NPL REPORT COAM 16

Report on: Force repeatability in imaging forces and force vs distance spectroscopy using an atomic force microscope

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January 2003

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ABSTRACT

The issues affecting the force repeatability in imaging forces and force vs distance spectroscopy using an atomic force microscope (AFM) have been assessed. The major contributing factors that affect repeatability are, the drift in the piezo-electric tube scanner position when performing force displacement spectroscopy and the drift in the alignment of the optical lever. These are most acute for very soft cantilevers which heat under the action of absorbed laser radiation. It is recommended that the instrument is left on and aligned for two hours prior to accurate measurements. With care, the repeatability in determining the applied forces may be reduced to approximately 1.6% for a pre-cleaned silicon wafer substrate. When determining polymer modulus, repeatabilities of 6.5% can be achieved by AFM and Hysitron indentation but these are probably limited by the material homogeneity at the nanoscale.

ISSN 1475-6684

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1 Introduction

The ability to perform highly repeatable quantitative measurements lies at the heart of a good instrument. By understanding the repeatabilities of different aspects of the measurement process, techniques can be refined and improved. By recording the repeatability of force measurements in the atomic force microscope (AFM), experimental conditions can be found where quantification is most accurate. Defining the repeatability of measurements in the AFM enables comparison with other force methods such as nanoindentation. It also allows judgements to be made as to the suitability of using such a technique for routine analysis.

Common measurements undertaken by the AFM are force – displacement curves[1], the determination of modulus for materials which are generally less than 5 GPa[2-4] and mapping of material stiffness.[5,6] This range of materials includes most organic polymers and soft biological samples, which hitherto have been very difficult to measure by traditional indentation equipment. The advantage in using AFM is the increased sensitivity with soft materials because of the availability of soft microfabricated cantilevers and the ability of measuring forces down to 10 pN, i.e. below the force required to break a single chemical bond. This leads to the possibility of measuring bond breaking forces in single molecules under certain conditions. The drawback to using AFM is the brittle nature of the single crystal silicon tip. The yield point is much lower than for traditional diamond indenters which are capable of indenting samples up to several hundred GPa but with poor spatial resolution. Using a combination of AFM and nanoindentation it is possible to measure modulus properties for all classes of materials. In this study, the AFM is assessed for its repeatability in making force measurements and in making modulus measurements.

An essential concern for AFM is to consider how repeatabilities vary with time. A main issue for the analyst is, how the force measurement drifts over a few hours given the same experimental conditions and the same operator. Small drifts in the alignment of optical levers and drifts in the piezo-electric scanner can affect final measurements considerably. Therefore, careful instrumental design is a critical factor. In this study, the repeatability and quantification of noise levels and hysteresis of key measurable parameters have been studied. The results show that the development of stable close loop feedback systems and careful design of instruments electronics are essential to ensure the best quality of images and of force spectra.

In Figure 1, the necessary measurements in the AFM are linked together via a simple representative measurement tree. This tree maps the accrual of uncertainty including repeatability and also calibration dependencies of the modulus and force measurements. Here, AFM measurement starts with the z axis calibration. This is used, in turn, to calibrate the deflection scale and the spring constant for the cantilever. For optimum repeatability of the force or modulus measurement, it is important to select the cantilever spring constant to be in an appropriate range.

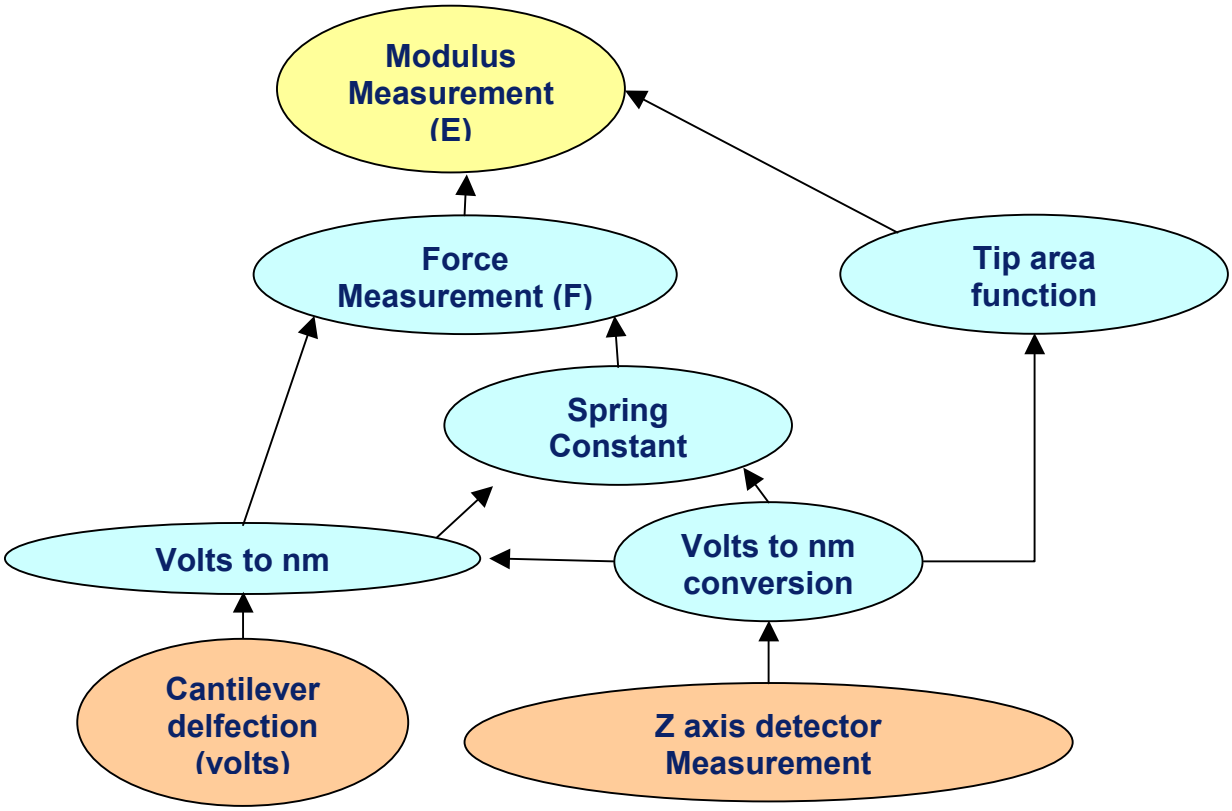


Figure 1:Measurement tree for nanoscale mechanical measurements used in AFM - each line represents a calibration and an uncertainty

Repeatabilities for differently commercial AFMs are different because of the wide variety of designs and configurations for each instrument, especially of the optical beam. In this report, a common method of evaluating all AFM instruments and recommendations to improve the repeatability in imaging forces and force-distance curve spectroscopy is given. The structure of the report uses the measurement tree to model the repeatability in force and modulus measurements.

