A Virtual Reality Visualization Tool for Three-Dimensional Biomedical Nanostructures

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Abstract. Nanoscience is the source of many advanced techniques and applications, the areas of life sciences and materials sciences included. In the field of materials, the latest technological developments have enabled the emergence of innovative materials. There are numerous of nanomaterials in the field of healthcare (nano-, bio- electronic, tools for medical diagnostics, theragnostics, etc.). It also can be noted that the involvement of nanotechnology to problems of medicine and health care is causing a revolution in the biomedical field, including nanomedicine with the emergence of new disciplines such as nanobiomechanics and nano-biology (mechanics of cells, cancer, microbiology, virology, etc.). The paper describes our new approach to creating 3D nanostructures on the SEM frame base. The impact of our research is demonstrated on the 3D digital model of nanostructures which includes 3D coordinates of nanostructures and is ready for the next research simulation process and also for virtual reality (VR) of the research results.

1. Introduction

In addition to the latest developments in small-scale imaging we observe also a significant technical and technological progress in order to better understand and identify the structures and their implications in biochemical and biophysical phenomena, as well as interactions with surfaces and materials. There are various approaches and results concerning the design of three-dimensional (3D) nanostructures for biomedical applications. Since the beginning the costs for many of 3D nanostructures models have been high.

For example, researchers from the North Carolina State University (NC State) have developed a new low-cost lithography technique that can create three-dimensional (3D) nanostructures for biomedical, electronic, and photonic applications, replacing laborious stacking of two-dimensional (2D) patterns to create 3D structures. Most conventional lithography uses a variety of techniques to focus light on a photosensitive film to create 2D patterns. These techniques rely on specialized lenses, electron beams or lasers — all of which are extremely expensive. Other conventional techniques use mechanical probes, which are also costly.

The NC State researchers took a different approach, using nanoscale polystyrene spheres. The nanospheres are transparent, but bend and scatter the light that passes through them in predictable

ways, according to the angle that the light takes when it hits the nanosphere. They are using the nanosphere to shape the pattern of light, which gives us the ability to shape the resulting nanostructure in three dimensions without using the expensive equipment required by conventional techniques. Just such approach allows them to create 3D structures all at once, without having to make layer after layer of 2D patterns. The researchers have also shown that they can get the nanospheres to self-assemble in a regularly-spaced array, which in turn can be used to create a uniform pattern of 3D nanostructures.The slanted hollow structures can be fabricated in an ordered periodic array, which can find applications in nanoneedles for medical nanomaterial drug delivery, nanoscale "inkjet printers" for printing electronics or biological cells, antennas, or photonic components. The work was published online Dec. 8 in the *Small* journal and supported by the grant from the NASA Early Career Faculty and by the Nanosystems Engineering Research Center for Advanced Self-Powered Systems of Integrated Sensors and Technologies at the NC State, under a National Science Foundation grant [1].

Xu A. Zhang et al. [2] sculpting asymmetric, hollow-core, 3D nanostructures using colloidal particles is also one of the research approaches. By Zhang, colloidal elements have historically played a key role in "bottom-up" self-assembly processes for nanofabrication. However, these elementary components can also interact with light to generate complex intensity distributions and facilitate "top-down" lithography. Here, a nanolithography technique is demonstrated based on oblique illuminations of colloidal particles to fabricate hollow-core 3D nanostructures with complex symmetry. The light–particle interaction generates an angular light distribution as governed by Mie scattering, which can be compounded by multiple illuminations to sculpt novel 3D structures in the underlying photoresist. The fabricated geometry can be controlled by the particle parameters and illumination configurations, enabling the fabrication of a large variety of asymmetric hollow nanostructures. The proposed technique has high pattern versatility, low cost and high throughput, and it can find a potential application in nanoneedles, nanonozzles, and materials with anisotropic properties.

K.H.A. Bogart et al. [3] are interested in simulation and fabrication of large-area 3D nanostructures. They developed a model that predicts the phase mask required to generate a specifically desired nanostructure. We have compared this inverse model with experimental 3D structures to test the validity of the simulation. We have transferred the PnP fabrication process to a class-10 commercial cleanroom and scaled-up the processed area to >2000mm^2, tested photopolymer additives designed to reduce resist shrinkage, incorporated atomic layer deposition (ALD) to coat the 3D patterned resist with metals/metal-oxides improve structure robustness, and generated quasi-crystal patterned 3D nanostructures.

