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Electron Tomographic Reconstruction of Soft Nanomaterials for Morphometry Studies

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Electron tomography has been widely used to image and quantify materials systems with complex and irregular morphologies, especially with the ever-increasing demand to understand three-dimensional (3D) nanoarchitectures of soft material and colloidal assemblies for energy [1], separation [2] and catalysis [3] applications. Electron tomography on soft materials, such as polymers and biomaterials composed of low atomic weight (low-Z) elements, is challenging due to their low contrast and sensitivity to damage when exposed to the electron beam. Our previous work used strategies of defocusing (to create a Fresnel fringe) and a low dose of the electron beam to tomographically reconstruct the 3D morphology of biological and synthetic soft materials with nanometer resolutions [2, 4]. Moreover, using the 3D reconstructions we developed a morphometric workflow to quantify the nanomorphology, followed by relating the nanomorphology to the morphogenesis and mechanical properties [2].

Building on our previous work [2], using polyamide (PA) polymer membranes as a model system, here we demonstrate parameter extraction (such as surface area and void sub-sections) at nanometer resolutions using transmission electron microscopy (TEM) tomography. Furthermore, we present a 3D watershed analysis on nanovoid architectures to understand the morphogenesis of nanovoid clusters. PA membranes are synthesized as described by Karan *et al* [5]. Three membranes are synthesized with 5 w/v% *m*-phenylenediamine monomer, and 0.05 w/v% (PA1), 0.1 w/v% (PA2) and 1 w/v% (PA3) trimesoyl chloride monomer. All PA membranes are imaged using a JEOL 2100 Cryo TEM at an acceleration voltage of 200 kV, electron dose rate of 4–7 $e^{-}Å^{-2}s^{-1}$, and defocus of −2048 nm. The PA membrane samples are tilted from 0° to −60° and 0° to +60° to collect a series of TEM images at 2° intervals. The tomogram and 3D reconstructions are generated using IMOD 4.9.3 [6], OpenMBIR [7] and Amira 6.4. Using the TEM tomographic reconstructions at voxel resolutions of (3.5×3.5×3.5) $Å^3$, we are able to successfully extract the surface area of PA membranes (Fig. 1A). The surface areas of the three PA membranes are calculated by enveloping the reconstructed 3D volume in a triangular mesh surface. The results from tomographic reconstructions are compared with atomic force microscopy (AFM)— a more conventionally used method of surface area measurement (Fig. 1B). Quantitative comparison of the percentage increase in surface area (SA_i=[(SA–SA_p)/ SA_p], where SA is the area from tomography or AFM, and SA_p is the projected area; SA_i(AFM)= 78.2%±8.5%, 71.4%±5.4%, and 13.3%±0.6%, SA_i(tomography)= 82.7%±3.5%, 74.6%±2.5%, and 29.8%±0.6% and for PA1, PA2 and PA3, respectively) shows that the surface area from tomographic reconstruction is higher than that of AFM, especially for PA3, which has the smallest nanoarchitectures out of the three samples under study (Fig. 1C). Unlike the AFM cantilever tip which limits the resolution and detection of nanoscopic surface features, tomographic 3D reconstruction can capture the details of the PA membrane surface at nanometer resolutions, to provide a more accurate measurement of the surface areas for soft nanomaterials. Furthermore, our recent efforts show that PA membranes have interconnected inner nanovoid clusters (Figs. 2A,B). Expanding on our efforts of analyzing nanovoids within soft materials using electron tomography, we subjected the reconstructed internal nanovoids of PA membranes to 3D watershed segmentation (Figs. 2C,D). The segmentation separates interconnected nanovoid clusters into sub-voids. As shown in Figs. 2E,F, we foresee these segmented watersheds being used to understand the connections between sub-void sections during the morphogenesis of interconnected nanovoid clusters [8].