2 Experimental set-up

Experiments were performed using a commercial AFM, a TM Microscopes Autoprobe CP. The configuration of this AFM utilises a scanning sample with a detection head which is lowered to the sample stage via stepper motors, as shown in Figure 2. An optical knife edge sensor monitors the position of the *z* piezo. This provides a more accurate measure of the *z* displacement, rather than assuming a displacement from an applied voltage to the piezo-electric scanner. Other manufacturers use other devices to provide accurate measures such as Linear Variable Differential Transformer (LVDT) and capacitance sensors.[7,8] Each of these techniques has their own performance criteria, all being similar to that of an optical knife edge.

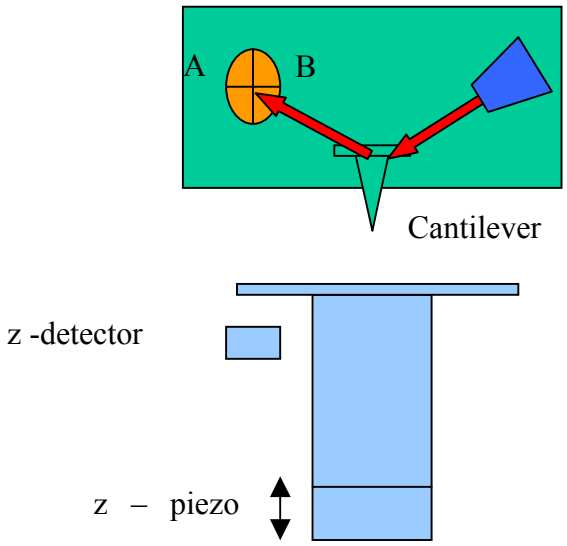


Figure 2: AFM configuration of Park Auto probe CP instrument used in this study

To monitor the performance of the machine independently, data were collected using a signal access module coupled to the AFM and an independent data acquisition program utilising National Instruments acquisition cards (PCI - 6052E, 16 Bit 333 kHz sampling and PCI - MIO – 16E, 12 Bit 1.25 MHz sampling) and Labview software (Version 6.0). Signals monitored from the four quadrant detector were the A-B, A+B (position sensitive diode) and the *z*-detector signals. These were carefully checked and calibrated against those displayed in the proprietary AFM acquisition software for drift studies. Data were taken at 1 second intervals for both the position of the piezo-electric scanner using the *z* detector and the deflection of the cantilever using the position sensitive diode (PSD), A-B. The deflection scale constant was calibrated using the gradient when a non-indenting force distance curve was taken against silicon (E = 125 GPa) with a cantilever whose spring constant was nominally 0.04 Nm^{-1}. This method is commonly used to find the sensitivity of the optical lever to displacement. An alternative method is to use a known step height which is traced over in 'error mode' to provide the deflection constant, however, this has the risk of breaking and wearing the tip and thus was not used in this study.

2.1 Sample preparation

The first sample used for the loading and unloading studies was a small piece of Si wafer that had been pre cleaned using a swabbing of excess absolute ethanol with a cotton bud followed by multiple CO_2 snow jet cleaning followed by a rinse of excess absolute ethanol and inspection for particulates by light microscopy. When the cleaning had been performed well, a very small number of particulates were observable by light microscopy. This produced a surface in which the carbonaceous contamination had been reduced significantly down to approximately 0.16nm thickness with only negligible reduction of the native oxide covering the Si substrate as measured by XPS.

Four polymers were used in the modulus experiments. 1 cm^2 areas were cut from preformed polymer sheets obtained from Goodfellows. The polymers used were poly(propylene) - PP, poly(methylpentene) - PMP. Low density poly(ethylene) - LDPE and poly(tetrafluroethylene) – PTFE. The squares were washed in ethanol, with which they are immiscible, and allowed to dry.

Indentation studies by AFM and Hysitron on the polymers were carried out by a series of differing loads. In each experiment, indentations were made approximately 5μm apart. This was enough to avoid perturbation of the substrate by other adjacent experiments. The AFM tip shape was deduced using a tip characteriser specimen as supplied by NT-NDT (Cat No.TGT01). The tip area function for the Hysitron was calculated using a fused quartz reference sample (69 GPa). The indenter was attached to the AFM base using an interface plate and control was by a separate computer acquisition system. Typical loads used in the experiments were approximately 10 μN for the AFM and 200 μN for the Hysitron indentation studies.

2.2 Data acquisition and post analysis

For the AFM force-distance studies, the proprietary software, supplied by the manufacturer was used to set-up and run the instrument. Analysis routines were developed in Labview to set new origins for the force versus distance curves according to the criteria as described in Appendix 1 and to find the maximum and minimum forces and integrate the work of adhesion. For AFM indentation studies, the voltages versus displacement data were converted to force-displacement data by calibrating the displacement sensitivity. The spring constant of the cantilever was measured using the reference cantilever on test cantilever method as originally described by Tortonese and Kirk[9] but adapted by us.[10] This was then converted to load versus indentation depth by subtraction of the cantilever deflection signal. Data were fitted to a cylinder model as given by Equation (1) below,

$$E^* = \frac{F}{2R\delta} \tag{1}$$

where E^* is Youngs' modulus, F is the applied force, R is the radius of the AFM tip and δ is the indentation depth

The comparative data from the Hysitron nanoindenting unit were analysed using MATLAB to calculate the modulus. This employed the Oliver and Pharr method using the equation described below.[11]

$$E^* = \frac{S_{init}\sqrt{\pi}}{2\sqrt{A_c}} \tag{2}$$

where S_{init} is the initial stiffness from the unloading curve and A_c is the area of contact from the initial point of unloading.

