Three-dimensional visualization of contact networks in granular material
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This chapter describes the different techniques that are used in the visualization of particles in granular material. The main technique that is used in this respect is confocal microscopy, a high resolution imaging method that provides high three-dimensional optical resolution and contrast. In addition, numerous other techniques have been employed in this project, like absorption and fluorescence spectroscopy and AFM studies. The pressure sensitivity of the fluorescent probes has been investigated with a specially designed high pressure set-up, in which hydrostatic pressure can be applied to the fluorescent probe solutions.
3.1. Steady state spectroscopy

Steady state spectroscopy uses radiation to obtain information on the structure and properties of matter. The basic principle is to shine a beam of electromagnetic radiation onto a sample, and observe how the sample responds to such a stimulus. Generally, this response is recorded as an absorbance spectrum or emission spectrum, wherein the intensity (amount of absorbed or emitted light) is plotted as a function of radiation wavelength. These spectra reveal the spectral and electronic properties of the investigated sample.

The theory behind absorption and fluorescence is discussed in section 2.1. In the following sections, the details on the employed apparatus and methods in this project are given.

3.1.1. Steady state absorption

Absorption measurements of solutions usually obey the Lambert-Beer law (equation 3.1).[1] The molar absorption coefficient \( \varepsilon \) is a measure of the extent to which a molecule absorbs light at a given wavelength. It is a characteristic parameter of a molecule and can be derived from the absorbance \( A \) at that wavelength via the Lambert-Beer law:

\[
A(\lambda) = \varepsilon(\lambda) \times c \times l \tag{Eq. 3.1}
\]

In this formula, \( l \) is the path length of the light through the sample in cm and \( c \) is the concentration of the compound under investigation in solution. \( A \) is dimensionless, \( \varepsilon \) is usually given in the units of \( \text{L mol}^{-1} \text{cm}^{-1} \).

In this project, electronic absorption spectra were recorded on a double beam Cary 3E spectrophotometer (Varian) with a spectral range of 190 – 900 nm, using a scan speed of 100 nm/min. The compounds were dissolved in spectrograde quality solvents and measured in a quartz cuvette with a path length of 1.0 cm (Hellma).

3.1.2. Steady state fluorescence

The efficiency of fluorescence is given by the fluorescence quantum yield \( \Phi_f \), which is defined as the ratio of the number of emitted photons to those absorbed. The quantum yield for fluorescence can also be expressed in terms of rate constants of radiative and non-radiative decay (equation 3.2).[2]

\[
\Phi_f = \frac{k_F}{k_F + k_{ISC} + k_{IC}} \tag{Eq. 3.2}
\]
Here, $k_F$ is the rate constant of fluorescence, and the non-radiative decay includes intersystem crossing ($k_{ISC}$) and internal conversion ($k_{IC}$). For weakly luminescent compounds, $k_F \ll k_F + k_{ISC} + k_{IC}$, and thus $\Phi_F \approx 0$, while $\Phi_F$ approaches 1 for the best chromophores, as $k_{ISC}$ and $k_{IC}$ approach 0.

Because of the difficulty in determining the correct quantum yields in an absolute fashion, the quantum yield is commonly determined relative to a chromophore with known quantum yield, the reference compound designated as REF in equation 3.3.

$$\Phi_F = \Phi_{\text{REF}} \times \frac{l}{l_{\text{REF}}} \times \frac{A_{\text{REF}}}{A} \times \frac{n^2}{n_{\text{REF}}}$$

(Eq. 3.3)

In equation 3.3, $l$ is the integrated emission intensity, $A$ is the absorption factor at the excitation wavelength and $n$ is the refractive index of the medium. These are determined for the compound under investigation and the reference compound. $\Phi_{\text{REF}}$ is the quantum yield of the reference compound. In this thesis, two different reference compounds are used; perylene red in chloroform ($\Phi_F = 0.96$) and perylene orange in acetonitrile ($\Phi_F = 0.98$).

