



1 *Type of the Paper (Article, Review, Communication, etc.)*

2 **TEM Tomography for the Identification of** 3 **Nanopores in Silicon Nanomembranes**

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10 **Abstract:** We [modified](#) a TEM sample holder to accept 5.4x5.4 mm silicon nanomembrane chips and
11 imaged porous silicon nitride films (50-100 nm thick, NPN, SiMPore Inc). After imaging and
12 reconstruction (Fiji, TomoJ, SEG3D2), we used COMSOL Multiphysics to simulate the flow profile
13 of constrictions inside a nanopore ($\lambda = 725$ nm). These techniques are adaptable to other structures
14 that provide sufficient contrast under TEM imaging.

15 A single paragraph of about 200 words maximum. For research articles, abstracts should give a
16 pertinent overview of the work. We strongly encourage authors to use the following style of
17 structured abstracts, but without headings: 1) Background: Place the question addressed in a broad
18 context and highlight the purpose of the study; 2) Methods: Describe briefly the main methods or
19 treatments applied; 3) Results: Summarize the article's main findings; and 4) Conclusions: Indicate
20 the main conclusions or interpretations. The abstract should be an objective representation of the
21 article, it must not contain results which are not presented and substantiated in the main text and
22 should not exaggerate the main conclusions.

23 **Keywords:** TEM; tomography; nanomembranes

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25 **1. Introduction**

26 [Electron tomography \(ET\) is a powerful technique used for resolving three dimensional](#)
27 [structures of nanometer-scale objects. Most frequently performed with transmission electron](#)
28 [microscopy \(TEM\), ET has been used to determine the structure of nanoparticles, gel embedded](#)
29 [objects, and even biological structures \(nanoparticle in agarose, 3D dislocations, synapse references\).](#)
30 [The resolution limit of this technique is typically between 5-20 nm with the potential to image features](#)
31 [much smaller.](#)

32 [Electron tomography is typically performed in standard TEM systems, therefore the samples are](#)
33 [constrained to requirements of the sample holder and system. This means that samples are mounted](#)
34 [on 3 mm circular copper mesh grids \(Fig. 1\), where the imaging is performed in the free space of the](#)
35 [mesh. Nanoparticles are typically imaged on 'lacey carbon' versions of these copper grids, which](#)
36 [have carbon fibers that span the free space allowing for the attachment and retention of the particles](#)
37 [in these spaces. Biological samples are typically sliced to 100 nm thick by ultramicrotomes and then](#)
38 [placed on the copper grid without any fixation. However, larger samples must be reduced to fit](#)
39 [within the constraints of the standard grid size and sample holder geometry. This prevents users](#)
40 [from imaging larger structures in whole.](#)

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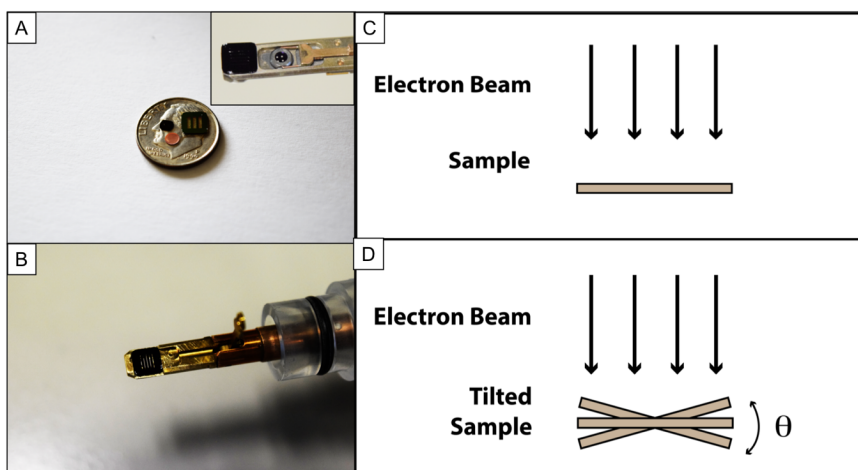
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44 Transmission electron microscopy (TEM) is a powerful imaging method that allows for high
 45 magnification imaging of ultrathin samples. This technique allows for high resolution analysis of
 46 structures ranging from 100 nm to the level of single atoms.