3 Results

3.1 Z axis displacement (*z*) repeatability

The typical translation stage used in an AFM is a piezo-electric tube scanner. The vertical displacement is controlled by an applied voltage to the appropriate piezo-electric segment. It is well known that piezo-electric materials exhibit creep, hysteresis and aging in their performance.[12] This can result in large errors in measuring the *z* displacement accurately. The development of capacitive, optical knife edge and LVDT sensors has greatly improved matters. These have greater accuracy and linearity but at the cost of precision and are therefore not used when high resolution scanning is required, typically below 4 μm field of view. The scanner hysteresis can be shown readily below. The repeatability of the open loop displacement will vary according to the maximum extent of the displacement. Typical hysteresis can be up to 20%, translating into a repeatability of the same amount. The typical noise level of the detector is approximately 6 nm rms. It is advised, therefore, when performing force-displacement studies, that the position of the sample or the tip is monitored by a displacement detector and not just from the applied voltage of the piezo-electric tube.

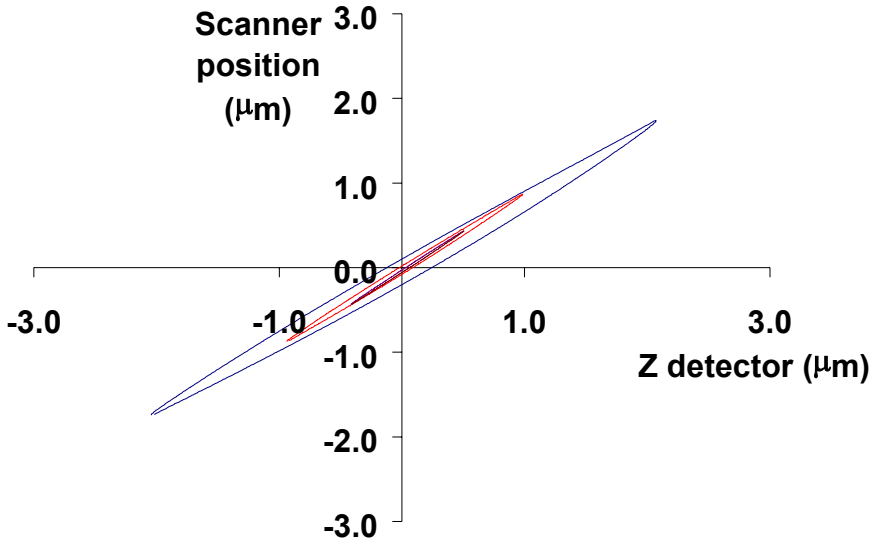


Figure 3: Hysteresis of a 4 μm scan using a large area scanner with 9 micron total z axis movement

The description of piezo-electric behaviour is further compounded by the problem of creep described earlier. If the piezo-electric tube is left in the middle of its displacement range, the tube will gradually creep upwards as the internal stress is relieved with time. For scanning sample and tip configurations, the sample and tip will be driven towards each other. The effect of this is that the analyst has to be careful in performing longer time experiments. The typical timeframe for a force versus displacement experiment is 0.1-10 seconds and, in that time, the piezo-electric tube can drift up to 0.7 nm. It has been noted that, in nanoindentation experiments, the measured indentation depth can be affected by the drift. Therefore, force-versus distance experiments are performed several minutes after the tip has engaged the sample. Figure 4 demonstrates this drift effect over time. After 1 hour, the scanner has risen by 250 nm and tailing off after 2

hours from the initial set point. The tube is already set to the middle position of 4.5 μm so repeatability in displacement over time is always better than 1 nm for experiments of less than 14 seconds but may be less over 1 minute after 1 hour warm-up. If different piezo-electric tubes are used the proportion is likely to remain constant but not the absolute amplitude. In topographic imaging, the effect of z piezo drift means that scans which may be several minutes in acquiring may have a slope introduced in the slow scan direction. This is detrimental to metrological measurements where there may be a requirement to assess flatness. The way to minimise this drift is to set the instrument up and leave it with a scan size of 0, say, up to 1 hour after setting up the sample. It is important to note that the effect occurs after setting up a sample, not simply after switching on the instrument. In this way it has been found that stable atomic imaging can be reproducibly obtained. However this may not always be appropriate for a high throughput in samples and therefore imaging software carries extensive routines to correct for this drift at the cost of some unknown image distortion.