Fluorescence spectra were recorded on a Spex Fluorolog 3 spectrometer (HORIBA Jobin Yvon), which was provided with double grating monochromators in the excitation and emission channels. The excitation monochromators have a grating groove density of 1200 grooves per mm and those for the emission have 600 grooves per mm. The excitation light source was a 450W Xe arc lamp (Osram) and the detector a Peltier cooled R636-10 photomultiplier tube (Hamamatsu). A scan speed of 1 nm/s was used. Fluorescence spectra were corrected for the wavelength response of the detection system and the intensity of the source lamp.

The fluorescence of the compounds in solution is detected in the right angle mode in 1.0 cm quartz cuvettes. All solvents were of spectrograde quality and the concentration was chosen such that $A < 0.10$, so correction for the inner filter effect is not necessary. For detection of the fluorescence of the cover slips, the front face mode was used. The excitation wavelength ($\lambda_{\text{exc}}$) was 525 nm for the solvatochromic compounds and 495 nm or 475 nm for the rigidochromic compounds.

3.2. Confocal microscopy

Confocal fluorescence microscopy is a high resolution imaging technique that provides improved three-dimensional optical resolution and contrast compared to conventional microscopy. This is accomplished by selective illumination of a small part of the sample and suppressing any light coming from out of focus-planes. In this way, fluorescence can be recorded from a very small volume (‘single point’) within the sample. By varying the x- and y-positions of the focus spot while keeping the z-position constrained, two
dimensional images can be obtained by series of single point measurements. Stacks of such x,y-images, while changing the z-position in steps, together ultimately form a three dimensional reconstruction of the sample. By scanning through the sample in this fashion, very sharp images can be obtained. The images in figure 3.1 exemplify the striking improvement of the image obtained by confocal microscopy (B) compared to one recorded by a conventional microscope (A).\[9\]

The concept of confocal microscopy was developed by Minsky in 1957.\[10\] This first trial version did not yet give an improvement of the quality when compared to the images obtained by conventional microscopy. However, the idea of confocal microscopy was born and a few years later, Egger and Petran designed the first optimized purely analog confocal microscope, in which laser light and a oscillating objective were used to scan the sample.\[11\] In the 1970s, a specimen scanning confocal microscopy was developed by Cremer and Cremer, in which the sample is moved and the laser beam is kept in place.\[12\] Nowadays, specimen scanning is replaced by laser scanning. Such Laser Scanning Confocal Microscopy (LSCM)\[13\] is a popular tool in industry, in life science research\[14-18\] and material sciences.\[19-22\] LSCM uses a pair of mirrors to scan the laser light over the sample, and in this way visualizes each point of it.

3.2.1 The basic concept of confocal microscopy

Single point measurements in a sample are accomplished by placing a pinhole in front of the detector, which blocks all light coming from out-of-focus planes. Only light that is in-focus can pass through the pinhole. This is in marked contrast with conventional microscopy, where everything in the optical path is excited and all the fluorescence is detected. In confocal microscopy, only the fluorescence from (very close to) the focal plane can be detected.

In a confocal microscope, a laser is used as light source to illuminate the sample. The light from the laser passes through the excitation pinhole or a single mode optical fiber and via a dichroic mirror, the light is focused by a microscope objective in a small spot in the sample, where it can excite a fluorescent probe.\[23\] In figure 3.2, a schematic representation of the confocal microscopy set-up is depicted.
The emitted light (fluorescence) travels back from the sample to the detector via the objective and the dichroic mirror, after which it hits the detection pinhole. This pinhole filters all out-of-focus emission light, i.e. from a different depth in the sample. Only the rays of light from exactly the focal plane are able to pass through the pinhole (see figure 3.2). In this way very small volumes of detection are obtained.

The excitation volume of the sample is determined by the lateral resolution in the x,y-plane on the one hand and the axial resolution in the z-direction on the other hand, and is limited by the wave nature of light. Due to diffraction of the wave, radiation of a point light source is detected as a three dimensional pattern, which is referred to as the point-spread function (PSF) and is shaped as a so-called Airy disk. Such Airy disks consist of a central bright spot and progressively weaker concentric dark and bright rings (figure 3.3 (A)).

