi) without deformation. SICM is used in an electrolyte-containing solution, so can be used in physiological media and image living cells and tissues, and monitor biological processes while they are taking place. In hopping mode, it is able to correctly determine profiles with steep slopes and grooves.

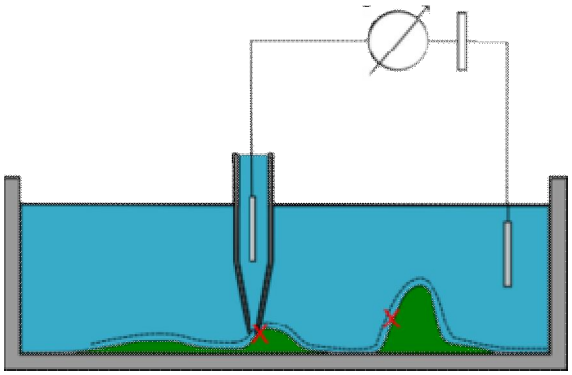
Imaging modes

There are four main imaging modes in SICM: constant-z mode, Direct current (constant distance) mode, alternating current mode, and hopping/backstep/standing approach mode.



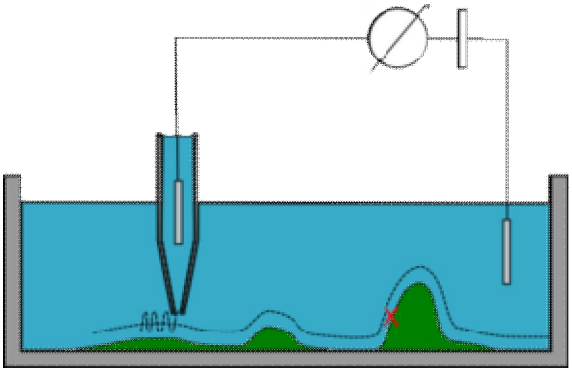
Trajectory of a SICM probe in constant-z mode.

In constant-z mode, the micro-pipette is maintained at a constant z (height) while it is moved laterally and the resistance is monitored, its variations allowing for the reconstitution of the topography of the sample. This mode is fast but is barely used since it only works on very flat samples. If the sample has rugged surfaces, the pipette will either crash into it, or be too far for imaging most of the sample.



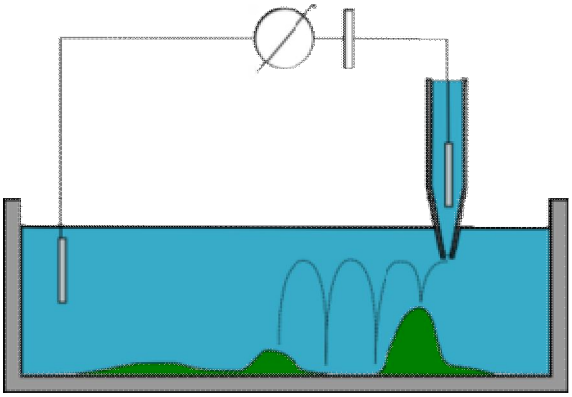
Trajectory of a SICM probe in DC mode

In direct current (DC) mode (constant distance mode), the micro-pipette is lowered toward the sample until a predefined resistance is reached. The pipette is then moved laterally and a feedback loop maintains the distance to the sample (through the resistance value). The z-position of the pipette determines the topography of the sample. This mode does not detect steep slopes in sample, may contact the sample in such cases and is prone to electrode drift.



Trajectory of a SICM probe in AC mode

In alternating current (AC) mode, the micro-pipette oscillates vertically in addition to its usual movement. While the pipette is still far from the surface the ionic current, and the resistance is steady, so the pipette is lowered. Once the resistance starts oscillating, the amplitude serves as feedback to modulate the position until predefined amplitude is reached. The response of the AC component increases much steeper than the DC, and allows for the recording of more complex samples.



Trajectory of a SICM probe in hopping mode.

In hopping mode, the micro-pipette is lowered to the sample until a given resistance is reached, and the height is recorded. Then the pipette is dragged back, laterally moved and another measurement is made, and the process repeats. The topography of the sample can then be reconstituted. Hopping mode is slower than the others, but is able to image complex topography and even entire cells, without distorting the sample surface.