High-resolution, conformable phase masks provide the means to fabricate, in an experimentally simple manner, classes of 3D nanostructures that are technologically important but difficult to generate in other ways. In this approach, light passing through a phase mask that has features of the relief comparable in dimension to the wavelength, generates a 3D distribution of intensity that exposes a photopolymer film throughout its thickness. Developing this polymer yields a structure in the geometry of the intensity distribution, with feature sizes as small as 50 nm. Rigorous coupled-wave analysis reveals the fundamental aspects of the optics associated with this method; a broad-range 3D nanostructures patterned with it demonstrates its technical capabilities. A nano-porous filter element built inside a microfluidic channel represents one example of many types of functional devices that can be constructed.

2. Virtual Reality Tool of 3D Nanostructures for Biomedical Applications

Our approach is focused on creating the 3D model of nanostructures to be used for simulation process in bio medical spheres. We use them to develop a digital 3D model of nanostructure which we are able to use for many simulation processes and also for virtual reality of research simulation results. There are five steps to be handled to obtain the correct result for our next research.

- Observations of surface of the fibers of synthetic polymeric nanofibrous membrane

- 3D filter structure model
- Digital 3D model of nanostructure
- Virtual reality tool
- Using synthetic polymeric nanofibrous membranes for fabrication skin substitutes

2.1 Observations of the Surface of Synthetic Polymeric Nanofibrous Membrane Fibers

Scanning electron microscopes are widely used for material characterization because of the high-resolution image, three-dimensional images, observation abilities and topographical, morphological and compositional information.

Surface and topography of fibers were observed using a high-resolution scanning electron microscope (SEM) with the field emission cathode Quanta FEG 250 (FEI, USA). While the image in secondary electrons mode using Everhart-Thornley detector (ETD) was not usable for generating the 3D structure model image with an in-house developed software (UTB soft Filtration), therefore the backscattered electron detector (BSED) in high-vacuum mode was used for imaging. In comparison to ETD mode (see Fig. 1a), the following advantages were observed using BSED mode:

The fibers exhibited homogenous color around the diameter and the whole picture, the borders of fibers were darker, and the fibers on the top showed a lighter color value then the more deep in fibers.

The best parameters of imaging for BSED mode were found: acceleration voltage 5 kV, spot 7, magnification 10 000x, BSED detector in Z contrast mode see ‘figure 1’.

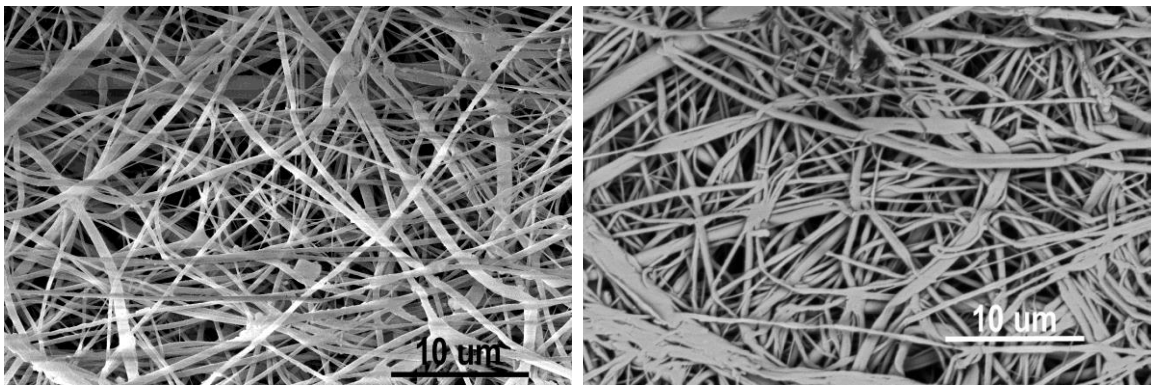

Figure 1. Comparison of SEM images generated using Everhart-Thornley detector (ETD) a), and backscattered electron detector (BSED) in Z Contrast mode b).

2.2 3D Filter Structure Model

Backscattered electron detector (BSED) in high-vacuum mode was used for imaging. Such prepared SEM images were usable for the next process to generate 3D structure model.