The lateral resolution of a confocal microscopy measurement, i.e. the ability to distinguish two separate points as distinct entities, is dependent on the radius $r$ of the Airy disk, which is defined as the distance from the center of the bright spot to the first minimum (the first dark ring, figure 3.3 (B)). According to the Rayleigh Criterion, two spots are resolved when the maximum of one coincides with the first minimum of the other (see figure 3.3 (C)). Thus, emitters that are spaced apart by a distance $r$ are considered resolved.
The radius $r_{x,y}$ of the Airy disk depends on the wavelength of the laser ($\lambda_{\text{exc}}$) and the numerical aperture of the objective ($NA_{\text{obj}}$), as given by equation 3.5. $NA_{\text{obj}}$ in turn is dependent on the refractive index of the medium ($n$) between the objective and the sample and the angular aperture $\alpha$ of the lens in the objective (equation 3.6).

$$r_{x,y} = 0.61 \times \frac{\lambda_{\text{exc}}}{NA_{\text{obj}}} \quad \text{(Eq. 3.5)}$$

$$NA_{\text{obj}} = n \times \sin(\alpha) \quad \text{(Eq. 3.6)}$$

In practice, the lateral excitation width is approximated by the full width at half maximum (FWHM) of the Airy distribution ($w_{x,y}$; equation 3.7), about 84% of the value for $r$.\textsuperscript{[23]}

$$w_{x,y} = 0.51 \times \frac{\lambda_{\text{exc}}}{NA_{\text{obj}}} \quad \text{(Eq. 3.7)}$$

Similar to the Rayleigh Criterion in the lateral direction, the axial resolution can be determined by the minimal distance at which two emitters are resolved. This radius $r_z$ of the PSF in the z-direction is given by equation 3.8. Again, in practice the full width at half maximum of the Airy distribution of the PSF in the z-direction is used (equation 3.9), about 88% of the value for $r_z$.\textsuperscript{[23]}

$$r_z = 2 \times \frac{\lambda_{\text{exc}} \times n}{(NA_{\text{obj}})^2} \quad \text{(Eq. 3.8)}$$

$$w_z = 1.76 \times \frac{\lambda_{\text{exc}} \times n}{(NA_{\text{obj}})^2} \quad \text{(Eq. 3.9)}$$

Thus, the crucial factors that determine the size of the excitation volume are the numerical aperture of the objective, the refractive index of the object medium and the wavelength of excitation. Looking ahead, using an objective with $NA_{\text{obj}} = 1.4$ and oily object medium with $n = 1.516$, gives values for $w_{x,y} = 178$ nm and $w_z = 664$ nm (for $\lambda_{\text{exc}} = 488$ nm) and $w_{x,y} = 204$ nm and $w_z = 762$ nm (for $\lambda_{\text{exc}} = 560$ nm).
Using an objective with an as high as possible value for $NA_{\text{obj}}$ not only gives rise to small excitation volumes, but also increases the collection efficiency. Part of the fluorescence is collected by the objective and transmitted to the detector. Here, the detection pinhole, which is placed in front of the detector, ensures free passage of the in-focus fluorescence, as graphically depicted in figure 3.2.

Reducing the size of the pinhole results in filtering of the background light, originating from out-of-focus planes in the z-direction. A too large pinhole would allow too much out-of-focus light to reach the detector, while with a too small pinhole valuable photons are disregarded. When determining the size of the pinhole, one has to take the magnification $M$ of the objective into account. Thus, an Airy disk with a radius of 500 nm results in a projection of 50 $\mu$m when an objective with a magnification of 100 is used. The optimal pinhole diameter $d$ is determined by equation 3.10:

$$d = \left(2.5 \times \frac{\lambda_{\text{exc}}}{\pi}\right) \times \left(\frac{M}{NA_{\text{obj}}}\right)$$  \hspace{1cm} (Eq. 3.10)

Thus, for a $\lambda_{\text{exc}}$ of 488 nm, the optimal pinhole diameter $d$ is 28 $\mu$m, and $d$ is 32 $\mu$m for a $\lambda_{\text{exc}}$ of 560 nm. The size of the pinhole not only determines the detection volume, but also the amount of photons that reach the detector. For faintly fluorescing molecules, the optimal pinhole size would result in too dark images. Thus, the resolution of the confocal microscopy image can be enhanced by using brightly fluorescing probes, enabling a small detection pinhole.