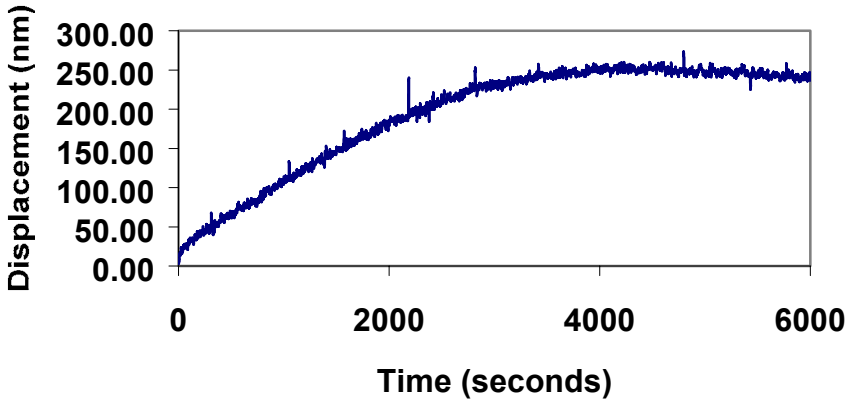
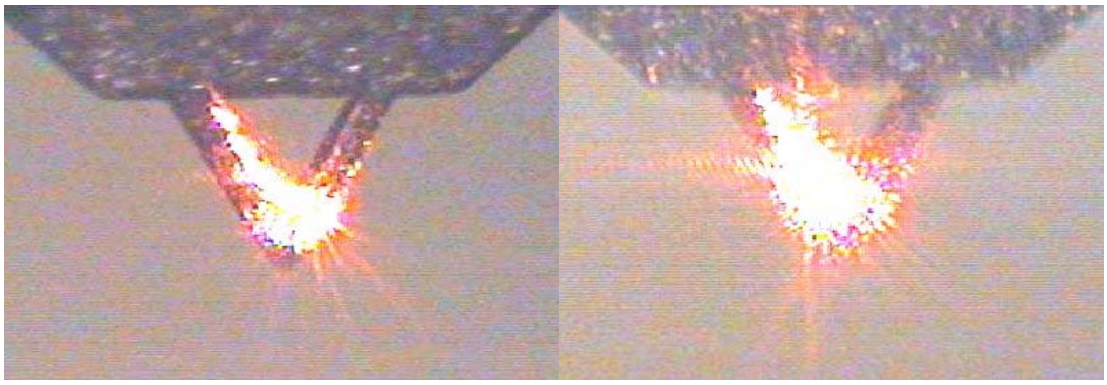


Figure 4: z piezo-electric drift of a large area scanner with 9 μm z range

3.2 Cantilever deflection (*d*) repeatability

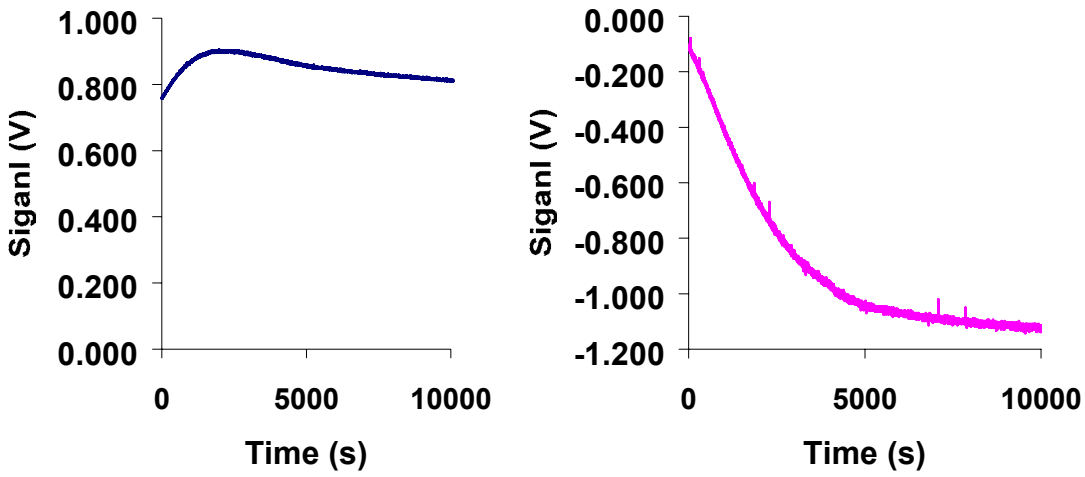
The cantilever deflection (*d*), given as a voltage output can be measured with extreme precision toward sub-Angstrom displacements using optical beams and position sensitive diodes. The measurement of cantilever deflection is prone, however, to instrument drift due to several effects, these include cantilever heating arising from imperfectly reflected light resulting in bending with time and also drift in the laser alignment with time. In the first case, cantilevers can be coated with a thin layer of gold to increase the reflectivity. This overlayer forms an inconvenient bimetallic strip which will bend when heated because of the differing coefficients of expansion of the components. This causes the cantilever to bend downwards inducing a change in the position of the laser spot hitting the PSD. This will introduce an offset in the apparent cantilever deflection that is usually accommodated by fine adjustment of the detector by the user when setting up the AFM for imaging. Soft cantilevers are affected most by this because the heat induced expansion stress is equalised by a greater displacement ($F = kx$) of the cantilever. In the second case, thermal heating of the head assemblies and natural drift and settling of the alignment screws means that over the course of a few hours, the laser spot can move across the cantilever affecting both the total intensity and the deflection constant. As an example, a stiff cantilever (k_c = 3 Nm^{-1}) was left for 2 hours. The laser spot moved across the cantilever by 15 μm during this time, causing

the measured *z* position to change by approximately 100nm. This was the most critical drift component when assessing all the repeatabilities in force measurement because for a stiff cantilever a drift of 2000 nN was observed.



Start Finish

Figure 5: Drift of a laser spot on a stiff cantilever (k ~20 Nm^{-1}) over 2 hours



(a) (b)

Figure 6: Graph to show the drift in (a) A+B and (b) A-B signals as a function of time for a stiff cantilever

The A+B signal was also recorded simultaneously with the A-B signal. Any variation will contribute to the deflection constant force repeatability as well. In this case, the drift in A+B signal was calculated to be in the region of 0.8% over the final 20 minutes. Comparing this to the drift in the A-B signal, the contribution was negligible.

3.3 Mean cantilever deflection constant (ε) repeatability

For determining the force for the force-displacement plot, we used the constant, ε, to convert cantilever deflection from Volts to nanometres. To determine ε, the cantilever deflection (*d*) and the piezo scanner displacement (*z*) are required along with Equation 3 below.

$$\varepsilon(nmV^{-1}) = \frac{z(nm)}{d(V)} \qquad (3)$$

The method for calibrating this parameter was a loading and unloading cycle on a sufficiently stiff sample whilst avoiding indentation of the sample which would lead to erroneous results. The repeatability of ε is important for other calibrations such as the cantilever spring constant. The repeatability of this measurement was performed on a silicon wafer using a 0.6 μm thick Microlever with a spring constant of 0.04 Nm^{-1}. Two experiments were performed involving eight force distance curves on one spot followed by eight force-distance curves on different spots. This will allow separation of the instrument repeatability from the sample repeatability. It is noteworthy to record that a systematic variation in values for the load and unload deflection scales is apparent. This is thought to arise from the scanner tube hysteresis.

