

Scanning Probe Recognition Microscopy Investigation of Nanoscale Mechanical and Surface Roughness Properties Along Nanofibers

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ABSTRACT

Scanning Probe Recognition Microscopy is a new scanning probe microscopy technique which enables selective scanning along individual nanofibers within a tissue scaffold. The Scanning Probe Recognition Microscopy system can automatically detect the nanofiber region of interest and collect a series of force curves and surface roughness measurements inside just this region, which saves operation time. More importantly, the nanofibers are curved surfaces while conventional AFM surface roughness and elasticity analyses are designed for flat surfaces. Deconvolution is used to identify a true curvature region. A Kasa circle fit method is then used to project the true nanofiber curvature. In a conventional surface roughness analysis of a non-planar object, the variation of height data would include the (false) variation caused by the shape in addition to the (true) variation in its surface roughness. In the present work, this inaccuracy is resolved. Furthermore, through development of an algorithm that identifies the top (center line) of the nanofiber, force curves are taken with the tip at its most normal to the nanofiber surface during elasticity measurements.

The region of interest auto tracking capability of Scanning Probe Recognition Microscopy results in the acquisition of far more data points than is typical in scanning probe investigations. Statistical methods were developed and used for surface roughness and elasticity measurements along the nanofiber surfaces. Surface roughness and elasticity maps were generated. Histograms showing mean values, ranges and variances were generated and used for analysis of nanoscale mechanical and tribological properties. The Scanning Probe Recognition Microscopy approach can be extended to other systems with non planar regions of interest.

INTRODUCTION

Tissue scaffold engineering is an active and successful research field [1-3] in biological studies. The field of tissue engineering merges the expertise of life sciences, physical sciences, and engineering to develop functional tissues that can maintain, restore, or improve damaged organs [4]. Cells are 'seeded' into an artificial structure capable of supporting three-dimensional tissue formation. These artificial structures, usually referred to as scaffolds, allow the cell attachment and migration. The scaffolds deliver entrained growth and can even exert certain mechanical and biological influences to modify the behaviour of the cell phase. Successful tissue engineering has led to clinical implant successes within complex tissues such as bladders [1] and cartilage [2]. However, despite such successes much fundamental understanding is still needed to design scaffolds with the most appropriate mechanical, topographical and chemical properties for particular cells or cell classes.

Actin-based cells can actively probe their environment through lamellipodia and filopodia extension. The leading edge of such extensions corresponds to a tens of nanometers sensing area at the cell's extending tip. The environmental triggers that cause extension/retraction/re-direction through actin polymerization/depolymerization should therefore be assessed on a comparable scale. Atomic force microscopy is suitable for use in investigations

of minimally conductive biomimetic tissue scaffold surfaces with the required nanometer-scale resolution [5].

In conventional atomic force microscopy, surface roughness information is acquired through manual application of a rectangular region of interest box. Conventional elasticity measurements are performed using force volume imaging over a whole scan area. There are several problems with these conventional approaches when the sample is a tissue scaffold. First, the region of interest is just along the nanofibers. Second, the nanofibers are curved surfaces while the conventional analyses are designed for flat surfaces. In a surface roughness analysis, the variation of height data in a non-planar sample includes not only its surface roughness variation, but also the variation caused by the shape. In an elasticity analysis, when the tip hits anywhere other than the top of a curved nanofiber surface, a special vector decomposition would be needed for accurate force interpretation.

In the present studies, we use atomic force microscopy operated in a new mode, Scanning Probe Recognition Microscopy, to investigate the environmental triggers for cell response to a series of tissue scaffolds fabricated from electrospun polyamide nanofibers. Scanning Probe Recognition Microscopy is a new scanning probe microscopy technique which allows us to adaptively follow along individual nanofibers within a tissue scaffold. The geometric inaccuracies discussed above are greatly ameliorated to start with, and we have also developed deconvolution-based analyses to further increase data accuracy. Statistically significant data for surface roughness and elasticity properties can be collected and combined by fine-scanning over a region of interest that can include the entire nanofiber mesh. Using Scanning Probe Recognition Microscopy, we have investigated the surface roughness and elasticity of a tissue scaffold sample.

EXPERIMENT

Tissue Scaffolds

The tissue scaffold samples were fabricated from electrospun [6, 7] carbon nanofibers obtained from the Donaldson Company. The nanofibers were electrospun using an adapted electrospinning procedure described in Reference [8].