### 3.2.2. Confocal imaging of particles with a fluorescent surface

When an image is recorded from a certain height in a sample (say at $z = 0$ $\mu$m), light is collected from the focal plane at $z = 0$ $\mu$m, but also from slightly above and below this focal plane. The fraction of the light collected from the focal plane is highest, and diminishes when going away from the plane via the Airy disk-shaped PSF, with the value for $w_z$ as determined by equation 3.9 as the FWHM value. This is graphically depicted in figure 3.4 for a fluorescent particle.

The theoretical cross section of the fluorescent spot of a particle with $\phi = 10$ $\mu$m, which appears in the x,y-image obtained at exactly $z = 0$ $\mu$m is depicted in figure 3.5 (turquoise line). Changing the focus from $z = 0$ $\mu$m to $z = -2$, -1, -0.2, 0.2, 1, 3 or 4.8 $\mu$m.
results in the cross sections as given in figure 3.5. Clearly at \( z > 0 \) \( \mu m \), two peaks appear, changing the spot into a circular perimeter of the particle. The intensity at the center of the spot does not drop immediately to zero when \( z \) rises slightly above \( 0 \) \( \mu m \), but a valley appears between the two maxima. This is already apparent at \( z = 0.2 \) \( \mu m \). Likewise, when \( z \) drops slightly below \( 0 \) \( \mu m \), some fluorescence is still observed from the bottom of the particle. Thus, when the focal plane is at exactly \( z = 0 \) \( \mu m \), a flattened Gaussian cross section is expected, while slightly out-of-focus imaging gives rise to either two maxima with a small valley (\( z > 0 \) \( \mu m \)) or a Gaussian without flattening and with lower intensity (\( z < 0 \) \( \mu m \)).

![Figure 3.5](image)

**Figure 3.5.** Fluorescence intensity cross sections of a particle (\( \varnothing = 10 \) \( \mu m \)) at various values for \( z \).

The cross sections given in figure 3.5 are calculated using an experimentally determined value for \( w_z \) of 1.0 \( \mu m \) for the Olympus confocal microscope on the PicoQuant set-up used in this project (see section 3.2.3) at \( \lambda_{\text{exc}} = 560 \) nm,\[27\] which is somewhat increased compared to the theoretical value obtained from equation 3.9. Notably, the PSFs in the x- and y-direction are not taken into account in figure 3.5. Those are much smaller compared to \( w_z \), but this may account for an increase in the diameter of the fluorescent spot. In this model, the expected diameter (FWHM value) of the fluorescent spot is 5.6 \( \mu m \), in case the focal plane is aligned at exactly \( z = 0.0 \) \( \mu m \), excitation occurs at 560 nm and no deformation of the particle occurs.

### 3.2.3. Confocal microscopy experiments

In this project, two confocal microscopy set-ups were used. Experiments were performed on a Confocal Line Scanner Microscope (Zeiss, LSM 5) or a MicroTime 200 apparatus (PicoQuant GmbH). In the latter, experiments were performed with excitation wavelengths of 560 nm and 488 nm, while on the Zeiss microscope an
excitation wavelength of 532 nm was used. On the PicoQuant set-up, the confocal fluorescence images were created using an inverted light microscope (IX71, *Olympus*), equipped with an oil immersion, 100x 1.4NA objective (UplanSApo, *Olympus*), mounted on a piezo-scanning stage (*Physik Instruments GmbH*). For excitation a Titanium:Sapphire laser (*Chameleon Ultra-II, Coherent*) was used, operating at a repetition rate of 80 MHz, with pulse widths of ~150 fs. The PicoQuant set-up is schematically represented in figure 3.6.

![Diagram of experimental setup](image)