To calculate the mean deflection constant (ε), the gradient excluding the lower and upper quartile of the loading and unloading curves was found by a simple linear least squares fit. The mean of the eight individual deflection values was found and the repeatability computed as the average of the standard deviations for each set. Figure 7 shows a bar chart detailing the mean deflection constant for loading and unloading curves at each position. The average repeatability of all the loading and unloading curves on silicon is 1.6% calculated from the standard deviations in each of the four sets of data. The differences between the averages of the deflection constant for two experiments in Figure 7 shows an effective repeatability of 0.7%. It is the instrument which is the main contributor to repeatability and thus a Si wafer is useful as a reference sample to measure instrument performance.

For general calibrations of Volts to nanometres the average of the two values is sufficient for careful indentation experiments it is recommended that the two constants are applied separately to each curve in turn for greater accuracy in the final results.

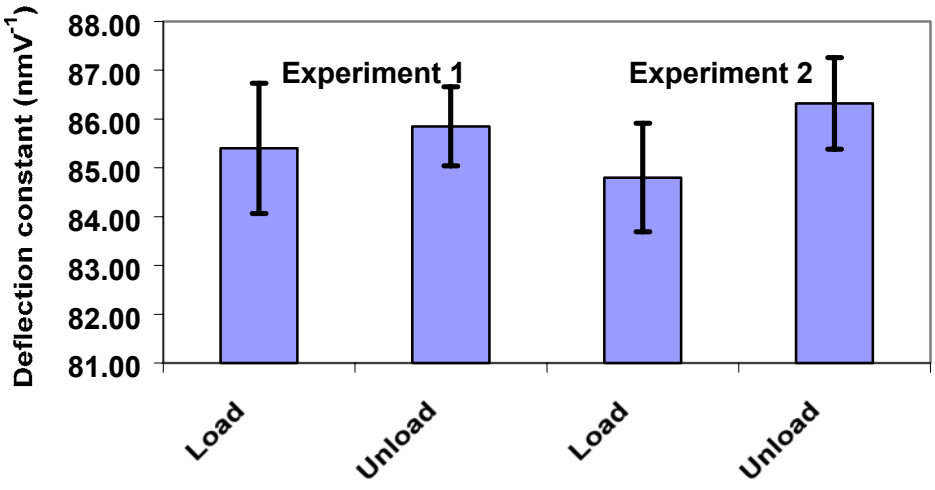


Figure 7: Repeatability of deflection constant determination on Si wafer

3.4 Imaging force (F_{set}) repeatability

When imaging a sample in the contact mode, it is often difficult to know the force repeatability. This can result in surface damage by the tip[13,14] or in extreme cases, the tip being damaged by the sample. The force applied to the surface in contact mode, F_{set}, and is calculated from Eq. 5, where I_{norm} is the normalised intensity of laser light falling on the position sensitive diode (PSD) from Equation 4. The deflection constant and the spring constant are predetermined from other calibrations and are stored in the computer. The control loop maintains a constant voltage of cantilever deflection so that a corresponding force remains constant if the spring constant and the deflection do not drift during the experiment. It is assumed that the voltage is nulled prior to experimentation so that a correct offset can be applied to the force.

$$I_{norm}(Volts) = \frac{(I(A) - I(B))}{(I(A) + I(B))} \tag{4}$$

$$F_{set}(nN) = I_{norm}(V) \times \varepsilon\ (nmV^{-1}) \times k_c(Nm^{-1}) \tag{5}$$

The components of repeatability of force measurement in this case are, cantilever deflection (d), the mean cantilever deflection constant (ε) and the spring constant (k_c). The mean deflection constant repeatability is around 1.5% and for the purposes of experiments using the same cantilever, the repeatability of k_c is ignored. If multiple cantilevers are used to image a sample then the repeatability will be worse as there is deviation on determining the spring constant between two levers. In this case study, the imaging force repeatability therefore, when one operator is performing imaging on a sample with one cantilever is dependent on how the cantilever deflection constant drifts with time as shown in Section 3.2. The cantilever spring constant will remain the same within measurable limits and thus does not contribute to the repeatability. If the optical lever is left for around two hours and the drift allowed to diminish then the repeatability in F_{set} is excellent but the accuracy is still approximately 25%.

Other factors affecting the force arise from the nature of the tip sample interaction. These include electrostatic and capillary forces. Electrostatic forces exerted by the surface on the cantilever can extend over several microns. Charging of cantilevers is not well understood and depends on specific operating environments with humidity being involved. This affects the tip approach, depending on the polarity, opposite charges pull the tip onto the sample harder than necessary or if the charges oppose, disengagement of the tip from the sample. This disrupts the nulled set point and leads to false or abortive engagements of the sample. Care must therefore be taken to avoid charging of the sample. It was noted that premounted cantilevers can be supplied on insulating substrates and therefore, to avoid electrostatic phenomena, discharging of the chip may be required. Another effect is capillary forces from the surface to the tip which is a function of the humidity and exact chemical nature of the sample. This force is dependent on the size and shape of the tip and typically this can be in the region of 10 - 20 nN.

3.5 Force and displacement repeatability

Force versus displacement experiments form the basis of molecular interaction studies and nanoindentation studies. The tip and sample are brought together and move apart while the cantilever deflection (d) is measured along with the position of the sample stage (z). As described before in section 3.4, the repeatability of F_{set} is known – 25% and the repeatability of z displacement is known (5%). This allows us able to predict the repeatability of force-distance measurements.

