Quantitative, Interactive Measurement of Tissue Engineering Scaffold Structure in an Immersive Visualization Environment

Feature

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Abstract
We describe a software system that enables the measurement and analysis of tissue engineering scaffold materials from three dimensional images that were generated with X-ray micro-computed tomography (μCT), segmented and converted to a polygonal representation. We use this system to compare an 'as designed' scaffold with a manufactured scaffold to determine differences in strut properties. Essential to this work is the use of an immersive visualization (virtual reality) system that gives the researcher the ability to interact directly with data representations in ways that are not possible with desktop systems. Structures can be inspected and measurements can be made and analyzed during the immersive session. Using these measurements, researchers can assess the fidelity of actual scaffolds to the design model and evaluate scaffold manufacturing processes. We describe future directions for more automatic measurement techniques for three dimensional images, and the role of immersive visualization in understanding and evaluating these techniques.

Introduction
Tissue engineering is an emerging interdisciplinary field that has evolved because of the dire need for compatible, replacement organs and tissues in light of the shortages of transplantable organs and the problems associated with biomaterial implants. Four issues critical to the success of tissue engineering were identified in a recent review.[1] One of the issues is the optimization of the matrix, or scaffold, for cell proliferation, differentiation and tissue remodeling. It is widely recognized that factors that influence cell response to scaffolds include chemistry, surface roughness, elastic modulus and structure. The structure also influences media transport through the scaffold. In order to understand how the structure influences cell response, structural descriptors such as porosity, pore size distribution, tortuosity, and connectivity are generated through analytical or computational means.

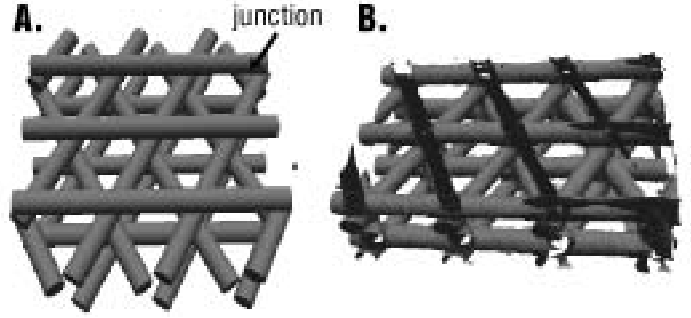

Figure 2. As-designed scaffold, synthetically constructed based on the design specifications (A.), 3D reconstruction of the scaffold made by SFF (B.).

Figure 1. A user in the NIST immersive visualization environment.

We are pursuing the processing of 3D images of scaffold materials in the context of an immersive (virtual reality) visualization environment (IVE). We use the IVE to measure scaffold descriptors and to present them in a clear and interactive manner.

An immersive visualization environment provides the researcher with the illusion that visual data representations are present in a volume of space within which the user can move. The user has the experience of being immersed in a virtual scene where he or she can view and manipulate elements of the virtual world. We prefer to use the term immersive visualization (IV) rather than virtual reality in order to emphasize our use of the technology for data visualization and to highlight the user's sense of being present in the midst of the data space. At the National Institute of Standards and Technology (NIST), we have created an IV environment which is shown in Figure 1.

The user can move around, look in different directions, and even interact with the data representations as if they were present. Interactions are often accomplished with a hand-held device (a wand) that is also motion-tracked. This environment provides the user with three dimensional (3D) visual and kinesthetic cues that are impossible to achieve with desktop displays. The IV environment provides perceptual cues (both visual and kinesthetic) that are extremely advantageous in understanding, measuring, and analyzing 3D structures.

Previous work applied measurement and 3D image analysis in an immersive environment to the understanding of microscopic biological structures.[2,3]

We are pursuing the use of the IV environment as a framework for more easily measuring scaffold descriptors, for support in developing consensus definitions of scaffold descriptors, for understanding automatic descriptor measurement methods, and for qualitatively evaluating and validating scaffold manufacturing techniques.

In this initial effort, we apply IV techniques to a straightforward manual linear measurement task to derive quantitative structural information from a digital 3D image of a tissue engineering scaffold.

3D Image Generation and Processing

The poly(E-caprolactone) (PCL) scaffold examined in this work was manufactured by a process called solid freeform fabrication (SFF).[4] The struts are designed to be 400 µm in diameter and are laid down in a 0o-60o-120o layer pattern. The gap width is 1.0 mm.

The µ-CT images of the scaffold were generated by a Skyscan 1072 micro-computed tomography scanner with voxel spacing of 12.9 µm in each direction. The images were output as bitmap files.