**Figure 3.6.** Confocal microscopy set up (Microtime 200).

### 3.2.3.1. Excitation at 560 nm

For the experiments on the Microtime 200 with an excitation wavelength of 560 nm (for the solvatochromic probe), the Titanium:Sapphire laser tuned to 832 nm was used to pump an OPO (Optical Parametric Oscillator, *Mira OPO, Coherent*), which delivered 380 mW at a wavelength of 560 nm. Excitation light was coupled into the adapted confocal unit via polarization maintaining monomode glass fiber (PMC-620-4-NA011-3- XPC-P, *Schaffer + Kirchhoff*) and filtered using an excitation filter (560/40x, *Chroma Tech*.) A dichroic mirror (595DCLP, *Chroma Tech.*) reflected excitation light to the sample and collected back the emission light from it. A pinhole with diameter of 50 μm was applied. Residual excitation light was filtered from emission light by applying a long wave pass filter (LP665) and a notch filter (561NF, *Semrock*). The filter set applied for the measurements is shown in figure 3.7. Emission light passed through a 50/50, 80/20 or 20/80 beam splitter, which directs part of the light to the SPAD (single photon avalanche diode, SPCM-AQR-13, *Perkin Elmer*) and the rest to the spectrograph (Spectra Pro-150, *Acton Research Corp*), equipped with a grating blazed at 500 nm, 150 g/mm with optimal wavelength range from 330 – 950 nm. Spectra were recorded from the light from the spectrograph, as captured by a high sensitivity EMCCD camera (PhotonMax 512B, *Roper Scientific*).
3.2.3.2. Excitation at 488 nm

For the experiments with an excitation wavelength of 488 nm (for the rigidochromic probe), the output of the laser was tuned at 976 nm. This was frequency doubled by an SHG (second harmonic generator, APE) to generate excitation pulses of 488 nm. The laser power was 170 mW. Via a polarisation maintaining monomode fiber (PMC-620-4-NA011-3-XPC-P, Schafter + Kirchhoff) the excitation light was coupled into the confocal unit. In front of the excitation light, an excitation filter (HQ480/40x, Chroma Tech.) and a dichroic mirror (Z488 RDC, Chroma Tech.) were placed, which reflects the excitation light into the sample. Emission light passed through the dichroic mirror, a notch filter (488NF, Semrock), a pinhole with a diameter of 50 μm and an emission filter (HQ510LP, Chroma Tech.) to a SPAD (SPCM-AQR-13, Perkin Elmer). The filter set
applied for the measurements is shown in figure 3.8. Emission light is led to the SPAD and the spectrograph with EMCCD camera identically to the 560 nm excitation set up.

3.2.3.3. Excitation at 532 nm
Both the solvatochromic particles and rigidochromic cover slips were imaged using a confocal microscope (Zeiss Axiovert 200M) and a microscope control system (Zeiss LSM 5 LIVE). A laser at a wavelength of 532 nm with power of 75 mW was used. An oil immersion 25x 0.8 NA or 63x 1.4 NA objective (Zeiss Plan APOCHROMAT) were used during those measurements. Emission filter was a bandpass filter (BP700-750). The scaling factor in the x,y-direction was 0.52 μm per pixel for the 25x objective, and 0.21 μm per pixel for the 63x objective. Images have been taken with a detector gain of 30 with a scan speed of 4 frames per second and the pinhole was set to 13.8 μm.

3.2.4. Sample preparation for PMMA particles (Ø = 10 μm)
For the confocal microscopy measurements with the 10 μm PMMA particles, a specially made sample holder was designed (figure 3.9). A glass cylinder was glued with silicon glue on top of a microscope cover slip. A plunger that fits perfectly into the glass cylinder was used to put a force on the sample. This plunger is made of porous material (glass filter), in such a way that the particles remain in the sample holder, but the solvent is able to pass through. As such, the particles experience pressure from a weight placed on top of the plunger. The weight of the plunger itself is 2.0 g. A 100 mg/ml solution of NaI in DMSO is used to match the refractive index of the PMMA particles. Locations within the sample are described using x-, y- and z-coordinates, with z = 0 μm corresponding to the bottom of the sample cell.

![Figure 3.9](image)

**Figure 3.9.** Schematic representation (A) and a picture (B) of the sample cell used in confocal microscopy. Sample cell dimensions: Ø_{inner} = 0.8 cm, h = 1.7 cm; plunger dimensions: Ø = 0.8 cm.
3.2.5. Sample preparation for rigidochromic cover slips

Besides the fluorescent particles, functionalized rigidochromic cover slips were used as well. To measure local pressure, the set-up depicted in figure 3.10 was used. With help of a rheometer (I), a glass bead was pressed with a known force on the cover slip. The glass bead (II) was glued to the upper rheometer plate, enabling us to measure the applied normal force. Because the rigidochromic probe does not work properly in air (see chapter 4), the cover slip has been kept wet with some DMSO during measurements. DMSO was put on both the cover slip of the microscope (IV) and the functionalized cover slip (III).

