Registration of Tapping and Contact Mode Atomic Force Microscopy Images

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***A*bstract— Atomic Force Microscopes can operate in multiple modes, such as contact, tapping, force-volume, etc., each mode providing different types of information about the test sample. AFM data therefore serves as an ideal candidate for data fusion applications. In practice however, different modes of scanning involve changing the scanning tip, which introduces severe mismatch between the scanned regions. Hence image registration is a key step for aligning the multiple mode data prior to data fusion. This paper presents the registration operation of data from contact and tapping modes with an aim to combine the information for investigating tissue scaffold properties from a cellular perspective.**

Keywords-atomic force microscopy (AFM); tapping mode; contact mode; automatic image registration

I. INTRODUCTION

The family of scanning probe microscopy techniques has revolutionized studies of semiconductors, polymers and nanostructures, with recent dramatic impact in nanobiology. The key capability of scanning probe microscopy (SPM) is that, through a combination of detection and piezoelectric actuation with controlling feedback loops, direct near-field interactions with atomic to nanometer scale phenomena are enabled such as those encountered in cellular and molecular imaging. The inherent resolution of SPM is Angstrom (atomic) level for scanning tunneling microscopy and nanometer (macromolecular) level for atomic force microscopy.

The original nano-amp scanning tunneling microscope (STM) [1] was not suitable for imaging largely nonconductive biological samples and this led directly to the invention of the atomic force microscope (AFM) for nanobiological research [2]. In AFM a sharp tip is brought within nanometers of a sample surface by the z-motion of a piezoelectric scanner and held there by a z-feedback loop. The two most used AFM imaging modes are contact mode and tapping mode. In both modes a non-zero force between the tip and sample is maintained to a preset constant value by the feedback loop. When scanning a soft sample this force introduces sample surface deformations which cause distortions in the information that represents some given aspect of the sample surface. Efforts made for enhancing accuracy of height measurements include the work by Grimellec et al [3], for reducing the effect of direct tip-sample interactions. Jiao and Schaffer use additional information about the sample's mechanical properties taken from the spatially resolved force curve at each pixel to obtain accurate height and volume information on soft samples [4].

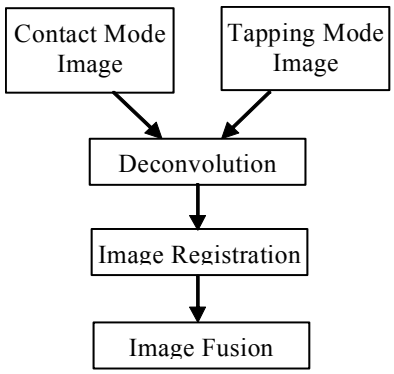

Figure 1. Flow chat of the image fusion

Tissue scaffolds with and without living cells attached are a current focus within our research group. Tissue scaffolds that enable the entrained re-growth of cells into damaged areas are of great medical importance. Sophisticated tissue scaffolds with properties designed for the re-growth of specific cell types while maintaining maximum biocompatibility, would greatly increase the quality and number of applications. Such developments require an understanding of the tissue scaffold's properties from the macromolecular perspective of cellular receptors and actuators.

Scanning probe microscopy (SPM) techniques are ideally suited for the scale of these investigations. Like the cell itself, a scanning probe microscope tip maintains a macromolecular-scale near-field interaction with a scaffold surface. To mimic a realistic cellular perspective, it is necessary to investigate particular combinations of properties.

Scanning Probe Recognition Microscopy is a new SPM modality under development by our group in partnership with Veeco Instruments [5]. It allows us to adaptively track and scan along individual nanofibers within a tissue scaffold and investigate properties of interest. High resolution statistically significant data for multiple properties can be collected and combined. Cell sensing of rigidity (elasticity), curvature, and

surface roughness are all known to trigger cell responses. Using Scanning Probe Recognition Microscopy, we investigate these multiple properties. Data fusion techniques are then utilized to combine mechanical, topographical and curvature information into a composite picture representative of a cell's perception of its environment.

In this paper we propose an algorithm for automatic registration of data obtained using tapping and contact mode AFM scans, for enhanced analysis of the tissue scaffold topography.

II. Materials and Methods

A. Data Collection

Tissue scaffolds were prepared by electrospinning as described in Reference [6]. AFM measurements were performed in an ambient environment using a Nanoscope IIIa Multimode instrument (Veeco Metrology, Santa Barbara, CA).

The tissue scaffold samples were scanned in two different imaging modes. First the tapping mode technique was used to scan the sample. In tapping mode, the tip is excited by a constant amplitude vibration. When the tip is brought close to the sample surface, the amplitude and phase of oscillation are changed. The change in amplitude is fed back via the z-feedback loop to maintain constant amplitude, thereby generating the topography of sample. A topographic image of the same sample can also be obtained using contact mode information.

In the AFM image, tip shape, surface roughness, surface environment, and yielding surfaces can all result in information artifacts [7]. In this paper we propose an image analysis system as shown in Fig. 1. The overall system has 3 major components, namely, deconvolution, registration and data fusion. A review of deconvolution techniques is discussed in [6]. This paper addresses the second step of registration of images obtained using distinct tips in tapping and contact mode scans. Once the different mode images are registered, they can be applied to the data fusion module for enhancing the accuracy of tissue scaffold topography.