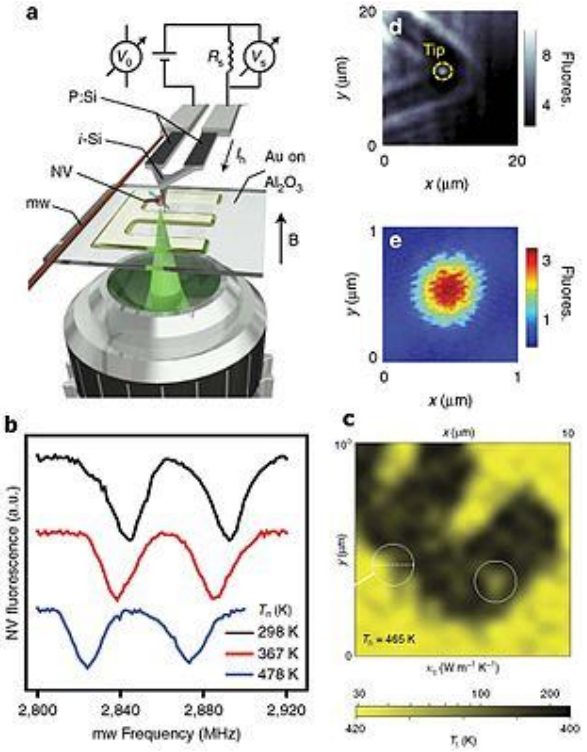
Combinations with other techniques, and alternative uses

- SICM was used to image a living neural cell from rat brain, determine the life cycle of microvilli, observe the movement of protein complexes in spermatozoa.
- SICM has been combined with fluorescence microscopy and förster resonance energy transfer.
- SICM has been used in a "smart patch-clamp" technique, clamping the pipette by suction to the surface of a cell and then monitoring the activity of the sodium channels in the cell membrane.
- A combination of AFM and SICM was able to obtain high resolution images of synthetic membranes in ionic solutions.
- Scanning near-field optical microscopy has been used with SICM; the SICM measurement allowed for the tip of the pipette to be placed very close to the surface of the sample. Fluorescent particles, coming from the inside of the micro-pipette, provide a light source for the SNOM that is being continuously renewed and prevent photobleaching.
- FSICM (Fast SICM), improving notably the speed of hopping mode has recently been developed.

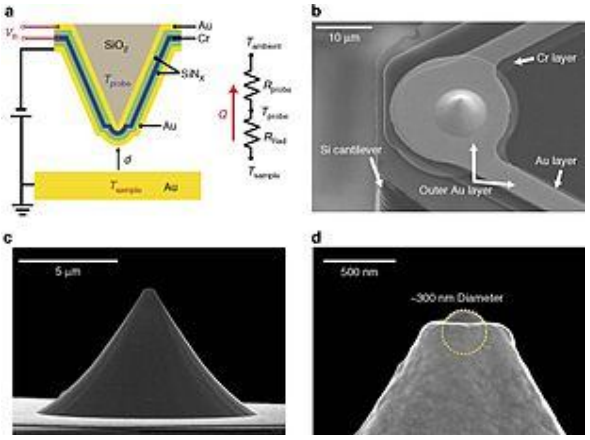
Scanning Thermal Microscopy (SThM)

Scanning thermal microscopy (SThM) is a type of scanning probe microscopy that maps the local temperature and thermal conductivity of an interface. The probe in a scanning thermal microscope is sensitive to local temperatures – providing a nano-scale thermometer. Thermal measurements at the nanometer scale are of both scientific and industrial interest.

(a) Schematics of experimental setup. An electric current is applied to the arms of an AFM cantilever (phosphorus-doped Si, P:Si) and heats up the end section above the tip (intrinsic Si, *i*-Si). The bottom lens excites a diamond nanocrystal with a green laser light and collects photoluminescence (PL). The crystal hosts an N-V center and is attached to the AFM tip. A wire on the sample surface serves as the microwave source (mw). The temperature of the cantilever T_h is determined from the applied current and voltage. (b) Optically detected magnetic resonance spectra of the N-V center at three temperatures. (c) Thermal conductivity image of a gold letter E on sapphire. White circles indicate features that do not correlate with the AFM topography. (d) PL image of the AFM cantilever end and tip where the diamond nanocrystal appears as the bright spot. (e) Zoomed PL image of the N-V center in d.