47 Sample preparation for TEM has been standardized with no variation in technique. Because of
 48 the nature of the technique, samples are typically suspended on a copper mesh grid, where the
 49 material is fixed over the empty spaces in the mesh. This method bypasses the need for a solid
 50 substrate, which would then make transmission imaging impossible. The standard TEM grid size is,

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 52 Many software tools have been developed to aid the reconstruction of electron tomography data,
 53 such as TomoJ¹, Etomo. While not atomistically thin as graphene
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55 **2. Discussion**



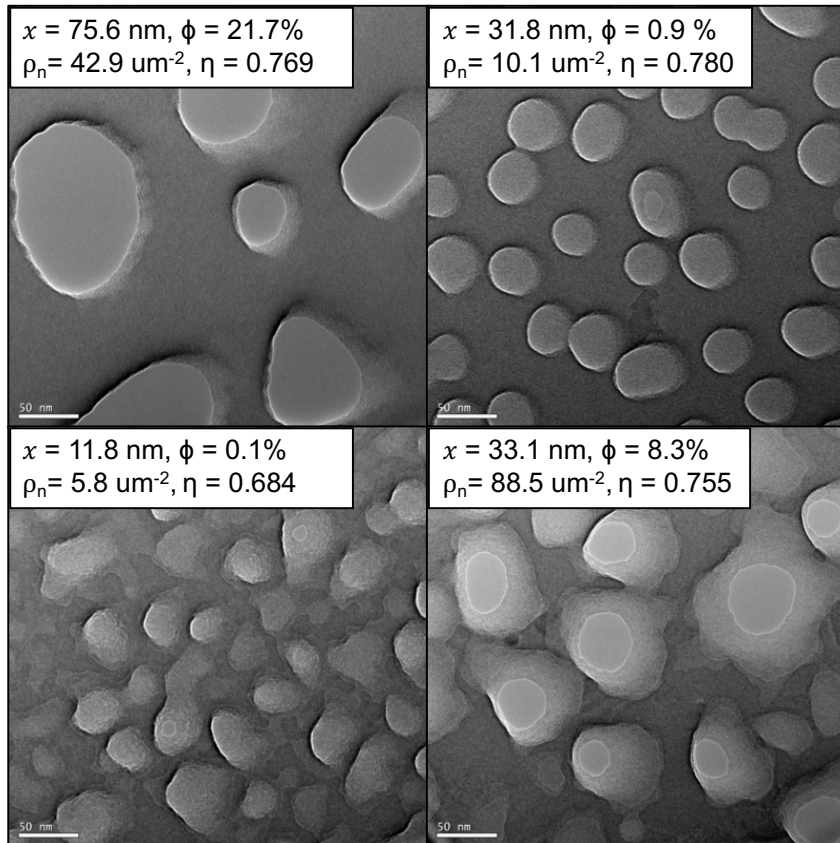
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 57 **Figure 1.** Experimental Setup. (A) Common TEM substrates are 3 mm circular copper grids or 3 mm
 58 silicon chips. Other larger substrate formats could be imaged (square chip), but TEM holders are
 59 designed for 3 mm diameters. (B) Image of modified FEI Tecnai G2 TEM holder containing 5.4x5.4
 60 mm NPN chip. The viewing area is still limited to the 3 mm circular aperture. (C, D) The modified
 61 holder facilitates gathering tilted views of the sample, important for reconstruction.