Scanning Probe Recognition Microscopy (SPRM)

A Veeco Instruments NanoScope IIIa MultiMode scanning probe microscopy system has been specially adapted by our group to auto-track regions of interest through the incorporation of recognition-based tip control. The adaptation is described as Scanning Probe Recognition Microscopy (SPRM). The recognition capability is realized using algorithms and techniques in computer vision, pattern recognition and signal processing fields. Adaptive learning and prediction are also implemented to make detection and recognition procedure quicker and more reliable. The integration of recognition makes the SPRM system more powerful and flexible in investigating specific properties of samples. Figure 1 shows conventional AFM image of the tissue scaffold. Figure 1 shows the sequence of SPRM scanning result of the same tissue scaffold inside the red dot line in figure 1(a). Three nano fibers are picked up during a coarse scan (large step size) and scanned individually using a fine scan (small step size).

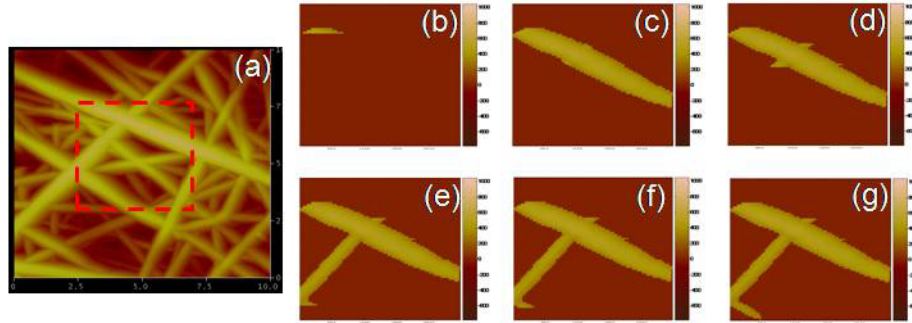


Figure 1. SPRM scan image of the nanofiber. (a) Conventional AFM image of the nanofiber (b) Coarse Scan picks up the first nanofiber; (c) Finish high resolution fine scan only on the first nanofiber and return to coarse scan to find another region of interest; (d) Coarse Scan picks up the second nanofiber; (e) Finish the high resolution fine scan only on the second nanofiber and return to coarse scan to find another region of interest; (f) Coarse Scan picks up the third nanofiber; (g) Finish the high resolution fine scan only on the third nanofiber

Curvature

In atomic force microscopy, electrostatic interactions are summed along the physical structure of the tip and over a local region of the sample surface. These interactions can also introduce characteristic distortions into the AFM measurements such as the width of the sample features. Contact at any point other than the tip end will result in an image smearing or dilation artifact. Additionally, significant artifacts in the form of physically missed region information are introduced due to the shape and scale of the nanofiber relative to the shape and scale of the tip. The usually accurate height measurement in z is also problematic because the flat substrate is not accessible through the layers of tissue scaffold nanofibers.

One approach for data restoration or eliminating the “smearing” effect of tip sample interaction is to assume that the observed signal is a convolution of the true image and the probe response function (PRF). We use deconvolution methods to extract the true image from the knowledge of measured data and PRF[9, 10]. These techniques essentially serve to reconstruct, from the measured image, the sample surface if the tip geometry is known, or the tip geometry if the sample surface is known. Our group has developed an iterative 3-D blind deconvolution algorithm for estimating the AFM tip shape taking advantage of prior knowledge provided through the manufacture specifications which includes tip radius and height. Figure 2(b) shows a typical AFM ‘section’ measurement across a nanofiber, black curve. The missed region artifact in x and y is prominent. The result of blind deconvolution by mathematical morphology is shown as red curve, and indicates that a tip dilation artifact was present as well.

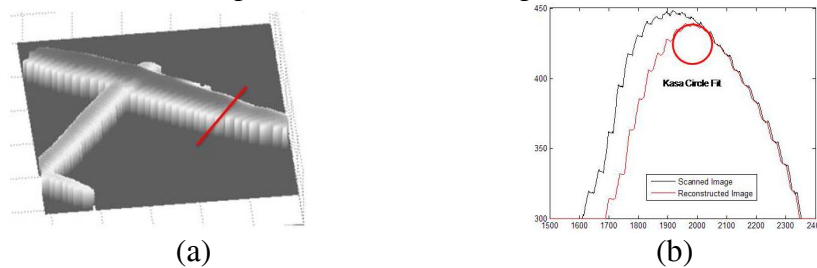


Figure 2. Curvature image of nano fiber. (a) Reconstructed 3-d SPRM image of nano fiber without line/frame fitting; (b) Section image of both scan image and reconstructed image

The intersection of the original and deconvolved curves is used to identify an arc of “true” curvature. The Kasa Circle Fit[11] procedure is then used to provide an accurate estimate of the nanofiber diameter by fitting to the “true curvature” arc. Using these approaches we extract both a direct curvature measurement (the arc) and a much better estimated nanofiber diameter (the Kasa circle fit) than is available based on just AFM data. The true curvature measurements can also be used for identification of the truly reliable data regions for the surface roughness and elasticity investigations described next.