![Figure 3.10](image)

**Figure 3.10.** Schematic representation (A) and a picture (B) of the measurements with the rigidochromic cover slips. Rheometer rod (I) with a glued glass bead (II), approaches the functionalized rigidochromic cover slip (III), soaked between two layers of DMSO to keep it wet during measurements. Wet cover slip III is placed on top of a unfunctionalized cover slip (IV).

3.3 Streak camera

The solution phase life time measurements are performed using a streak camera. A pulsed diode laser LDH-P-C-440 (PicoQuant) emits 440 nm (<90 ps width) with a repetition rate of up to 40 MHz (PDL 800-B, PicoQuant). A Hamamatsu streak camera was used in the photon counting mode, containing a spectrograph (IS250, Chromex), an slow speed sweep unit (M5677), a trigger unit (C4792), a blanking unit (C5680) and a digital CCD camera (C4742-95).

In this device, fluorescence light enters via a narrow slit, where it is deflected perpendicularly in a time dependent fashion. Photons that enter the streak camera later end up at a position further away on the detector, thus forming a streak corresponding to a decay curve.
3.4 Measurements with the high pressure set-up
To measure the effect of solvent pressure on the fluorescence, we use a high pressure set-up. This set-up consist of an optical high pressure cell, depicted in figure 3.11, which is connected to a system for generating high liquid pressure (figure 3.12). A cuvette (8) is fixed in the middle of the cell block (dimensions 90 × 90 × 125 mm), containing two perpendicular optical paths intersecting at the cuvette and closed off at both ends with sapphire windows (14). The quarts sample cuvette consists of a lower square shaped part, where the measurement takes place with an optical path length of 5 mm, and an upper cylindrical part upon which a movable Teflon piston (5) exerts pressure. The high pressure generating system is connected to this piston via high pressure tubing (3).

Figure 3.11. Representation of the optical high pressure cell plus cuvette. The dashed lines (---) represent the optical paths. (A) Side view, (B) top view showing the perpendicular arrangement of the optical paths and (C) cartoon representation of the outside.

The pressure is applied to the sample using a high pressure generating system as depicted in figure 3.12. A high pressure screw piston pump (4) is capable of generating pressures up to 4.5 kbar, that are transmitted to the optical high pressure cell using a liquid for transmitting the pressure (n-hexane). A low pressure piston pump (3) is
present to fill the circuit with \( n \)-hexane and to apply a pre-pressure of 200 bar. The pressure is measured by two pressure gauges; a low pressure one (1; 0 – 1000 bar) and a high pressure one (2; 0 – 6000 bar). The connection between the different parts of the system can be controlled with several needle valves (5 – 14).

![Diagram](image)

**Figure 3.12.** High pressure generating system.\(^{[28]}\)

### 3.5. Atomic force microscopy

Surfaces of samples can be imaged with high resolution by Scanning Probe Microscopy (SPM), a group of microscopy techniques in which the surface of a material is scanned with a physical probe. Such an image of the surface is recorded by mechanically moving a sharp tip over that surface, in which the movement occurs in a raster (line by line), and the interaction of the tip with the surface is recorded as a function of position. Scanning Tunneling Microscopy, which was developed by Binnig and Rohrer in 1981, is the first SPM technique. For the first time, individual atoms could be imaged on the surface of several materials,\(^{[29-31]}\) which was rewarded with the Nobel Prize for Physics in 1986.

Over the years, other SPM techniques emerged. Atomic Force Microscopy (AFM) is such a high-resolution microscopy method, which allows to obtain images of materials in the order of nanometers. The main advantage of AFM, developed by Binnig, Quate and Gerber in 1986,\(^{[32]}\) is the wide variety of materials that can be imaged, including non-conducting surfaces.