62 Figure 2 displays a sample of NPN TEM images. A wide variety of nanotopography and pore
 63 shapes can be created from the NPN fabrication process. Specific nanomembrane properties are listed
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72 **Figure 2.** NPN Pores Show Widely Different Morphologies. TEM images (63 kx) of 4 different varieties

73 of NPN. The range of sizes and shapes can be largely open, underetched, pitted and underetched, or

74 pitted and open. Histograms of counted pores show the wide variety of porosities and diameters. x =Average Pore Size, ϕ = Porosity, ρ_n = Pore Density, η = Circularity.

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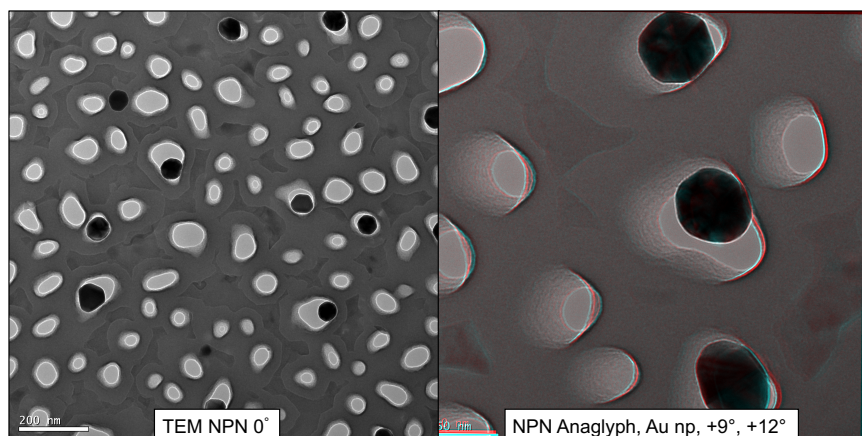
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Figure 3 displays a NPN membrane used for imaging as well as a red/cyan false colored image of gold nanoparticles inside some NPN nanopores. The red and cyan channels are images gathered at separate angles. By imitating the separation of a human's pupils ($\sim 3\text{-}4^\circ$) and wearing red/cyan glasses, a visual illusion is created where only one colored image is sent to one eye. This setup imitates the phenomenon of stereopsis, also known as binocular vision, recreating a perception of depth to the image. The nanofeatures displayed in Figure 3 display tiny Au nanoparticles (black ovals) lodged in a NPN nanopore, and it is clear that these particles appear at different heights within the nanopore. Similarly, the nanoparticles appear to have different adherent forces acting upon them; some are stuck in smaller diameter pores and others are cupped along the sidewalls. The major limitation of this technique in TEM imaging is that it requires electron transmissive samples, that is, samples that are sufficiently thin enough to be observed with the TEM. Gold is very electron-dense, and these particles are sufficiently thick in the center that it provides a strong contrast against the nanomembrane, however, the edges of the particles do provide some ability to image through the particle. Thicker amounts of gold are opaque using TEM (above ~ 20 nm). Thicker gold films could