The 3D model has been generated from SEM image with in-house developed software (UTBsoft Filtration) at the Faculty of Technology, Tomas Bata University in Zlín. The utilized procedure is based on the methodology originally proposed in [4-5], which enables to take into account the varying fiber diameter, curvature and possible structure defects as well as 3D features of the whole nonwoven structure correctly. In this work, back-scattered electron (BSE) detector has been used for electron microscopy scanning to get the SEM image for a given sample.

In the first step, proper threshold level is applied for the original slightly Gaussian blurred grayscale SEM sample image to filter the fibers at the medium and top surfaces for the resulting black/white image. Secondly, nanofiber centerline pixels are calculated. In the next step, the 3D shape of each fiber section is created by rotating into the depth of the local diameter circle fitted in the white fiber area along every corresponding centerline pixel. In the final step, 3D structure model was build up as the stack of the integer number of non-overlapping model layers, *N*, to reach the

same mass area as the real nonwoven structure. 3D model has been generated on a PC utilizing Windows 10 with the following configuration: Intel® Core™ I7-980X processor equipped with 6 cores able to run 12 threads, having each a clock speed of 3.33 GHz, and 24 GB DDR3 memory and a NVIDEA Quadra 2000 graphical card. See 'figure 2'.

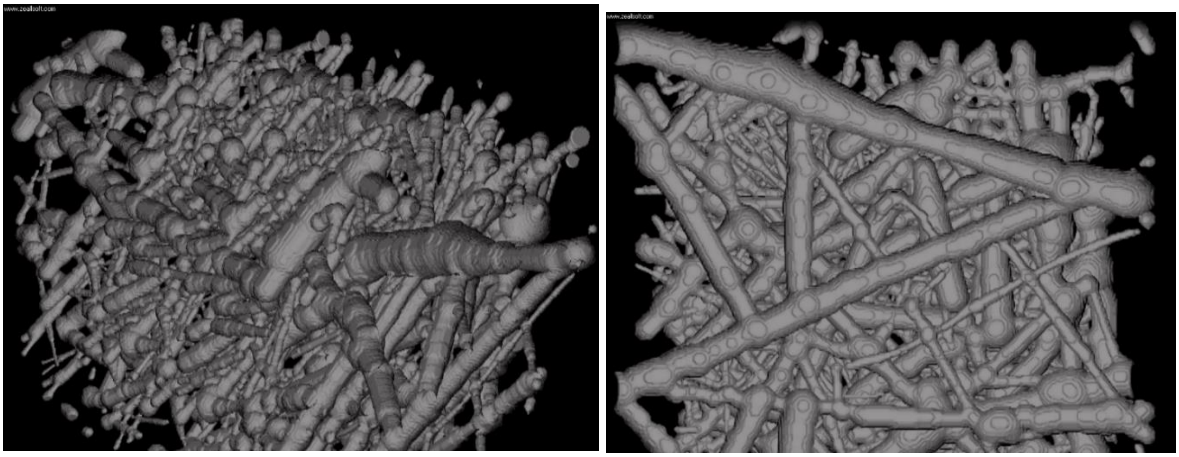

Figure 2. 3D filter structure model of nanofiber membrane

2.3 Digital 3D model of nanostructure

Three dimensional model of nanostructure provides a great decision in biomedical applications. There are many biological and medical applications in which digital 3D model of nanostructure is the first step for the entire process of the design. It is also a milestone in creating our virtual reality model. Asci code 'figure 3'of digital 3D model of nanostructure with 3D coordinates in VRML language provides the way how to schedule all simulation processes and produce them in Virtual Reality. We are able to incorporate c++ scripts and java and vrml scripts. We can combine several of 3D formats.

```
DEF default Transform {
     translation 0.0000000 0.0000000 0.0000000
     children[Shape {
          appearance Appearance {material Material {
                     ambientIntensity 1.0000000
                     diffuseColor 0.5880000 0.5880000 0.5880000
                     shininess 0.1450000
                     specularColor 0.0000000 0.0000000 0.0000000
                     transparency 0.0000000
               }}
          geometry DEF default-FACES IndexedFaceSet {
               coord DEF default-COORD Coordinate {point [
                                   458.8999939
258.5000000 -0.3968000,458.5000000 258.5000000
0.0000000,458.8999939 258.1000061 0.0000000,458.8999939
260.5000000 -1.2050000,
                                   457.7000122 260.5000000