Surface roughness map

In conventional atomic force microscopy, the surface roughness information is acquired through manual application of a rectangular region of interest box. The surface roughness within the box is then calculated, usually as the Root Mean Square. There are several problems with the conventional approach to surface roughness investigation when the sample is a tissue scaffold nanofiber. The shape of the region of interest (ROI) may not be rectangular, necessitating the application of several small ROI boxes which follow the curvature of the nanofiber. Only a single value is provided for each time of operation. Therefore, in order to get surface roughness along a nanofiber, this operation would need to be repeated many times. In the SPRM system, a recognition-based scan plan is generated for auto-tracking tip motion along an individual nanofiber, followed by identification and movement to a second nanofiber, and so on. Therefore, each individual nanofiber, and then the whole nanofiber mesh, becomes the region of interest. We use a local neighborhood method in which the surface roughness is calculated on each pixel based on a local neighborhood region. The shape and size of the local neighborhood region can be adjusted, which makes the method adaptable to different samples.

We have experimented with a rectangular box around each pixel approximately on the order of the nanofiber diameter. Multiple sets of overlapping surface roughness information were generated, with the provision that any box that extended outside the nanofiber boundaries was automatically truncated. A surface roughness map along individual nanofibers was then generated as shown in Figure 3. More importantly, a histogram based on 564267 data points from the analysis of many individual nanofibers was generated. It shows the distribution in the surface roughness values as well as a mode value of 4.56nm.

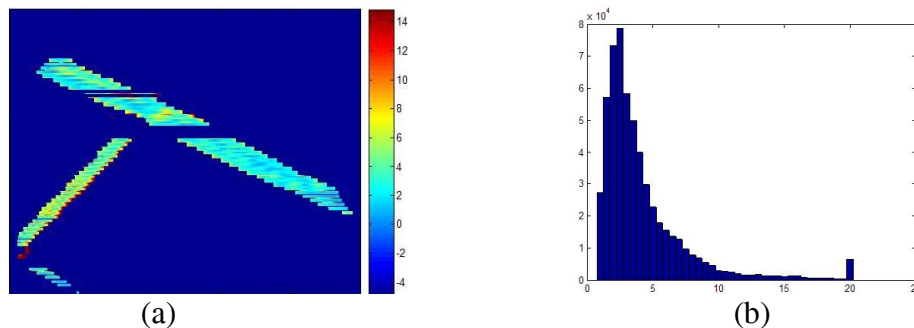


Figure 3. Surface roughness map (units: nm) and histogram image

Elasticity Map

Atomic force microscopy can be used to measure elastic properties by collecting force curves over points on the surface of the sample. The force curve plots the force applied to the tip as it is approached the indented into the sample surface until the force meets the presented trigger value. An array of force curves can be collected across the sample surface at regular intervals and this is known as force volume imaging. In conventional atomic force microscopy, the force

volume imaging will scan the full AFM scan area no matter the shape of the region of interest which is slow and time wasting. There are further difficulties for conventional force volume imaging applied to tissue scaffolds. Force curves acquired at regular intervals may be anywhere on a nanofiber or not on a nanofiber at all. The cross-section of the nanofiber section is a circle which means that the tip may hit the side of the nanofiber where the sample surface normal is at an angle to the force of the tip. In order to get a reliable measurement of the elasticity, the force curve should be collected at the top of the nanofiber where the force vector is exactly normal to the sample surface as shown in Figure 4.