B. Image Deconvolution

The approach based on mathematical morphology for surface reconstruction derived by Villarrubia [8] takes into account the effects of a finite probe tip size. In this approach, it is assumed that the image distortion is due to dilation of the image features by the finite size of the probe tip. The algorithm for 'deconvolving' the distortion due to the tip size can be obtained using a grayscale morphological erosion (inverse of dilation) operation defined as:

$$S_r(x,y) = \min_{u,v}[i(x+u, y+v) - p(u,v)] \qquad (1)$$

where $S_r(x, y)$ is the reconstructed surface, $i(x, y)$ is the measured surface and $p(x, y)$ is the tip surface.

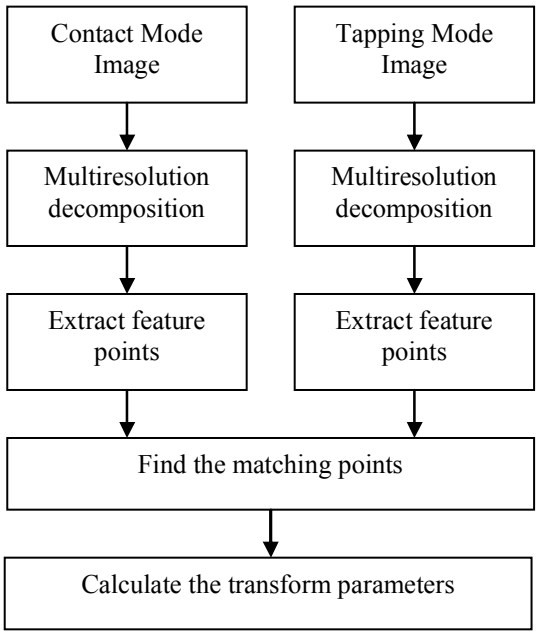

Figure 2. Flow chart of the image registration

C. Image Registration

The next step is a precise alignment of the images involved, such that the corresponding pixels in different images represent the same physical coordinate of the sample surface. This task is usually referred to as image registration. In the approach presented in this paper, it is assumed that the coordinate transformations between two images are rigid and composed only of scaling, rotation, and translation. The relation between the pixel $\boldsymbol{q_i}(q_x, q_y)$ in image Q and pixel $\boldsymbol{p_i}(p_x, p_y)$ in image P is defined as:

$$q_i = sRp_i + T \qquad (2)$$

where s is the scaling constant;

R is rotation operation defined as:

$$R = \begin{bmatrix} \cos\theta & -\sin\theta \\ \sin\theta & \cos\theta \end{bmatrix} \qquad (3)$$

and T is translation vector in the x and y directions

$$T = \begin{bmatrix} t_x \\ t_y \end{bmatrix} \qquad (4)$$

A schematic of the overall approach for registration is shown in Fig. 2. The key idea in registration is to identify distinctive features in the two images that can be used for determining the transform parameters. In this paper we use the multiresolution wavelet transform for detecting the edge-based

features. Once the feature vectors $\boldsymbol{p_i}$ *and* $\boldsymbol{q_i}$ are detected in both images, the matching pair will be defined based on their normalized cross-correlation. The matching pairs are then used to estimate the image transformation parameters.

The images are first decomposed using a discrete wavelet transform. We choose the multiresolution kernel as:

$$\Psi^1(x,y) = \frac{\partial s(x,y)}{\partial x} \tag{5}$$

$$\Psi^2(x,y) = \frac{\partial s(x,y)}{\partial y} \tag{6}$$

where s(x, y) is any smoothing (Gaussian) function.

The image multi-resolution decomposition at each scale 2^j is calculated as:

$$W_j^1(x,y) = IM(x,y) * \Psi_j^1(x,y) = IM(x,y) * (\frac{1}{2^j}\frac{\partial s_j(x,y)}{\partial x}) \tag{7}$$
$$= \frac{1}{2^j}\frac{\partial}{\partial x}(IM(x,y) * s_j(x,y))$$

$$W_j^2(x,y) = IM(x,y) * \Psi_j^2(x,y) = IM(x,y) * (\frac{1}{2^j}\frac{\partial s_j(x,y)}{\partial y}) \tag{8}$$
$$= \frac{1}{2^j}\frac{\partial}{\partial y}(IM(x,y) * s_j(x,y))$$