The effect of scanner hysteresis when performing force versus displacement experiments is demonstrated in Figure 8. Here z axis detection is not used, rather the scanner displacement is deduced solely using the applied voltage. The most hysteresis, 16%, was observed when the piezo-electric tube was extended to its maximum, 9 μm range. This hysteresis reduced to 4.8% for a 1.2 μm range. It is recommended that this mode of operation is not used for quantification unless very small ranges of z are used, typically 100 nm. Fortunately at very small displacements in the range of less than 100 nm the hysteresis is very low. In this range, the accuracy is best with the z-axis detector off as that eliminates the noise in the detector signal.

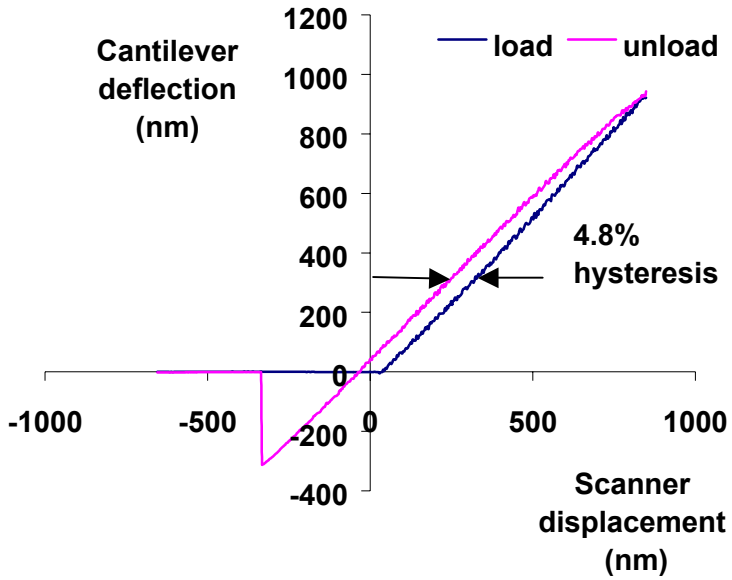


Figure 8:Hysteresis of a 9 μm scanner without z-axis detection

As described earlier, the implication of piezo-electric scanner drift is also important for force-distance studies. If a probe is left on the surface with the vertical control loop off, the force will steadily drift with time as shown in Figure 4 and may damage the sample. In this instance it is recommended that the instrument is left for an hour before performing force distance studies. The value of F_{max} will also change with time. This may be important when controlling the maximum indentation depth and force. To observe this effect, two repeatability experiments were performed to monitor F_{max}, F_{min}

and the work of adhesion (W_{adh}) as defined in Figure 9 for a cleaned Si wafer surface and a microlever with a pyramidal tip. One experiment performed 8 loading and unloading cycles on the same spot to assess the repeatability of single experiments. The second experiment was performed on 8 different spots to gauge the consistency of the sample. It is predicted that F_{max} will change given drift in the piezo-electric tube scanner causing the sample to creep upwards over time. F_{min} however, will remain more consistent and is related more to the surface properties rather than the instrument factors. The work of adhesion is simply the integral of the force and distance in the attractive regime of the unloading curve as shown in Figure 9 and again is not affected by a drift in the z displacement or cantilever drift.

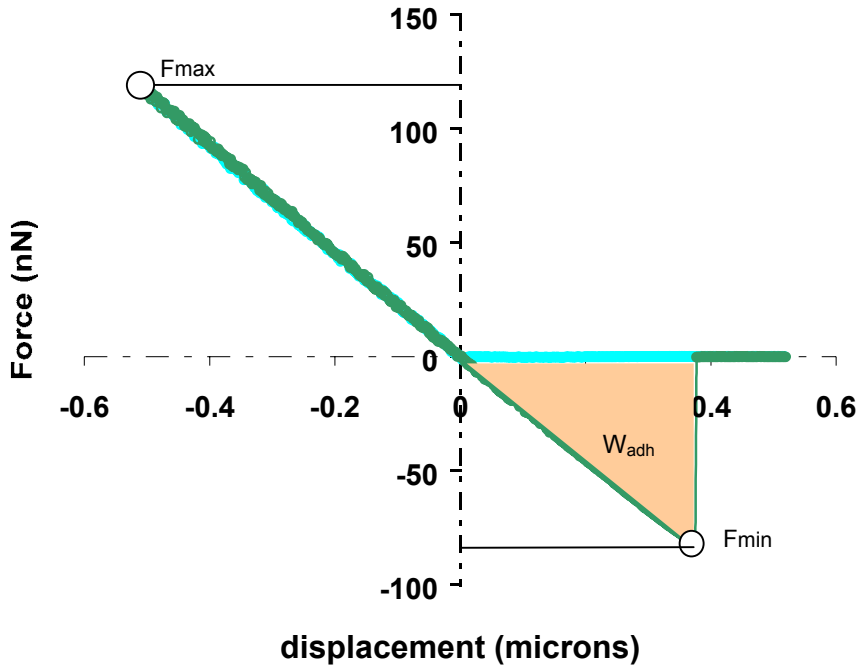


Figure 9: Illustration of the definition of F_{max} and F_{min} in force distance curve analysis for a typical load/unload cycle on a Si wafer with z axis displacement deflection

The average results for the two experiments show that the values of F_{max}, F_{min} *and* W_{ahd} have a repeatabilities of 1.25%, 0.5% and 1.5% respectively. The table below shows the values obtained.

Experiment	F_{max} (nN)	F_{min}(nN)	W_{adh} (mJ)
Single spot samples (1)	9.65 ± 0.12 (1.2%)	-26.44 ± 0.20 (0.7%)	9.18 ± 0.14 (1.5%)
Multiple spot samples (2)	9.40 ± 0.12 (1.3%)	-26.22 ± 0.11 (0.4%)	9.01 ± 0.15 (1.6%)

Table 1: Data to show the repeatabilites in single and multiple sample point force distance curve analysis

The individual results for W_{adh} are shown in Figure 10 and exhibit a systematic upward drift for the single spot. This is thought to be due to a loosening of the residual organic contamination through gradual 'kneading' of the organic layer the disruption of the surface structure probably leads to a quasi stable state in which the surface energy is raised from exposure of more polar groups. It appears that single shot experiments on different locations of the sample have a more random component to their repeatabilities. It is therefore recommended that for investigative studies of surface adhesion, experiments are performed on different areas and not on the same location.