SThM using the N-V center in diamond.



Schematic and SEM images of a conventional SThM tip based on a Au–Cr thermocouple.

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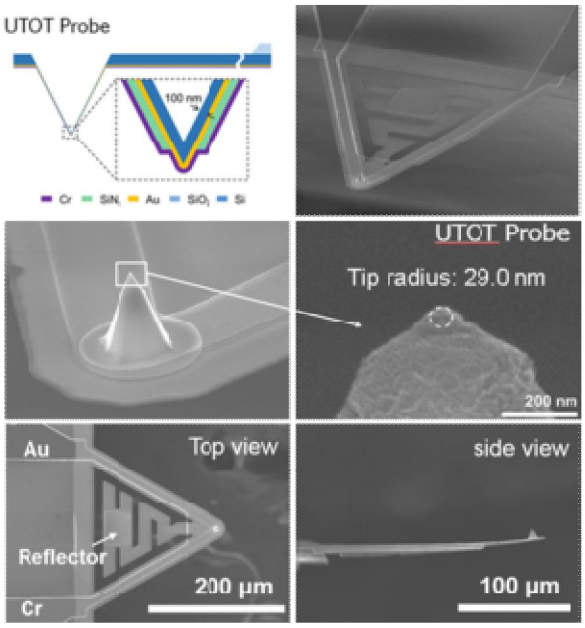

Schematic and SEM images of commercial SThM probe

Applications

SThM allows thermal measurements at the nano-scale. These measurements can include: temperature, thermal properties of materials, thermal conductivity, heat capacity, glass transition temperature, latent heat, enthalpy, etc. The applications include:

- Ultra large-scale integration (ULSI) lithography research and cellular diagnostics in biochemistry.
- Detecting such parameters as phase changes in polymer blends.
- Joule heating
- Measuring material variations in semiconductor devices
- Subsurface imaging
- Near-field photo thermal micro-spectroscopy
- Data storage
- Calorimetry applications
- Hot-spots in integrated circuits
- Low temperature scanning thermal microscopy
- Magnetic spectroscopy in combination with the ferromagnetic resonance realized in the SThM-FMR technique
- Other applications

Technique

SThM requires the use of specialized probes. There are two types of thermal probes: Thermocouple probes where the probe temperature is monitored by a thermocouple junction at the probe tip and resistive or bolometer probes where the probe temperature is monitored by a thin-film resistor at probe tip. These probes are generally made from thin dielectric films on a silicon substrate and use a metal or semiconductor film bolometer to sense the tip temperature. When the tip is placed in contact with the sample, heat flows from the tip to sample. As the probe is scanned, the amount of heat flow changes. By monitoring the heat flow, one can create a thermal map of the sample, revealing spatial variations in thermal conductivity in a sample. Through a calibration process, the SThM can reveal the quantitative

values of thermal conductivity. Alternately the sample may be actively heated, for example a powered circuit, to visualize the distribution of temperatures on the sample.
Tip-sample heat transfer can include

- Solid-solid conduction. Probe tip to sample. This is the transfer mechanism which yields the thermal scan.
- Liquid-liquid conduction. When scanning in non-zero humidity, a liquid meniscus forms between the tip and sample. Conduction can occur through this liquid drop.
- Gas conduction. Heat can be transferred through the edges of the probe tip to the sample.

Scanning Probe Microscopy (SPM)

Scanning probe microscope (SPM) is a branch of microscopy that forms images of surfaces using a physical probe that scans the specimen. The scanning tunnelling microscope is an instrument for imaging surfaces at the atomic level. The first successful scanning tunneling microscope experiment was done by Binnig and Rohrer. The key to their success was using a feedback loop to regulate gap distance between the sample and the probe. Many scanning probe microscopes can image several interactions simultaneously. The manner of using these interactions to obtain an image is generally called a mode.

The resolution varies somewhat from technique to technique, but some probe techniques reach a rather impressive atomic resolution. This is due largely because piezoelectric actuators can execute motions with a precision and accuracy at the atomic level or better on electronic command. This family of techniques can be called "piezoelectric techniques".

Image formation

To form images, scanning probe microscopes raster scan the tip over the surface. At discrete points in the raster scan a value is recorded (which value depends on the type of SPM and the mode of operation, see below). These recorded values are displayed as a heat map to produce the final STM images, usually using a black and white or an orange color scale.