89 be reconstructed using this tomogram reconstruction technique with SEM, but the resolution is worse
90 and might not capture the important texture of the nanomembrane.
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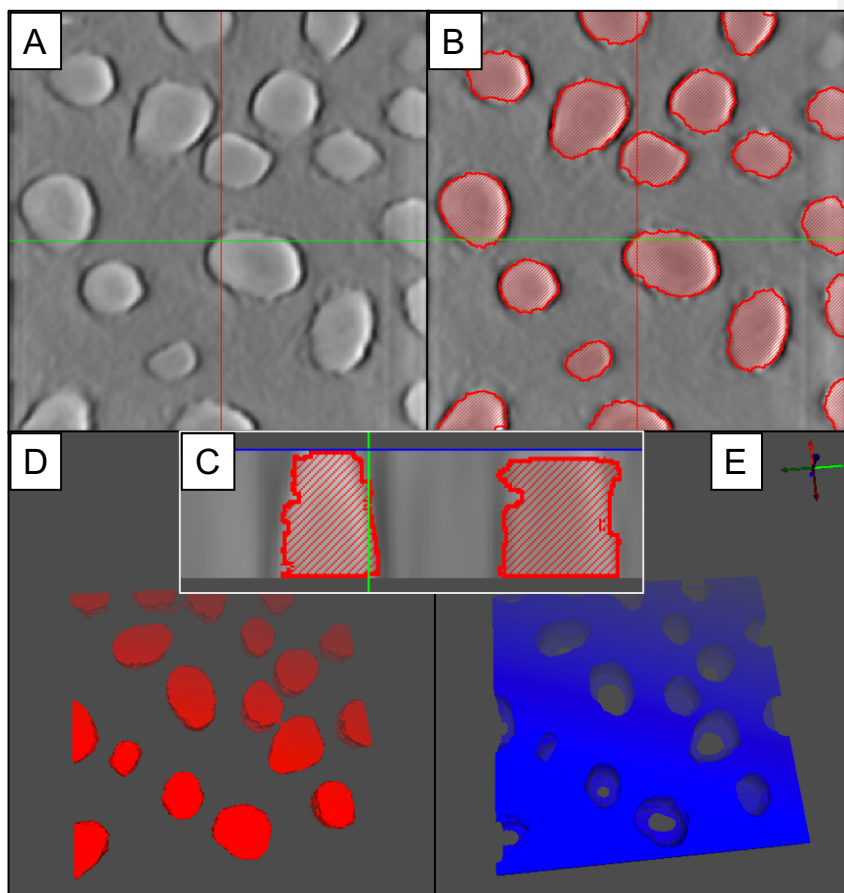
93 **Figure 3.** NPN Anaglyph with Gold Nanoparticles. NPN (100 nm thick) was exposed to 50 nm gold
94 nanoparticles and imaged from 0° to +15°, in 3° increments. The anaglyph was formed by taking the
95 +12° and +9° images, aligning them, and assigning them to red and cyan color channels. Viewed with
96 appropriate lenses, these images can provide the illusion of depth.

97 While stereoscopic images are useful to visualize the nanotopography of the NPN and Au
98 nanoparticle, they are reliant on our own visual processing system to ascribe depth to elements in the
99 image. There exist techniques for reconstructing 3D surfaces from stacks of tilted images, known as
100 electron tomography. Figure 4 describes a method for generating a 3D model from these tilted layers.
101 TOMOJ¹, an ImageJ² plugin (<http://www.cmib.fr/en/download/software/TomoJ.html>), was used to
102 map the tilted images into a gray-scale height stack of images, using a OS-SART algorithm. The
103 reconstructions can be large (~200-300 MB), therefore images were scaled down to 400 x 400 pixels
104 before tomogram computation. Each of the contours in the tomogram was then segmented into
105 different nanopores, using SEG3D2³ segmentation software (<http://www.sci.utah.edu/cibc-software/seg3d.html>), which was then exported into a 3-dimensional STL file. Assigning segments to
106 different heights in the stack and knowing the physical dimension of the NPN thickness allows us to
107 characterize the size and location of different contours in the nanopore.
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110 The STL file is then imported into free animation and modeling software (Blender 2.79c,
111 <https://www.blender.org/>) for visualization purposes.
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115 **Figure 4.** Method for Generating 3D models from Tilt Stacks. (A) As tomograms can be generated
 116 from the tilted images, (B) we can segment individual structures at layers within the stack. (C) A
 117 crosssection of the reconstruction highlights the nanopore walls. The thickness of the membrane
 118 provides a height for arbitrary structures within the tomogram. (D) Nanopores or the (E)
 119 nanomembrane itself can then be exported into other software for visualization or simulation
 120 purposes.

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123 **Figure 5.** Simulation of XXX Characteristic of Reconstructed Gold.