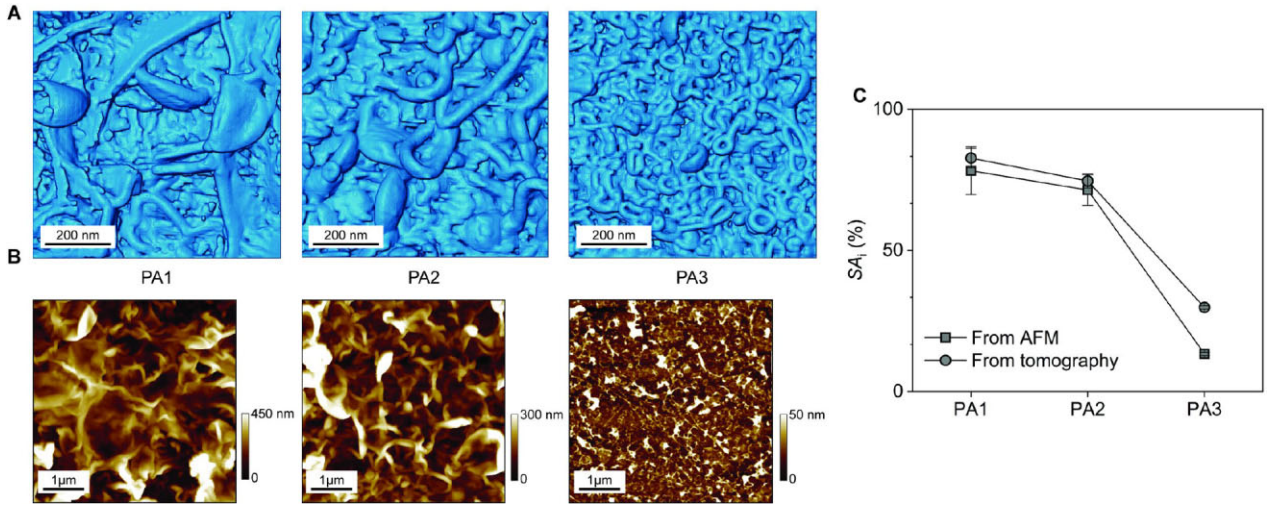

Fig. 1. (A) Surface area as characterized by electron tomographic reconstructions. (B) AFM height maps used for surface area measurements. PA1, PA2 and PA3 are depicted from left to right. (C) Comparison between the percentage increase in surface areas from tomographic reconstructions and AFM maps.

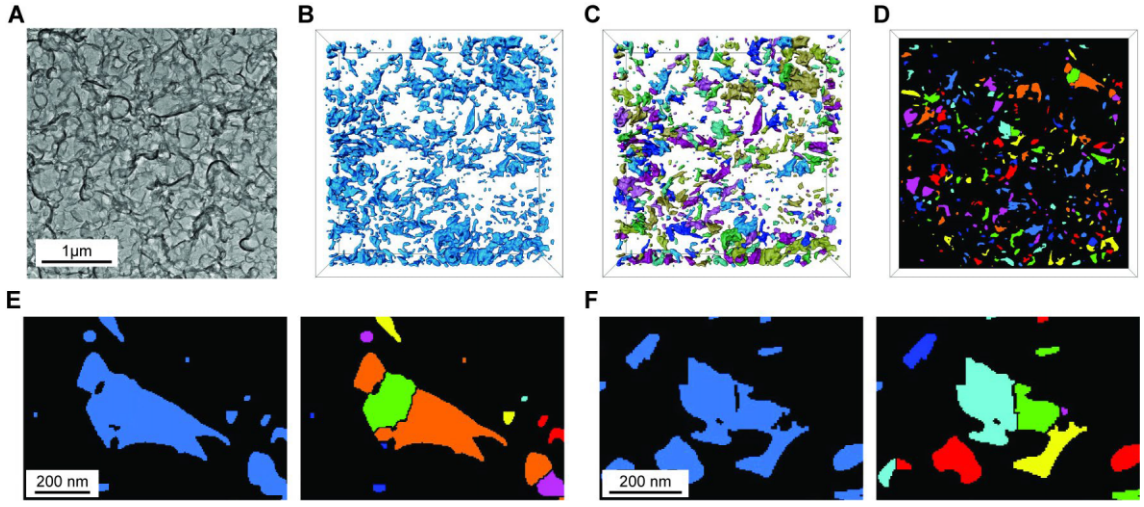

Fig. 2. (A) TEM micrograph of a PA membrane and (B) tomographic reconstructions of internal nanovoids. Note that the interconnected nanovoids can only be visualized using the tomographic reconstructions. (C) Watershed analysis result for the interconnected nanovoid clusters. The separated watersheds are colored randomly to show sub-voids. (D) A 2D slice showing a cross-section of separated watersheds and sub-voids. (E, F) Zoomed-in cross-sections of an interconnected nanovoid cluster (left) and sub-voids after watershed analysis (left). (B–D) Bounding box dimensions are 3580 nm×3580 nm×600nm.

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