0.0000000,458.8999939 262.5000000 -1.2050000,457.7000122
262.5000000 0.0000000,458.8999939 264.5000000 -1.2050000,
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266.5000000 0.0000000,458.8999939 268.5000000 -0.3968000,
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0.0000000,458.8999939 268.8999939 0.0000000,460.8999939
256.5000000 -0.9368000,460.0000000 256.5000000 0.0000000,
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260.5000000 -1.4710000,460.8999939 262.5000000 -1.4710000,
                                   460.8999939
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Figure 3. Asci code of 3D digital model

2.4 Virtual Reality Tool

Our 3D VT tool is composed of some modules. Each module can work separately as an independent tool or working with other modules together as an integrated tool. For our nano in bio example the major modules of our virtual reality tool are two modules.

- Module for receiving the third-dimension coordinates of nanostructure as a 1.level
- Module for receiving the third-dimension coordinates of - Fibrin mesh on membrane as a 2.level

2.5 Using Synthetic Polymeric Nanofibrous Membranes for Fabrication Skin Substitutes

It can be also noted that the involvement of nanotechnology into biology and medicine is causing a revolution in the biomedical fields, including nanomedicine with the emergence of new disciplines such as nanobiomechanics and nanobiotechnology. Our 3D model of nanostructure was developed for the researcher of Department of Biomaterials and Tissue Engineering, The Institute of Physiology, Czech Academy of Sciences, Czech Republic. Their research of the protein-coated biodegradable synthetic polymeric nanofibrous membranes seems to be promising for the fabrication of advanced skin substitutes. They prepared polylactic acid (PLA) nanofibrous membranes by needle-less electrospinning technology in collaboration with the Technical University in Liberec, Czech Republic. In order to enhance the cell attachment and growth, they coated membranes with biomolecules (e.g. fibrin, fibronectin and collagen I). They observed that fibrin either covered individual fibers in the membrane or covered individual fibers and moreover created a fine nanofibrous mesh on the membrane surface. In addition, fibronectin was predominantly adsorbed on the surface of the fibrin mesh and formed an additional nanofibrous structure. Fibrin nanocoating significantly improved the attachment, spreading and growth of human dermal fibroblasts. Moreover, fibrin nanocoating also improved the ultimate tensile strength of the nanofibrous membranes [6, 7]. Chemical and physical characterization of the synthetic materials is crucial for the preparation of suitable scaffold for cell cultivation and subsequently for the development of complete full-thickness skin substitutes ‘figure 4’. Many different techniques for the visualization and characterization of the structural properties of the materials and