Constant interaction mode

In constant interaction mode (often referred to as "in feedback"), a feedback loop is used to physically move the probe closer to or further from the surface (in the *z* axis) under study to maintain a constant interaction. This interaction depends on the type of SPM, for scanning tunneling microscopy the interaction is the tunnel current, for contact mode AFM or MFM it is the cantilever deflection, etc. In this mode a second image, known as the ″error signal" or "error image" is also taken, which is a heat map of the interaction which was fed back on. Under perfect operation this image would be a blank at a constant value which was set on the feedback loop. Under real operation the image shows noise and often some indication of the surface structure. The user can use this image to edit the feedback gains to minimise features in the error signal. If the gains are set incorrectly, many imaging artifacts are possible. If gains are too low features can appear smeared. If the gains are too high the feedback can become unstable and oscillate, producing striped features in the images which are not physical.

Constant height mode

In constant height mode the probe is not moved in the *z*-axis during the raster scan. Instead the value of the interaction under study is recorded (i.e. the tunnel current for STM, or the cantilever oscillation amplitude for amplitude modulated non-contact AFM). This

recorded information is displayed as a heat map, and is usually referred to as a constant height image.

Probe tips

The nature of an SPM probe depends entirely on the type of SPM being used. The combination of tip shape and topography of the sample make up a SPM image. However, certain characteristics are common to all, or at least most, SPMs. Most importantly the probe must have a very sharp apex. The apex of the probe defines the resolution of the microscope, the sharper the probe the better the resolution. For atomic resolution imaging the probe must be terminated by a single atom. For many cantilever based SPMs (e.g. AFM and MFM), the entire cantilever and integrated probe are fabricated by acid [etching]

Advantages

The resolution of the microscopes is not limited by diffraction, only by the size of the probe-sample interaction volume which can be as small as a few picometres. Hence the ability to measure small local differences in object height (like that of 135 picometre steps on <100> silicon) is unparalleled. Laterally the probe-sample interaction extends only across the tip atom or atoms involved in the interaction. The interaction can be used to modify the sample to create small structures. Unlike electron microscope methods, specimens do not require a partial vacuum but can be observed in air at standard temperature and pressure or while submerged in a liquid reaction vessel.

Disadvantages

The detailed shape of the scanning tip is sometimes difficult to determine. Its effect on the resulting data is particularly noticeable if the specimen varies greatly in height over lateral distances of 10 nm or less. The scanning techniques are generally slower in acquiring images, due to the scanning process. As a result, efforts are being made to greatly improve the scanning rate. Like all scanning techniques, the embedding of spatial information into a time sequence opens the door to uncertainties in metrology, say of lateral spacings and angles, which arise due to time-domain effects like specimen drift, feedback loop oscillation, and mechanical vibration. The maximum image size is generally smaller. Scanning probe microscopy is often not useful for examining buried solid-solid or liquid-liquid interfaces.

Visualization and analysis software

In all instances and contrary to optical microscopes, rendering software is necessary to produce images. Such software is produced and embedded by instrument manufacturers but also available as an accessory from specialized work groups or companies. The main packages used are freeware: Gwyddion, WSxM (developed by Nanotec) and commercial: SPIP (developed by Image Metrology), FemtoScan Online (developed by Advanced Technologies Center), MountainsMap SPM (developed by Digital Surf), TopoStitch (developed by Image Metrology).

Surface Plasmon Resonance Microscopy (SPRM)

Surface plasmon resonance microscopy (SPRM), also called surface plasmon resonance imaging (SPRI), is a label free analytical tool that combines the surface plasmon resonance of metallic surfaces with imaging of the metallic surface. The heterogeneity of the refractive index of the metallic surface imparts high contrast images, caused by the shift in the resonance angle. SPRM can achieve a thickness sensitivity of few tenths of nanometer and lateral resolution achieves values of micrometer scale. SPRM is used to characterize surfaces such as self-assembled monolayers, multilayer films, metal nanoparticles,

oligonucleotide arrays, and binding and reduction reactions. Surface plasmon polaritons are surface electromagnetic waves coupled to oscillating free electrons of a metallic surface that propagate along a metal/dielectric interface. Since polaritons are highly sensitive to small changes in the refractive index of the metallic material, it can be used as a biosensing tool that does not require labeling. SPRM measurements can be made in real-time. Wang and collaborators studied the binding kinetics of membrane proteins in single cells

Principle

Surface plasmons or surface plasmon polaritons are generated by coupling of electrical field with free electrons in a metal. SPR waves propagate along the interface between dielectrics and a conducting layer rich in free electrons. As shown in Figure 2, when light passes from a medium of high refractive index to a second medium with a lower refractive index, the light is totally reflected under certain conditions.

