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Numerical processing of CNT arrays using 3D image processing of SEM images

R.M. Dunn, M.G. Schrlau*

Department of Mechanical Engineering, Rochester Institute of Technology, Rochester, New York 14623, USA

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ABSTRACT

This paper outlines an image processing based technique to characterize carbon nanotube (CNT) array devices for enhanced cell transfection. Investigating how manufacturing parameters affects CNT array geometry, and how geometry affects transfection, requires the arrays to be measured. Obtaining a statistically sufficient number of measurements by hand is tedious and subject to human error. An automated system to characterize the arrays facilitates data collection of numerous pore properties. Scanning electron microscopy (SEM) micrographs are pre-processed to identify the location of CNTs which are then measured individually to obtain their characteristics. The data from single pores is aggregated to generate a numerical summary of the array parameters. Stereomicroscopy techniques are used to measure the heights of the CNTs using pairs of tilted images. The overall technique accurately measures the parameters relevant to cell transfection significantly faster than manual measurements while eliminating human error and bias.

1. Introduction

Cell transfection is an important tool for numerous biological research applications including disease study. Carbon nanotube (CNT) arrays are used to transfect thousands of cells simultaneously. The Nano-Bio Interface Lab (NBIL) at the Rochester Institute of Technology (RIT) has demonstrated effective manufacturing of CNT arrays by depositing carbon in anodized aluminum oxide (AAO) templates and etching away the AAO surface to expose the tips of the CNTs [1]. The process yields a porous surface through which cargo can be transfected into cells.

The performance of a CNT array is largely dependent on the geometric surface properties of the array [2,3]. The dimensions and shape of the tubes as well as their spatial density contribute to the effectiveness of enhanced transfection [4,5]. Bulk characterization of the arrays is extremely tedious if done manually and requires multiple measurements for every CNT. The accuracy of manual measurement is subject to human error and bias. Automatic characterization allows entire arrays to be analyzed accurately and quickly with added precision due to the elimination of human bias, eliminating a common bottleneck of CNT array experimentation.

CNT arrays are imaged using electron or scanning probe microscopy (SPM) because the resolution of the geometric features exceeds what can be imaged by traditional optical microscopy [6]. Scanning electron microscopy (SEM) is common for this application [7–9]. An SEM detecting secondary electrons generates contrast corresponding to geometric features. An SEM detecting backscattered electrons generates contrast corresponding to material properties as well. The primary concern of CNT array characterization is surface geometry so secondary electron detection is preferred.

There are a number of other nano-scale surface characterization methods relying on microscopy or spectroscopy techniques. SPM including atomic force microscopes (AFM) are particularly useful for measuring small geometric features. AFM is capable of producing three-dimensional data facilitating convenient geometric measurements. However, some experimentation requires capabilities that AFM does not allow, such as tilt. X-ray microtomograpy (micro-CT) has been used to characterize CNT scaffold porosity [10], but lacks the resolution for individual CNT measurement. Nano-CT is a promising technique for characterizing CNT arrays [11], but is not widely commercially available. Spectroscopy techniques including Raman spectroscopy and X-ray photoelectron spectroscopy (XPS) are more suited towards measuring non-geometric material properties. Optical profilers are non-destructive with sub-nanometer resolution making them well suited for 3D surface measurements. However, they are expensive and may require additional software to perform an analysis of a CNT array. Typically AFM would be the first choice for geometic CNT characterization including height but when SEM characterization is also required a software-based approach to geometric analysis eliminates both the need for additional expensive equipment as well as a post-processing analysis step. This

* Corresponding author.
E-mail addresses: rmd8337@rit.edu (R.M. Dunn), mgseme@rit.edu (M.G. Schrlau).

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study was partially motivated by the lack of availability of AFM equipment.

Surface characterization is beneficial at multiple steps in the template based CNT array fabrication process, notably to measure the geometry of the template and the finalized CNT array. The membranes used in our procedure, a Whatman Anodisc 13 (Cat. No. 6809–7023), have a surface region of variable depth in which pores are irregularly shaped and spaced. This region is refereed to as the lattice layer. In order to obtain desired surface characteristics this layer must be removed up to a point where the geometry is acceptable. Experiments relating to the removal of the lattice layer require surface characterization for each sample. Finally, as desirable CNT array geometry is identified, fabrication techniques can be evaluated based on surface characterization without having to test cell transfection and viability with every device.