biomolecules can be used [8]. Immunofluorescence staining and visualization by confocal microscopy are one of the most frequently used techniques, but they are not suitable for the simulation of the 3D structure. Scanning electron microscopy (SEM) and using the computer visualization tools bring more advanced approach in 3D material analysis. This novel technique allows visualization of real data obtained from SEM and to analyze them using a computer 3D virtual model. Porosity, density and average diameters of each fiber of nanofibrous membrane can be analyzed more accurately and the mechanical properties of the material can be better described from the point of view of the cell–materials interactions.

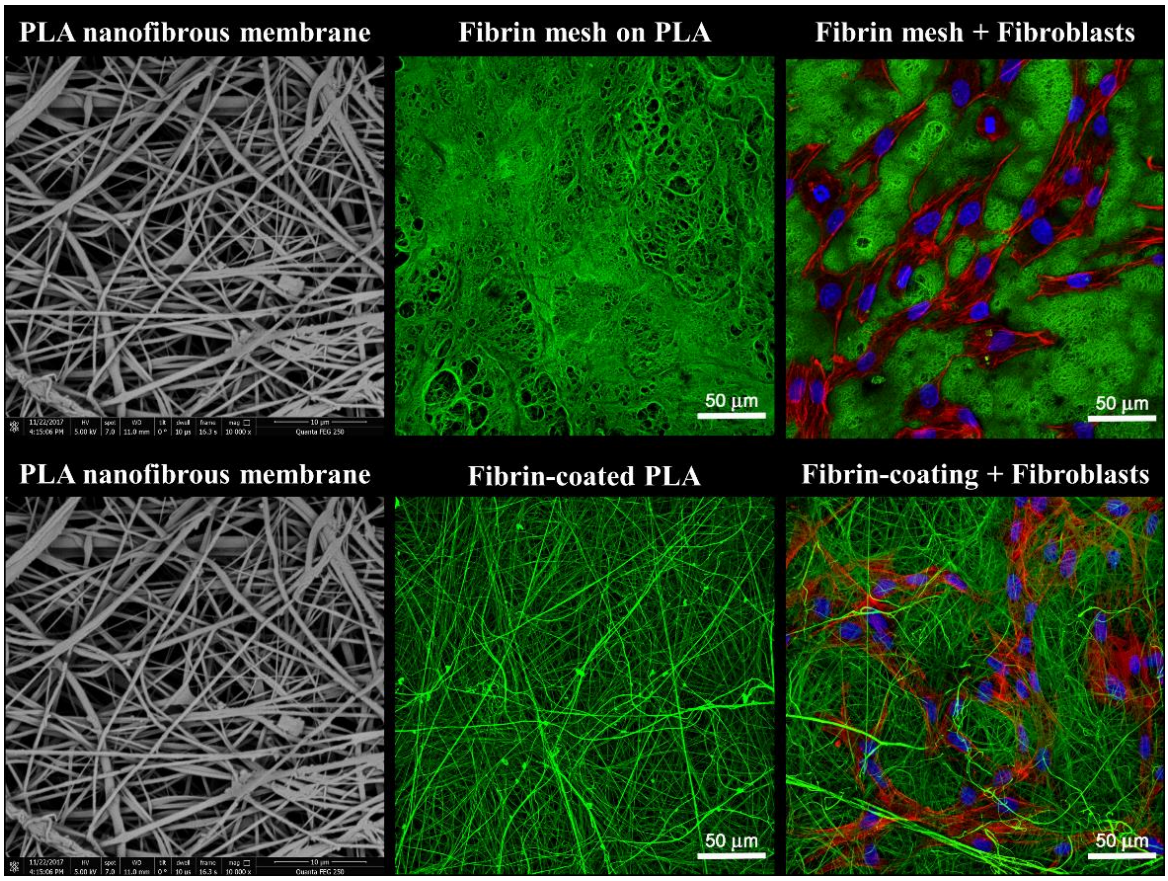

Figure 4. Fibrin-coated PLA membranes with human dermal fibroblasts. 1. Column – SEM of PLA nanofibrous membrane, 2. Column – immunofluorescent staining of fibrin mesh on PLA membrane and fibrin-coated PLA membrane, 3. Column – immunofluorescent staining of fibroblasts on fibrin Fibrin stained with primary and secondary antibodies (green, Alexa 488). F-actin of fibroblasts stained with Phalloidin-TRITC (red). Cell nucleus stained with Hoechst #33258 (blue). Leica TCS SPE DM2500 confocal microscope, obj. 40x/1.15 NA oil.

The current work has been contributed to the construction of 3D full-thickness skin substitute. Definitely, the 3D environment is more physiological for the cells especially for fibroblasts and adipose stem cells. At the current step it was clearly identified how to appropriately modify nanofiber membranes by fibrin homogenous mesh in order to enhance fibroblast proliferation. In addition, the researcher knows how to prepare collagen gel on the modified membranes with fibroblasts. He has already found that fibroblasts migrate into the gel and the morphology of the fibroblasts in collagen gel is completely different, as you can see in the bottom picture. Keratinocytes were primarily seeded on the surface of the collagen gel. We observed keratinocytes proliferation and cell stratification.

3. Conclusion

Using virtual reality tool for visualization of the full-thickness skin substitute require a number of steps. Currently we are able to use 3D virtual reality tool for the development of the digital 3D model of synthetic polymeric nanofibrous membranes, which is useful for analysis of structural properties of the membrane in the process of the fabrication of skin substitute. This 3D virtual reality tool can be used for those nanostructures that can be scanned by electron microscope. Our next steps depend on the research steps of our collaborators in the process of the fabrication of the skin substitutes. Our second step will be to develop additional part of virtual reality tool for biological samples, for

example fibrin, fibronectin and collagen I, which have to be fixed, dehydrated and dried before scanning by electron microscope.

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