In order to get Total Internal Reflection (TIR), the θ_1 and θ_2 should be within a certain range that can be explained through the Snell's law. When light passes through a high refractive index media to a lower refractive media, it is reflected at an angle θ_2, which is defined in Equation 1.

$$\theta_2 = \sin^{-1}((\eta_1/\eta_2)\sin\theta_1) \quad \textbf{Eq. 1}$$

Figure. Total Internal Reflection (TIR), an incident beam of light with angle θ_1, is passing through a media with refractive index η_1, the beam reflects back at angle θ_2, when the refractive index of the medium changes to a value η_2.

In the TIR process some portion of the reflected light leaks a small portion of electrical field intensity into medium 2 ($\eta_1 > \eta_2$). The light leaked into the medium 2 penetrates as an evanescent wave. The intensity and penetration depth of the evanescent wave can be calculated according to Equations 2 and 3, respectively.

$$I_z = I_0 \exp^{-z/d_p} \quad \textbf{Eq. 2}$$

$$d_p = \lambda/(4\pi\sqrt{\eta^2}_1 \sin^2\theta_1 - \eta^2_2) \quad \textbf{Eq. 3}$$

Figure 3 shows a schematic representation of surface plasmons coupled to electron density oscillations. The light wave is trapped on the surface of the metal layer by collective coupling to the electrons of the metal surface. When the electron's plasma and the electric field of the wave light couple their frequency oscillations they enters into resonance.

Figure. Cartoon of polaritons propagation along a metal dielectric interface, rich and poor electron density regions are referred as + and –, respectively.

Recently, the leakage light inside of the metal surface had been imaged. Radiation of different wavelengths (green, red and blue) was converted into surface plasmon polaritons, through the interaction of the photons at the metal/dielectric interface. Two different metal surfaces were used; gold and silver. The propagation lengths of the SPP along the x-y plane (metal plane) in each metal and photon wavelength were compared. The propagation length is defined as the distance travelled by the SPP along the metal before its intensity decreases by a factor of 1/e, as defined in Equation 4.

Plasmon excitation methods

Surface plasmon polaritons are quasiparticles, composed by electromagnetic waves-coupled to free electrons of the conduction band of metals. One of widely used methods uses to couple p-polarized light with the metal-dielectric interface is prism based coupling. Prism couplers are the most widely used to excite surface plasmon polaritons. This method is also called Kretschmann-Raether configuration, where TIR creates an evanescent wave that couples the free electrons of the metal surface. Waveguide coupling is also used to create surface plasmons.

Prism Coupling

Kretschmann-Raether configuration is used to achieve resonance between light and free electrons of the metal surface. In this configuration a prism with high refractive index is interfaced with a metal film. Light from a source propagates through the prism is made incident on the metal film. As a consequence of the TIR, some leaked through metal film, forming evanescent wave in the dielectric medium as in Figure. The evanescent wave penetrates a characteristic distance into the less optically dense medium where it is attenuated.

Figure 6 shows the Kretschmann-Raether configuration, where a prism with refractive index of $\eta 1$ is coupled to a dielectric surface with a refractive index $\eta 2$, the incidence angle of the light is θ. The interaction between the light and the surface polaritons in the TIR can be explained by using the Fresnel multilayer reflection; the amplitude reflection coefficient (r_pmd) is expressed as follows in Equation 5.

$$r_{pmd} = \{[r_{pm} + r_{md}\, e^{(2ik_{mxq})}]/[1+ r_{pm}\, r_{md}\, e^{(2ik\_mx\ q)}]\} \quad \textbf{Eq. 5}$$

The power reflection coefficient R is defined as follows:

$$R=\|r_{pmd}\|^2 \quad \textbf{Eq. 6}$$

In Figure, a schematic representation of the Otto prism coupling prism is shown. In the Figure, the air gap was shown a little thick just to explain the schematic although in reality, the air gap is so thin between prism and metal layer.