In order to find the edge points in the image, we calculate the energy map of the multi-resolution image in each scale j as:

$$M_j(x,y) = \sqrt{|W_j^1(x,y)|^2 + |W_j^2(x,y)|^2} \tag{9}$$

A criterion based on edge correlation [9] is used to choose the feature points and is defined as:

$$\Re_n(x,y,j) = \prod_{i=0}^{n-1} M_{j-i}(x,y) \tag{10}$$

where n is a positive integer indicating the number of scales involved in the multiplication, and j represents the initial scale for edge correlation. Considering the computation time, the number of the feature points should be restricted. In this paper, ten pixels in each image with maximum energy are chosen to represent the feature vectors $\boldsymbol{p_i}$ and $\boldsymbol{q_i}$. Normalized cross-correlation between two windows centered at feature points $\boldsymbol{p_i}$ and $\boldsymbol{q_i}$ is then calculated to determine the matching pairs of pixels to be used in image registration. Feature points with the highest correlation values are identified as the matching points pair $\{p_i \Leftrightarrow q_i\}_{i=1,2,...10}$ to estimate the transformation parameters as follows:

The rigid planar transformation parameters (scale, rotation, translation) are calculated using singular value decomposition (SVD) method [10] which is summarized below:

1. Calculate matrix $H = \sum_{i=1}^{N} \tilde{p}_i \, \tilde{q}_i{}'$,

   where $\tilde{p}_i = p_i - \bar{p}$; $\tilde{q}_i = q_i - \bar{q}$;

   $$\bar{p} = \frac{1}{10}\sum p_i \; ; \; \bar{q} = \frac{1}{10}\sum q_i$$

2. Find the SVD of H, i.e. $H = P\Lambda Q^T$
3. The rotation parameters are obtained as:

   $R = QP^T$
4. The scaling constant is obtained as:

   $$s = \frac{\sum_{i=1}^{10} \tilde{q}_i{}^T R \tilde{p}_i}{\sum_{i=1}^{10} \tilde{p}_i{}^T \tilde{p}_i}$$
5. The translation vector is calculated as:

   $$T = \bar{q} - sR\bar{p}$$

III. REGISTRATION RESULTS

Figure 3 shows the topography image collected in both contact mode (a) and tapping mode (b). Ten feature points were chosen and are indicated by red stars in Fig. 3 (a) and (b). Using the image registration procedure, the image transform parameters are determined as:

S = 0.9996;

R = [0.9660 0.2587
-0.2587 0.9660];

T = [-100.0000 200.0000];

Using the calculated parameters, the tapping mode image in Figure 3(b) is transformed to the coordinate system of the contact mode image in Figure 3 (a). It is also seen that in the process of the transformation (rotation and translation) there are regions in the resulting registered image where data is not available as indicated by the regions in dark blue in Figure 3(c). Once we get the registered images, the data can be used in a data fusion algorithm to get more accurate and reliable information related to topography and other properties of tissue scaffold.

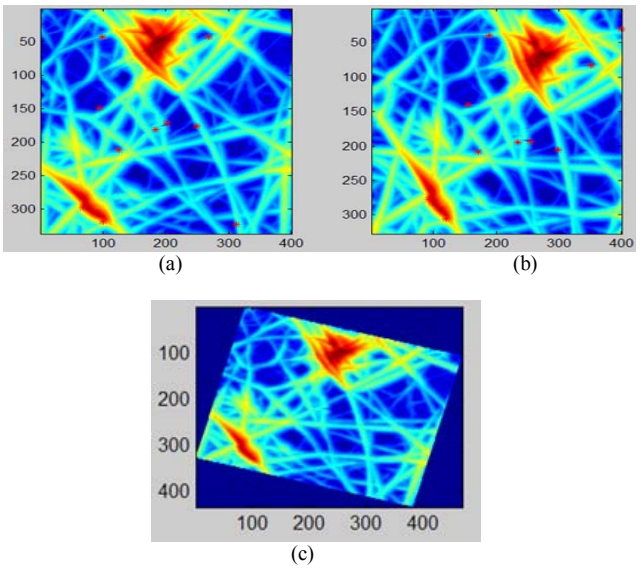

Figure 3. Image registration results. (a) Contact mode image; (b) Tapping mode image; (c) registered image

IV. DISCUSSION

The Atomic Force Microscope can operate in multiple modes, such as contact, tapping, force-volume, etc., each mode providing different types of information about the test sample. AFM data therefore serves as an ideal candidate for data fusion applications. In practice however, different modes of scanning involve changing the scanning tip, which introduces severe mismatch between the scanned regions. Therefore, a key step in Data Fusion is image registration.

This paper presents the registration operation for contact and tapping modes that enables us to combine information into a meaningful investigation of tissue scaffold properties from a cellular perspective. Continuing experiments are focusing on elasticity information acquired in contact mode, to be combined with accurate boundary and surface roughness information acquired in tapping mode. Our group investigates tissue scaffold elasticity using the Force Integration to Equal Limits (FIEL) mapping method to produce a robust measurement of relative elasticity [6, 11]. This method has the advantage of being independent of the tip-sample contact point, and of not requiring calibration of the AFM cantilever spring force constant. We are currently investigating curvature through AFM measurements with slope-based automated SPM tracking optimization, augmented by deconvolution [7], to include tip-shape broadening and angle-dependence. Confidence maps are then developed to determine reliable data as a function of curvature, and independent SEM and TEM diameter measurements are also used as checks. Surface roughness is investigated using a mapping technique developed by our group, in which the roughness is calculated within a user-defined local neighborhood region of each current pixel, entirely within the nanofiber boundaries.

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