The basic set-up of an AFM experiment is depicted in figure 3.13. Paramount is the cantilever with a sharp tip on the end. This sharp tip scans the surface of the material and produces an image of it. Especially the shape and sharpness of the tip determines
Experimental techniques

the lateral resolution of the image, which may be in the order of nanometers. When the tip is in close proximity to the surface, forces between the tip and the sample cause bending of the cantilever. Depending on the stiffness $k$ and the amount of bending of the cantilever (deflection $z$), Hooke’s law, which is given in equation 3.11, gives the force $F$ present between the tip and the sample.

$$F = -k \times z$$

(Eq. 3.11)

The inclination of a laser beam, which is reflected from the surface of the cantilever, determines the deflection $z$. Stiffness $k$ is determined by the material and geometry of the cantilever.

A piezoelectric scanner controls the movement of the cantilever, to avoid that the tip collides with the surface. This scanner ensures a constant force between the tip and the sample and thus moves the cantilever in the $z$-direction depending on the magnitude and direction of the force. In this way, the same distance can be maintained while the sample is scanned in the $x$- and $y$-direction.

More recent designs have the tip mounted on a vertical piezoelectric scanner, which directly influences the $z$-position of the tip, circumventing a cantilever altogether. Another piezo block governs the scan in the $x,y$-plane and the topography of the sample is given as a map of the surface area.

Several types of forces may act on the tip and the cantilever, graphically depicted in figure 3.14, which all contribute to its deflection. Attractive Van der Waals forces exists between the atoms of the tip and the atoms of the sample when the tip approaches the surface (grey line in figure 3.14). However, when the tip gets too close, it collides with the surface, resulting in repulsive forces between the electron clouds (black line). At a distance between tip and surface of a few tenths of a nanometer, the net force is zero and becomes repulsive at smaller distance.\(^\text{33}\)

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**Figure 3.13.** Schematic representation of an atomic force microscope.\(^\text{34}\)
The AFM can be operated in a number of modes, depending on the application. In general, those modes are divided into two groups: static (also called contact mode) and dynamic (non-contact or "tapping") mode.

In the static mode, the deflection of the cantilever is kept constant while the tip is scanning (figure 3.15, (A)). The cantilever moves up and down with the topography of the surface. In this case, the distance between the tip and the sample is less than a few tenths of a nanometer and thus the forces that are present are repulsive. Using this mode, a surface topography with many valleys, crests and ridges often gives rise to faults in the image and increased chance of sample and tip damage. The advantage of measuring in this mode is that the recorded image of the surface is not affected by any liquid, which may be present on the surface, enabling high resolution surface imaging in liquid.\[^{36}\]

The dynamic mode of AFM can be subdivided in two main measurement types; the non-contact mode and the intermittent contact mode. In both measurements the cantilever oscillates above the surface to be imaged. In the non-contact mode (figure 3.15 (C)), the magnitude of the attractive forces between the tip and the surface influences the vibrational amplitude of the cantilever, without the tip ever touching the surface. However, attractive forces may also exist with any liquid on the surface, so the surface of the liquid is imaged, instead of the surface under investigation. Using this method, there is no chance of tip or sample damage. The intermittent contact mode actually uses the same working principle, but the tip touches the surface during each vibrational cycle (figure 3.15 (B)). In this mode, any chance on damage is prevented, but the presence of liquid does not pose a problem on imaging of the surface.
AFM studies are performed on a Nanosurf Easyscan 2 (Nanosurf GmbH), operating in the contact mode. The setpoint, which is a certain measure of the force applied to the sample (deflection of the cantilever), for approaching and scanning is set to 10 nN, with an Integral Gain (IGain) value of 1000 and a Proportional Gain (PGain) value of 10000. The IGain and PGain regulate the sensitivity of the feedback loops and thus the accuracy with which the controller maintains the constant force value. The scan speed is set to 1 – 3 s/line.

AFM samples are prepared by placing a glass cylinder (Ø = 10 mm) on top of a clean cover slip, in which a few drops of suspension of PMMA particles in ethanol or DMSO were placed. The solvent was evaporated (2 h under a flow of air for ethanol; 24 h in vacuo for DMSO) and the glass cylinder was removed. The resulting monolayer of PMMA particles was then used to image surfaces of individual particles using AFM.

3.6. References


34. Science in your eyes, Atomarkraftmikroskopi, Syddansk Universitet http://scienceinyoureyes.memphys.sdu.dk/atomarkraftmikroskopi_en.php