Waveguide coupling

The electromagnetic waves are conducted through an optical waveguide. When light enters to the region with a thin metal layer, it evanescently penetrates through the metal layer exciting a Surface Plasmon Wave (SPW). In waveguide coupling configuration, the waveguide is created when the refraction index of the grating is greater than that of substrate. Incident radiation propagates along the waveguide layer with high refractive index. In Figure 8, electromagnetic waves are guided through a wave-guiding layer, once the optical waves reached the interface wave-guiding layer- metal an evanescent wave is created. The evanescent wave excites the Surface Plasmon at the metal-dielectric interface.

Grating coupling

Due to the periodic grating, the phase matching between the incident light and the guide mode is easy to obtain. According to Equation 7, the propagation vector (Kz) in the z direction can be tuned by changing the periodicity Λ. The grating vector can be modified, and the angle of resonant excitation can be controlled. In Figure 9, q is the diffraction order it can have values of any integer (positive, negative or zero).

$$K_z = q \{2\pi/\Lambda\} \quad \textbf{Eq.7}$$

Resonance measurement methods

The propagation constant of a monochromatic beam of light parallel to the surface is defined by Equation 8.

$$k_x = [2\pi/\lambda]\, n_p \sin\theta \quad \textbf{Eq. 8}$$

Where θ is the angle of incidence, k_sp is the propagation constant of the surface plasmon, and n_(p) is the refractive index of the prism. When the wave vector of the SPW, ksp matches the wave vector of the incident light kx, SPW is expressed as:

$$k_{sp} = [2\pi/\lambda] \sqrt{(\varepsilon_m+\varepsilon_d)/(\varepsilon_m+\varepsilon_d)} \quad \textbf{Eq. 9}$$

εd and εm represent the dielectric constant of dielectrics and the metal while the wavelength of the incident light corresponds to λ. kx and ksp can be represented as:

$$\theta_{sp} = \sin^{-1} \sqrt{[\varepsilon_m\varepsilon_d/(\varepsilon_p(\varepsilon_m + \varepsilon_d)]} \quad \textbf{Eq. 10}$$

The surface plasmons are evanescent waves that have their maximum intensity at the interface and decay exponentially away from the phase boundary to a penetration depth. The propagation of the surface plasmons is intensely affected by a thin film coating on the conducting layer. The resonance angle (θ) shifts, when the metal surface is coated with a dielectric material, due to the change of the propagation vector (k) of the surface plasmon. This sensitivity is due to the shallow penetration depth of the evanescent wave. Materials with a high amount of free electrons are used. Metal films of roughly 50 nm made of copper, titanium, chromium and gold are used. However, Au is the most common metal used in SPR as well as in SPRM.

Lateral resolution

The resolution of a conventional light microscopy is limited by the light diffraction limit. In SPRM, the excited surface plasmons adopt a horizontal configuration from the incident beam light. The polaritons will travel along the metal-dielectric interface, for a determined period, until they decay back into photons. Therefore, the resolution achieved by SPRM is determined by the propagation length ksp of the surface plasmons parallel to the incident plane. The separation between two areas should be approximately the magnitude of ksp in order to be resolved. Berger, Kooyman and Greve showed that the lateral resolution can be tuned by changing the excitation wavelength, the better resolution is achieved when the excitation energy increases. Equations 4 and 12 defines the magnitude of the wave vector of the surface plasmons.

$$k_{sp} = (2\pi/\lambda) \sqrt{[(n_2^2\, n_g^2)/(n_2^2 + n_g^2)]} \quad \textbf{Eq. 12}$$

Where n_2 the refractive index of medium 2 is, n_g is the refractive index of the metal film and λ is the excitation wavelength.

Instrumentation

The surface plasmon resonance microscopy is based on surface plasmon resonance and recording desired images of the structures present on the substrate using an instrument equipped with a CCD camera. In the past decade, SPR sensing has been demonstrated to be an exceedingly powerful technique and used quite extensively in the research and development of materials, biochemistry and pharmaceutical sciences.