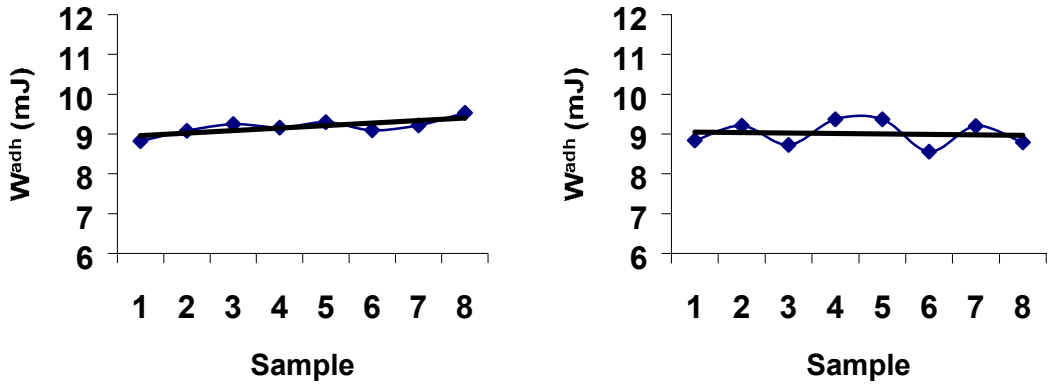


Figure 10: Graphs to show variation in W_{adh} as a function of sample order for single and multiple location experiments

3.6 Force repeatability of Modulus measurements

In the previous sections, the AFM has been characterised for its repeatability in making force measurements. The next investigation involved the assessment of the repeatability of making modulus measurements. The modulus can be calculated by knowing the applied force and by knowing the tip contact area as a function of indentation depth. In this study an evaluation of the repeatability was undertaken to investigate which is the dominant factor in making repeatable and valid measurements. It is thought that the repeatability is composed of two elements, sample and instrument repeatability. Given the excellent repeatability of a silicon wafer sample in Section 3.3, which shows a repeatability clearly below 1% and instrument repeatability of 1.6%, this value can be used as the reference base line for indentation experiments.

It has been shown that AFM is most suited in performing modulus measurements on soft materials. Polymers were therefore employed as samples and the repeatability was assessed across the surface. The upper limit of material modulus of the AFM, is around 5 GPa, with the stiffness of the AFM and of microfabricated cantilevers being the limiting factors. The stiffness of the cantilever used in indentation experiments was approximately 120 Nm^{-1}, and the tip was blunted before use to stabilise its shape and provide a more cylindrical shape for more accurate calculations. Typical load for AFM indentation were typically around 10 μN.

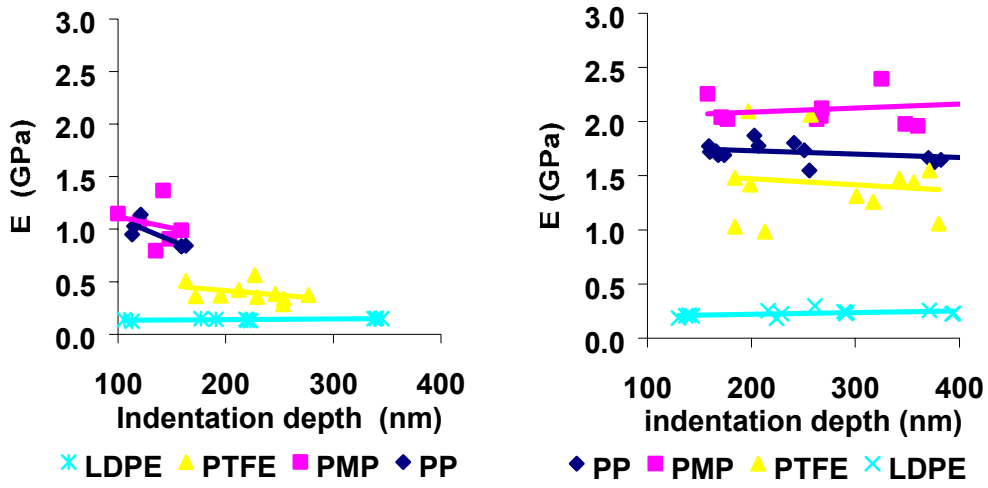
To compare the results of AFM measurement, we also used a Hysitron nanoindenter with a diamond Berkovich tip actuated through a three plate capacitor to indent samples. The Hysitron is interfaced with the AFM to enable fast analysis. The indentation depth was kept greater than 100 nm to enable consistent measurements to

be made in both sets of instrumentation as it has been noted that results in both cases deviate from average values close to the surface.

To compute a force-indentation curve for the AFM, the non-indenting response function is subtracted from the force-displacement curve. Using the cylindrical model, the modulus can be determined from Equation 1. The average data from loading and unloading cycles in the Hysitron were fitted to power law which yielded modulus values for each of the polymers. Figure 11 and Table 2 show the values for the moduli determined.

Polymer	*AFM (E* / GPa)*	*Hysitron (E* / GPa)*
LDPE	0.14	0.23
PP	0.94	1.71
PMP	1.04	2.13
PTFE	0.38	1.85

Table 2: Table of polymer modulus results



(a) (b)

Figure 11: Graphs to show recorded modulus values for polymer indentation using a) AFM b)Hysitron nanoindenter

From these sets of results it is simple to calculate the repeatability for the range of polymers and experimental techniques. Again the mean value was taken and the repeatability standard deviation calculated. Values for the AFM and Hysitron are similar. The most unrepeatable sample was PTFE – 23.1% and the best was Poly(propylene) at 6.5%. The main difference being the inhomogeneous nature of manufacture where PTFE is composed from colloidal spheres and LDPE and PP are rolled sheets of polymer.