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127 **Supplementary Materials:** The following are available online at www.mdpi.com/link, Figure S1: title, Table S1:
128 title, Video S1: title.

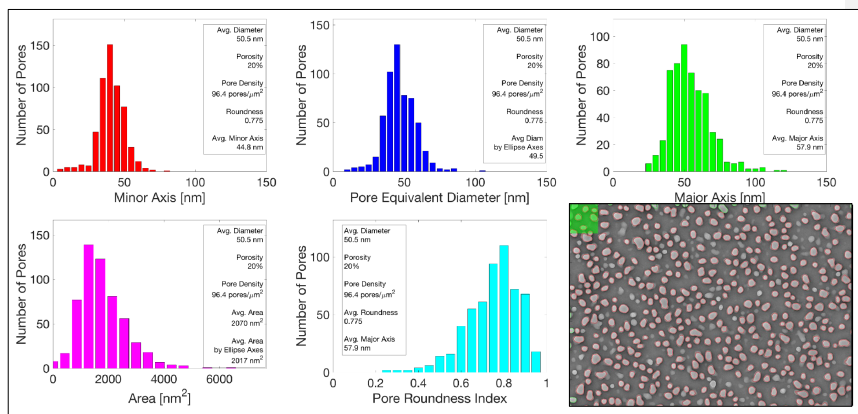
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135 reagents/materials/analysis tools; Y.Y. wrote the paper." Authorship must be limited to those who have
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143 the writing of the manuscript, and in the decision to publish the results".
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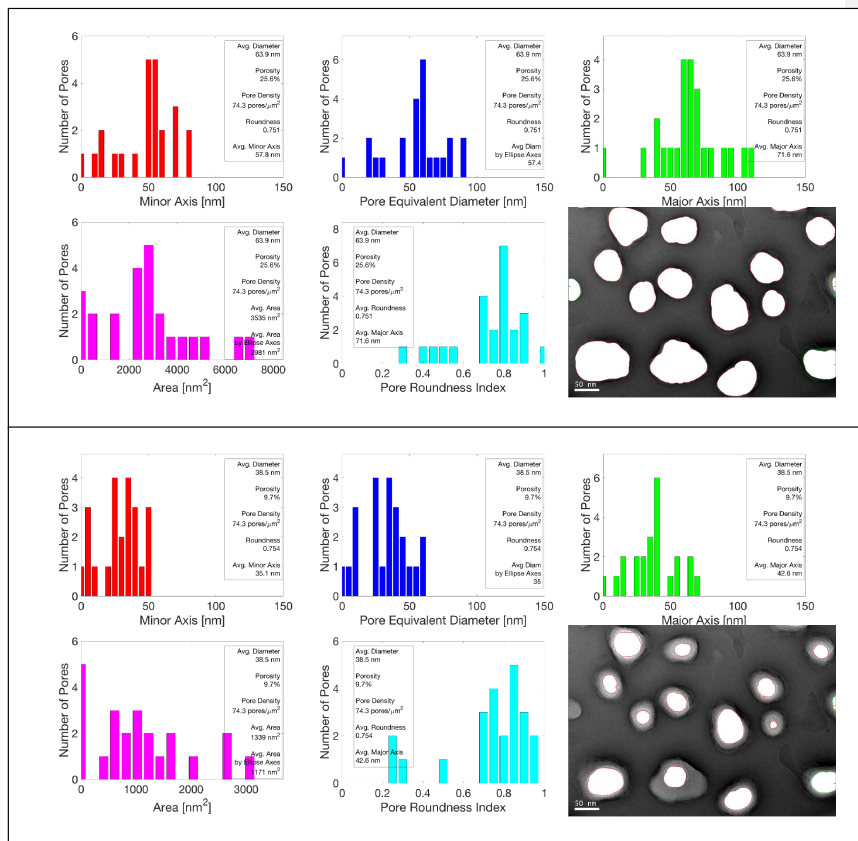
145 **Appendix A**