Because SEM is 2-dimensional, a single image with contrast corresponding to spatial geometry is not sufficient to accurately determine surface height. It is possible to obtain accurate measurements in the 3rd dimension by rotating or tilting the microscope stage and comparing the resulting images. Li et al. demonstrated that existing methods of 3D projections obtained through tilting can be utilized on SEM micrographs to obtain nanoscale height measurements [12,13]. Tafti et al. have created a successful point-cloud based surface reconstruction system for SEM imagery [14,15]. At minimum two images of the same sample separated by angular tilt in the plane of the sample can be used to obtain measurements along the direction of the electron beam.

This paper outlines the pre-processing techniques and numerical analysis used to complete a method of characterizing CNTs utilizing SEM micrographs. A number of established image processing techniques are utilized including morphological operations and region identification. Once identified, each tube is analyzed independently then aggregated to generate statistics about the CNT array geometry. The system was validated through a comparison to manual measurements and observation of false-positives and false-negatives.

2. System overview

The overall software flow of the system is shown in Fig. 1. The system utilizes an SEM micrograph as an input. Scaling information can also be provided if it is not present in the image. If no scaling information is provided the output unit will be pixels. To obtain height measurements, a set of two SEM micrographs of the same viewing area must be provided. The first image should be oriented horizontally, such that the surface is normal to the electron beam. The second image should be tilted by approximately 5°. The tilt, θ_s must also be specified as an input. A Tophat filter is utilized to obtain uniform regional intensity in the case that one region of an image is darker than the other edge. Dynamic contrast thresholding is applied to separate the image into three layers, each representing a geometric feature including the CNT walls, pores, and membrane surface. Pores are paired with their associated walls and treated as independent regions which are processed individually. Shape analysis identifies which regions are tubes, rather than other geometric features, and calculates several geometric properties. Each region corresponding with a tube is superimposed with the optional tilted image and features are matched to calculate their height based on lateral displacement. The resulting information for each region is aggregated for numerical analysis and the image is reconstructed.

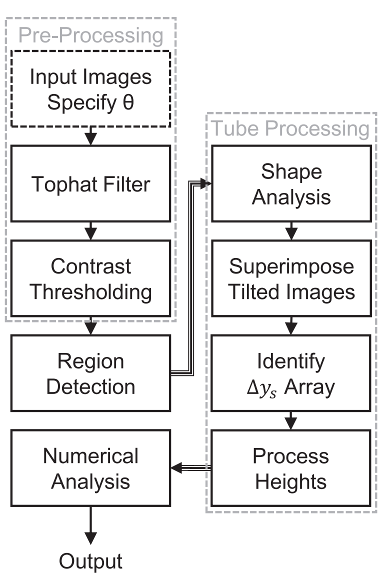

Fig. 1. Software flow of CNT-array characterization system.

3. Pre-processing

Before structural analysis takes place regions must be identified that are potentially pores. The pre-processing makes intensity adjustments to the image and converts the image to black-and-white based on contrast thresholding to identify candidate regions representing paired pores and CNT walls.

3.1. Tophat intensity filtering

Because intensity is the source of geometric information it is important for the intensity of the images to be uniform in order for the same intensity related parameters to be used throughout the whole image. Because a tilted SEM stage will cause one edge of the sample to be closer to the electron beam an intensity gradient is expected. Variable overall intensity can also occur in an SEM micrograph if a region is shadowed by a larger object. A Tophat filter adjusts the average intensity of large regions such that the regions have matching average intensities. The intensity of large regions change while maintaining contrast of fine geometric features. It is important to specify a structuring element[1] larger than the geometric features. A disk-shaped structuring element with a diameter, d_{st}, equal to 20% of the image's smallest dimension is used by default but d_{st} can be manually provided if pore diameters exceed d_{st}.

The filtering process is constrained such that it does not make an adjustment if it would over-saturate a region of the image as to not lose any geometric information. It is important that d_{st} exceed the size of the individual CNTs by a wide margin otherwise intensity adjustments will change the contrast of geometric features within a single tube. If downstream shape analysis identifies a CNT with $d_{CNT} > d_{st}$ the process starts over and the Tophat filtering is carried out with a structuring element 50% larger than the previous d_{st}. Fig. 2 illustrates an application of the Tophat filter.

3.2. Contrast thresholding

CNT Arrays have three major defining geometric features: tube walls, pores, and the membrane surface. These features, shown in Fig. 3 must be discreetly identified. The features are identifiable based on their relative intensity. In general the pores have the lowest intensity while the CNT walls have the highest intensity. The following assumptions are used to perform contrast thresholding:

1. The membrane surface takes up the largest area.
2. Pores consist of the lowest intensity.

[1] A structuring element is a shape used to probe an image. Many image processing techniques function pixel-by-pixel considering all neighboring pixels that fall within the structuring element [16].

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