The SPRM instrument works with the combination of the following main components: source light (typically He-Ne laser), that further travels through a prism that is attached to a glass side, coated with a thin metal film (typically gold or silver), where the light beam reflects at the gold/solution interface at an angle greater than the critical angle. The reflected light from the interface surface area is recorded by a CCD detector, and an image is recorded. Although the above-mentioned components are some important for SPRM, additional accessories such as polarizers, filters, beam expanders, focusing lenses, rotating stage, etc., similar to several imaging methods are installed and used in the instrumentation for an effective microscopic technique as demanded by the application. Figure 12 shows a typical SPRM. Depending on the applications, and to optimize the imaging technique, the researchers modify this basic instrumentation with some design changes that even include altering the source beam. One of such design changes that resulted in a different SPRM is an objective-type as shown in Figure 11 with some modification in the optical configuration.

Sample preparation

To perform measurements for SPRM, the sample preparation is a critical step. There are two factors that can be affected by the immobilization step: one is the reliability and reproducibility of the acquire data. It is important to ensure stability to the recognition element; such as antibodies, proteins, enzymes, under the experiment conditions. Moreover, the stability of the immobilized specimens will affect the sensitivity, and/or the limit of detection (LOD). One of the most popular immobilization methods used is Self-Assembled Monolayer (SAM) on gold surface. Jenkins and collaborators 2001, used mercaptoethanol patches surrounded by SAM composed of octadecanethiol (ODT) to study the adsorption of egg-phosphatidylcholine on the ODT SAM. A pattern of ODT-mercaptoethanol was made onto a 50 nm gold film. The gold film was obtained through thermal evaporation on a LaSFN 9 glass. The lipid vesicles were deposited on the ODT SAM through adsorption, giving a final multilayer thickness greater than 80 Å.

11-Mercaptoundecanoic acid-Self assembled monolayer (MUA-SAM) were formed on Gold coated BK7 slides. A PDMS plate was masked on the MUA-SAM chip. Clenbuterol (CLEN) was attached to BSA molecules through amide bond, between the carboxylic group of BSA and the amine group of CLEN molecules. In order to immobilize BSA on the gold surface, the spots created through PDMS making were functionalized with sulfo-NHS and EDC, subsequently 1% BSA solution was poured in the spots and incubated for 1 hour. Non-immobilized BSA was rinsed out with PBS and CLEN solution was poured on the spots, unimmobilized CLEN was removed through PBS rinse. An alkanethiol-SAM was prepared in order to simultaneously measure the concentration of horseradish peroxidase (Px), Human Immunoglobulin E (IgE), Human choriogonadotropin (hCG) and Human immunoglobulin G (IgG), through SPR. The alkanethiols made of carbon chains composed by 11 and 16 carbons

were self-assembled on the sensor chip. The antibodies were attached to the C16 alakanethiol, which had a terminal carboxylic group.

The micro patterned electrode was fabricated by gold deposition on microscope slides. PDMS stamping was used to produce an array of hydrophilic/hydrophobic surface; ODT treatment followed by immersion in 2-mercaptoethanol solutions rendered a functionalized surface for lipid membranes deposition. The patterned electrode was characterized through SPRM. In the Figure 14 B, the SPRM image reveals the size of the pockets, which was 100 um x 100 um, and they were 200 um apart. As is seen in the image the remarkable contrast of the image is due to the high sensitivity of the technique.

Applications

SPRM is a useful technique for measuring concentration of biomolecules in the solution, detection of binding molecules and real time monitoring of molecular interactions. It can be used as biosensor for surface interactions of biological molecules: antigen-antibody binding, mapping and sorption kinetics. For example, one of the possible reasons of Type 1 diabetes of children is the high-level presence of Cow's milk antibodies IgG, IgA, IgM (mainly due to IgA) in their serum. Cow's milk antibodies can be detected in the milk and serum sample using SPRM. SPRM is also advantageous to detect the site-specific attachment of lymphocyte B or T on antibody array. This technique is convenient to study the label free and real time interactions of cells on the surface. So SPRM can be served as diagnostic tool for cell surface adhesion kinetics. Besides its merits, there are limitations of SPRM though. It's not applicable for detecting low molecular weight molecules. Although it's label free but will need to have crystal clean experimental conditions. Sensitivity of SPRM can be improved with coupling of MALDI-MS. There are a number of applications of SPRM from which some of them are being described here.