These bitmap files were processed by custom software in conjunction with open source software to produce files suitable for input to the IVE. Segmentation was performed by applying a threshold, and a 3D polygonal representation was generated based on the threshold.

Image Measurement and Analysis

While the initial processing of the image data was relatively straightforward, the analysis and measurement of geometric descriptors was more challenging. The latter motivated the use of IV. We do not have algorithms for the automatic three-dimensional measurement and analysis of the image data, so we used the IVE to interactively measure the desired features.

Our objective was to build a software system within the IV environment that integrated the following tasks: 1. measurement of scaffold characteristics; 2. analysis of the collected measurements; 3. display of the analysis; 4. interactions with the data and analyses that will enable grouping of results. The goal of these tasks was to achieve greater understanding of the structural characteristics of the scaffold material.

The initial measurement task that we undertook was the manual measurement of linear distances. It was felt that this step would enable the understanding of several important scaffold characteristics, one of which is strut diameter distribution and any associated anisotropy. We decided that the specific scenario for this first implementation would be: 1. The user collects a set of linear measurements; 2. A simple statistical analysis is made; 3. The analysis, including the distribution of measurements, is presented to the user; 4. The user can interact with the measurement distribution in order to highlight measurements that fall within any selected range of values. All of these tasks are to be performed in real-time during the IV session.

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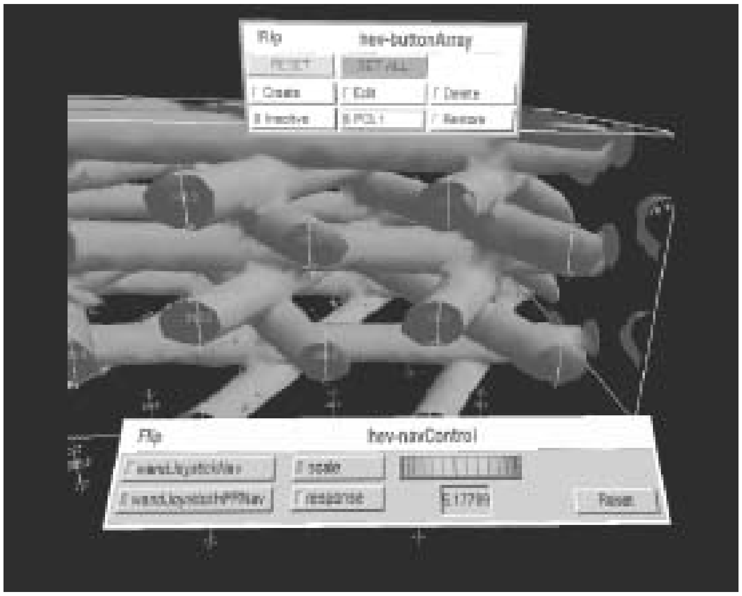

Figure 3. Cross-section of PCL scaffold with measurements of strut diameter.

The underlying software on which our immersive system is built is DIVERSE,[5] which provides a portable, modular, open source software platform that manages all aspects of the IVE. For the presentation of the data analyses and for some aspects of the user interaction, we used VEWL[6] which enables the use of standard desktop user interfaces within the IV environment. VEWL is a software subsystem that operates within the DIVERSE framework.

There are two main components to our implementation. The first component allows the user to manually make a series of linear measurements in the IV environment. The other component is a standard 2D user interface (displayed with VEWL) for displaying the measurement statistics and distribution in tabular and histogram form.

Our main objective in designing the user interface was to make the 3D measurement task direct and natural. The user makes a linear measurement simply by moving the hand-held wand to a point in the 3D virtual environment, pressing a button on the device, then moving to a second point and pressing the button again. Visual feedback is given at each step of the process and the user is able to adjust each end point simply by grabbing it with the wand and repositioning it. The process is fast, simple, and direct.

As a first demonstration of the quantitative IV, we compare the strut dimensions of the manufactured scaffold in Figure (2B) to the as-designed scaffold shown Figure (2A). We examine in detail the diameters at the strut junctions as compared to the inter-junction diameters.

We made two types of measurements: strut diameters and strut layer thickness. Strut diameters were measured directly at several points on each strut in a vertical direction in between the junctions (Figure 2). Strut layer thickness was found by measuring the vertical distance between the bottoms of struts on adjacent layers. We made layer thickness measurements at several junctions for each layer.