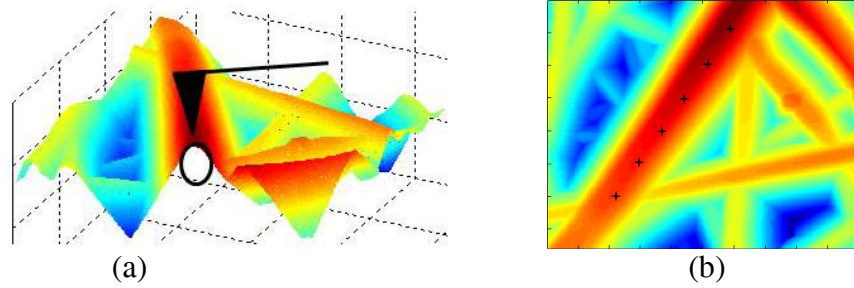


Figure 4. (a) Tissue scaffold surface the tip contact point (b) 6 points were acquired along the individual nanofiber

We replace force volume imaging across a full AFM scan area with SPRM-based force volume imaging directly on individual tissue scaffold nanofibers. The SPRM system will first recognize the left and right edge of the nanofiber, then detect the highest position in between. The tip is returned to this position. Then the tip is controlled to move in z direction and the force curve is generated. Figure 5 shows the sampled signal for one force curve. The tip was first moved to the highest position on the nanofiber. The SPRM force volume imaging system lifted the tip up for 300nm and then move tip down 302 nm in z direction. The trigger signal was set to 3.0V to indicate the tip was moving down corresponding to the extend part of the force curve. Once the end was reached, the tip was pulled back to the original position. The trigger signal was set to -1.0 V to indicate the tip was moving up, corresponding to the retraction part of the force curve.

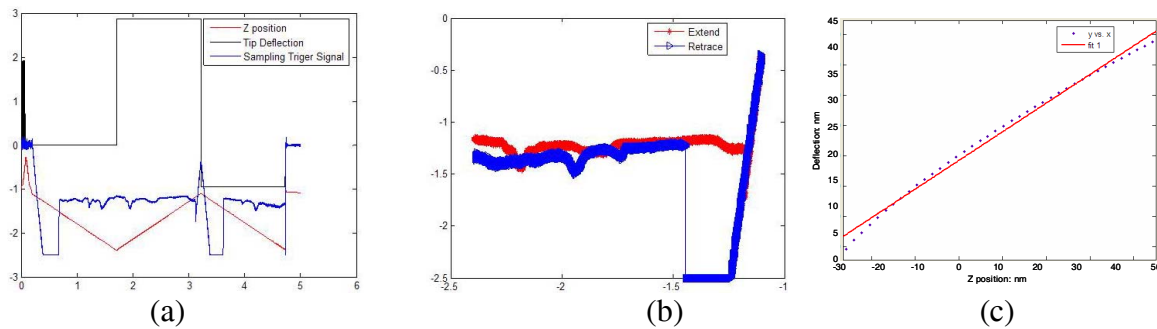


Figure 5. Force curve at the top of the nanofiber (a) Signal timing waveform (b) Force curve (units: voltage) (c) Experiment force curve and fitted force curve (contact part in extend curve)

To quantitatively assess the elasticity the force curves were fitted to the Hertz model[12, 13] to obtain the Young's modulus E and height of the sample at zero loading force z_0 . The tip-sample distance was calculated as follows:

$$\delta = (z - z_0) - (d - d_0) \tag{1}$$

Where z is the sample height, d is the cantilever deflection and d_0 is the deflection of the free cantilever when the cantilever is far from the sample surface. Assume the AFM tip end is spherical and the sample surface is flat, the Hertz model is defined as:

$$F = \frac{2E \tan(\alpha)}{\pi(1-\mu^2)} \delta^{\frac{3}{2}} \quad (2)$$

Where α is the half-opening angle of the conical AFM tip, μ is the Poisson's ratio, E is the Young's modulus. The experimental force curves were fitted using the equation 2 as shown in Figure 5(c). With the value of 18° for α and 0.5 for Poisson's ratio, the Young's modulus value along the nanofiber is about 3342 Pa.

DISCUSSION

SPRM provided the first capability to collect statistically meaningful surface roughness information extracted along many individual nanofibers using an automatic procedure that maintained uniformity of experimental conditions. More importantly, histograms showing the distribution in the surface roughness values as well as the mode value were generated from the analysis of many individual nanofibers. The usual AFM individually applied ROI box would be most likely to return the mode value. However, the variances and distributions between samples under investigation can prove to be the real difference between them[14].

SPRM also provided the first capability to acquire force curves directly along an individual nanofiber under conditions such that the applied force was exactly normal to the curved nanofiber surface, therefore fulfilling conditions for appropriate use of the Hertz model elasticity analysis. This method is presently being refined and calibrated by our group and will lead to the statistically meaningful elasticity information extracted along many individual nanofibers using an automatic procedure that maintains uniformity of experimental conditions

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