146 Nanopore statistics were generated using a custom MATLAB pore processing software⁴. TEM
 147 images were cropped, background corrected, and thresholded to generate open pore shapes. Pores
 148 were dilated and then eroded to merge edges (on average 2x), and then recorded. Aberrant pores
 149 were removed manually. Pore diameters were calculated using two methods 1. Converting the
 150 thresholded nanopore area into an equivalent circular diameter, or 2. Establishing an ellipse based
 151 on the major/minor axes of the thresholded nanopore area, and calculating an equivalent circular
 152 diameter from the ellipse.
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154
 155 **Figure A1.** Example Nanopore Statistics. Histograms of nanopore properties were generated from
 156 background corrected, thresholded TEM images of NPN membranes (red pore outlines). The pores
 157 on the outer edge of the image were omitted (green pore outlines). The green square indicates the size
 158 of the background correction, averaging over many nanopore areas.

159 As an alternative method of thresholding, nanopores can be manually painted to identify both
 160 the top and bottom orifices in the nanomembrane.
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163 **Methods**

164 TEM: A FEI TECNAI G20 holder was milled to accept 5.4x.5.4 mm chips. After centering the
 165 substrates at the eucentric point, images were gathered on nanoporous silicon nitride (NPN, SIMPOre
 166 Inc.) chips, tilting the chips between -14° and +14°. After each tilt, images were realigned using a
 167 software fiducial mark and manually moving the stage.

168
 169 ImageJ/TOMOJ: Images were then imported to ImageJ software
 170 (<https://imagej.nih.gov/ij/download.html>)², scaled to 400x400 pixels, and sorted in order from
 171 negative angle to positive angle. Tomograms were generated using the TOMOJ plugin (version 2.35,
 172 <http://www.cmib.fr/en/download/softwares/Tomoj.html>)¹. Blank pixels were filled with the average
 173 value of the image. Hot spots were removed at a radius of 3 pixels. The ordered stacks were
 174 background corrected using a 50 pixel sliding parabola. Stacks were aligned simply using a cross-
 175 correlation translation correction, then a landmarks-based 3D alignment was performed by mapping
 176 local minima, (200 seeds, 5 minimum chain length, 20 pixel patch size, 0.5 correlation threshold)⁵.
 177 Tomograms were computed using OS-SART (30 iterations, 0.1 relaxation coefficient, 300 pixel
 178 thickness).

179
 180 SEG3D2: Tomograms were imported into SEG3D2 software ([http://www.sci.utah.edu/cibc-](http://www.sci.utah.edu/cibc-software/seg3d.html)
 181 [software/seg3d.html](http://www.sci.utah.edu/cibc-software/seg3d.html))³. Tomograms were cropped to the thickness of the nanomembrane from x and

z projections, and then intensity corrected (2 polynomial order, 0.15 edge sensitivity). Individual nanopores were identified using the neighborhood connected filter, with 5-10 seeds placed on the feature of interest. As an individual feature was identified, seeds were cleared and another feature was then identified by placing seeds on the new feature and running the filter again. Once all features were identified, layers were merged into a single segmentation layer using boolean OR filters. Noise in the segmentation layer was removed by using a smooth binary dilate and erode filter (value = 2). Isosurfaces were then computed and exported to a STL file format.

References

References must be numbered in order of appearance in the text (including citations in tables and legends) and listed individually at the end of the manuscript. We recommend preparing the references with a bibliography software package, such as EndNote, ReferenceManager or Zotero to avoid typing mistakes and duplicated references. Include the digital object identifier (DOI) for all references where available.

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2. Author 1, A.; Author 2, B. Title of the chapter. In *Book Title*, 2nd ed.; Editor 1, A., Editor 2, B., Eds.; Publisher: Publisher Location, Country, 2007; Volume 3, pp. 154–196, ISBN.
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