These two types of measurements were made on the "as-designed" scaffold model (generated synthetically from the design) and on the image of the actual manufactured scaffold material. The measurements of the "as-designed" scaffold are intended to validate the measurement method. The measurements of the experimental data will be used to understand the scaffold structural characteristics and fabrication method.

Figure 3 shows the PCL scaffold as it appears within the IVE, including several menus that allow the user to control the system. The box around the image data enables the researcher to interactively access features of interest for measurement throughout the volume of the 3D image. The IVE also enables the scientist to inspect various features and size ranges within the dataset. In Figure 4, the scientist has specified a range in the histogram. The corresponding measurements are highlighted in the display, providing additional visual feedback on scaffold uniformity.

Results and Discussion

As mentioned above, the as-designed scaffold model was made with 400 μm diameter struts arranged in layers that contacted. This configuration results, of course, in strut layer thickness of 400 μm. Our measurements resulted in these statistics:

Strut diameter: mean 399.3 μm std dev 1.3 μm n=63
Layer thickness: mean 399.8 μm std dev 1.6 μm n=24

The numbers clearly indicate that the measurement procedure is capable of producing valid results. During the collection of these data, we noticed that the distribution of diameter measurements was skewed slightly to the low side of 400 μm, likely because the cylindrical struts were being represented by polygonal approximations.

The measurements of the data representing the actual scaffold material yielded these results:

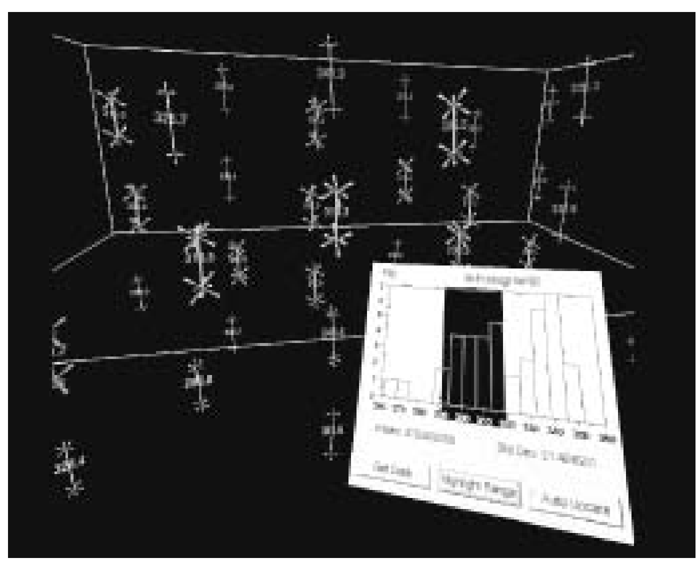

Figure 4. Selected histogram bins are highlighted in visualization showing distribution of strut diameters in the range of 200 to 320 Ìm. The struts have been removed from the scene in order to make the measurements more visible.

Strut diameter: mean 325.2 µm std dev 31.2 µm n=82
Layer thickness: mean 271.1 µm std dev 22.2 µm n=33

These manual measurements required the user to exercise judgment in selecting the measurement end points. Also note that, just as with the as-designed model, we perform our measurements using polygonal representations that are, inherently, approximations to the true form of the scaffold.

We see from the measurements of the actual scaffold that the mean inter-junction strut diameter is significantly more than the mean layer thickness (strut diameter) at the junctions. A qualitative sense of this effect can be seen in the IV environment; the struts appear to be fused and somewhat overlapping at the junctions. We also note, of course, that the actual strut diameter differs significantly from the as-designed model. The effect of these differences on the function of the scaffold is unclear and is the subject of future study. It is clear, however, that the IV environment has provided us with a way of making a meaningful quantitative characterization of scaffold structure. From this analysis, we find no anisotropy or gradients in inter-junction or at junction strut diameters.

Conclusion and Future Directions

We have found that IV is a technology that enables both qualitative and quantitative understanding of 3D structure of tissue engineering scaffolds that was not otherwise possible. The measurements made within the virtual environment would have been very difficult to make with typical desktop visualization techniques. We also plan to use the immersive environment in conjunction with automatic measurement techniques that we are developing to aid in understanding the action of the automatic algorithms and as a way of validating those methods.

The measurements and analyses enabled comparison of key scaffold descriptors across images. We have found that the inter-junction strut diameter is about 19% smaller than the as-designed model. The at-junction strut diameter (or layer thickness) is about 33% smaller than the as-designed model. IV measurement of these descriptors should be implemented for a further evaluation of the SFF manufactured scaffold: layer planarity, strut diameter uniformity, strut circularity, and strut location.

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