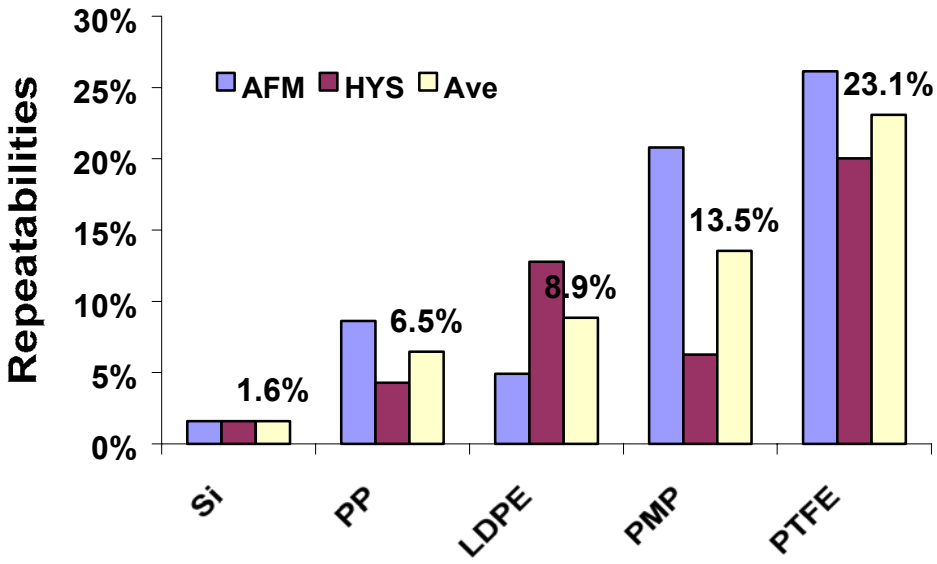


Figure 12: Repeatability of modulus measurement sorted by average

4 Conclusions and recommendations

Through the study of force repeatability, a great deal can be understood about measurements in the AFM. It has been surprising to note that the drift in laser alignment plays a significant role in imaging force repeatability. Careful control of engineering is required in AFM design to ensure that the optical path is stable and does not drift with time. It is important to perform experiments with machines that have *z* detection systems incorporated in their designs. The non-linearity of piezo electric transducers without a position sensitive detection system is not good enough to provide the quality of results needed for routine analysis. It is also noteworthy that instruments may need to be left for at least 2 hours after setting up the sample to ensure that drift of scanner tubes and the optical beam does not dominate the measurement. But this is only time if the experiment is likely to take more than 100 seconds.

The use of Si wafer as a test substrate is excellent for assessing the key parameters of instrumental repeatability as it has good consistency across the surface coupled with a very low surface roughness. Care must be taken, however, to ensure that sample is clean so that correct measurements can be made. Using this substrate it can be confirmed that the force repeatability of the instrument is around 1.6% in force distance curves and 25% repeatability in imaging. Finally, when the instrument has been properly calibrated and characterised it is possible to perform nanoindentation experiments which have a comparable repeatability to traditional nanoindenters. The two techniques complement each other, with AFM providing sensitivity for soft materials and traditional indenters for stiffer materials. The overlapping region for both AFM and nanoindentors covers most polymers. The ultimate reason for the upper limit of measurement by AFM is the material the indenter tip is made from i.e. silicon. Diamond which is used traditionally for indenters has a much higher yield point than single crystal silicon and can withstand higher loads thus if too much load is applied to a AFM tip it will shatter. Care also has to be taken in performing experiments with different shaped tips on polymeric materials as the sample volume is different. More plastic work is performed with pointed indenters and thus gives lower values for Young's modulus than expected.

5 Acknowlegements

This work forms part of the Valid Analytical Measurement programme of the National Measurement System Policy Unit of The Department of Trade and Industry.

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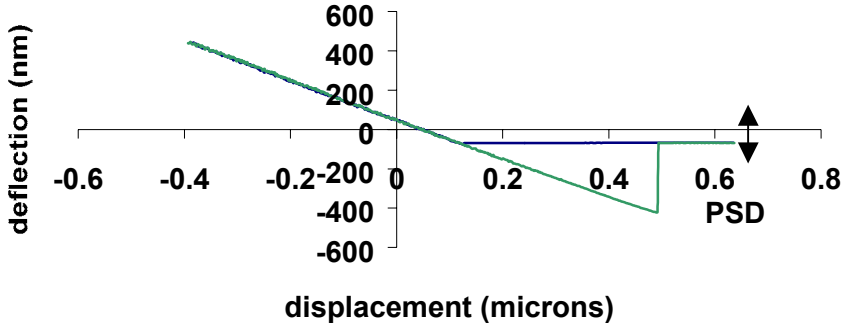
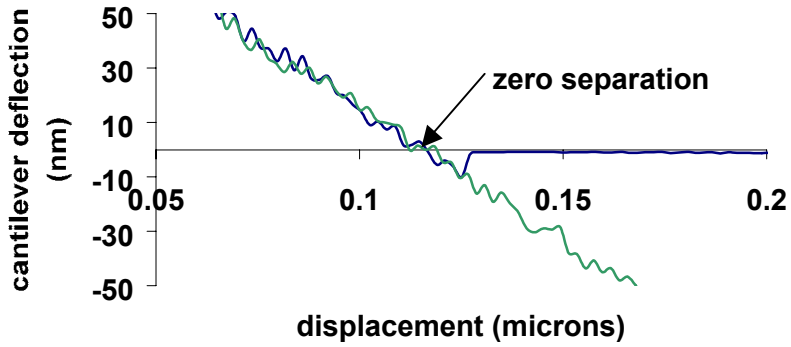
Appendix 1

Method to set the origin of force versus distance curves for AFM

1. Set the offset i.e. the vertical ordinate according to the net force on the cantilever in free space. This is done by finding the median of a selected region before the cantilever is in contact with the surface.



2. Locate the point at which the loading curve crosses the Y axis after it has gone through its snap in minima and zero the curve based on that position.



3. The final force distance curve will pass through the origin for further analysis