Supplementary Material for:

**Statistically rigorous silver nanowire diameter distribution quantification by automated electron microscopy and image analysis**

Clifford S. Todd 1,4, William A. Heeschen 1,3, Peter Y. Eastman 2,3, Ellen C. Keene 1

1 The Dow Chemical Company, 1897 Building, Midland, MI 48667 USA

2 The Dow Chemical Company, 400 Arcola Road, Collegeville, PA 19426 USA

3 Retired

4 Author to whom any correspondence should be addressed

# Experimental Procedures

## Reactor

Glucose and salts in water were sealed in a reactor and brought to ~150°C. About 20% of the planned AgNO3 and polyvinylpyrrolidone (PVP) were added, and the mixture was held at temperature for a few minutes. This is the silver nucleation phase. The temperature was then lowered to ~130°C and the rest of the AgNO3 and PVP were added [S1, S2]. Some reactor products were cross-flow filtered before wire diameter and yield characterization.

## SEM Sample Preparation

Based on previous experience, clean polished silicon wafer fragments were chosen as the SEM substrate because they are flat and featureless, have a large atomic number contrast with silver for Backscattered Electron imaging, and are conductive enough that no metal sputter-coating is necessary for SEM imaging. Image analysis measurement of features is typically easier if the objects of interest are easily distinguishable from the background (high contrast) and separated from other objects. Therefore, the concentration of wires on the substrate can impact the ease of image analysis. Too high a concentration and wires overlap extensively, making automated wire identification difficult. Too low a concentration and there are few wires in the field of view, perhaps even no wires in a randomly located 6-µm field-of-view image (Figure S1). That would make characterization inefficient. The following procedure produced appropriate silver loading for SEM imaging. A 13 ml aliquot of the reaction output was centrifuged for 15 minutes. After the supernatant was decanted away, the sample was re-diluted with 13 ml de-ionized water, then agitated. The sample was centrifuged for 15 minutes, and decanted again. This time the sample was diluted with 2 ml of DI water, then agitated. One drop (~0.1 ml) was placed on clean silicon wafer fragment and dried under vacuum at room temperature.

|  |  |
| --- | --- |
| 2-3b.tif (a) | 2-4b.tif (b) |

Figure S1: Wire concentration on SEM substrate. (a) The upper end of good concentration. (b) Too low concentration.

As a drop of suspension dries on a silicon wafer some amount of wire and particle movement may occur. This can be manifest as a “coffee ring” of higher silver concentration at the outer edge of the dried droplet, with lower concentrations inside this ring. This brings up the possibility that differential movement of the solids may occur. Sorting by wire diameter seems quite unlikely, but preferential sorting of wires versus particles is conceivable. No extreme sorting was qualitatively observed, but quantitative assessment was not attempted. This remains an unknown source of bias for yield.

## SEM Image Acquisition

Backscattered electron SEM images were acquired using an FEI Nova NanoSEM field emission gun scanning electron microscope using a solid state detector. FEI’s Automated Image Acquisition (AIA) program was used to drive the stage, auto-focus, and capture images. A single setting of brightness and contrast was used for each collection session. Therefore, the brightness of silver and of silicon substrate were consistent for that session. FEI Company developed custom software for Dow to enable automated SEM imaging of samples in an unattended fashion. The software runs on the Nova NanoSEM used in this study. The software interface is flexible allowing users to define imaging parameters, locations, and magnifications. The auto-focus routine is also flexible and tunable so that it can be made to work on diverse samples [S3]. Eighteen images per sample were collected for diameter and yield characterization. The time to acquire one image was about 2 min. Given some overhead time for stage movement and auto-focus, the result was about one hour per sample (18 images per sample). Therefore about 14 samples could be imaged overnight, unattended. With one or two batches per week, imaging could readily keep up with synthesis and filtering experimental output without having a negative impact on the other daytime microscopy work in the lab.

The two parameters that influence SEM imaging the most are accelerating voltage and choice of electron detector. The interplay of these two parameters has an impact on impact the spatial resolution and image quality. In general, a lower accelerating voltage gives better (smaller) interaction volume, but higher voltage generally gives a smaller beam diameter and higher detector signal [S4]. The detector choice falls into two main categories: secondary electrons (SE) and backscattered electrons (BSE). Imaging by SE gives slightly better spatial resolution and is more surface sensitive. The contrast mechanism of BSE imaging is dominated by differences in mean atomic number. At low accelerating voltage the efficiency of BSE detectors decreases. So, there is a practical limit to how low an accelerating voltage can be used for BSE image collection that is dependent on the make and model of electron microscope. Figure S2a shows an example SEM image collected at 5 keV using the vCD (manufacturer’s acronym for a low-voltage high-Contrast Detector), a solid-state BSE detector. Images of the same area were also collected at 3 and 10 keV using the vCD as well as the SE-TLD (Through-Lens Detector optimized for SE detection) and BSE-TLD (through-lens detector optimized for BSE detection). Results of manual diameter measurement of the thick wire indicated by the arrow are summarized in Figure S2. Accelerating voltage did not impact diameter measurement. However, the vCD images measured significantly smaller diameters than images using the two TLDs. Examination of the images at higher magnification (Figure S2d and S2e) shows that the SE-TLD image reveals a coating on the wire making it appear thicker, whereas the BSE image does not. The fact that the coating is not apparent in vCD images indicates that it is of low atomic number, likely polymeric in nature. Although it may be tempting to think that this may be the adsorbed molecular layer of PVP from the synthesis process, underpotential deposition electrochemistry experiments indicated that it likely is residual polymer in the fluid that dried onto exposed surfaces as the suspension droplet dried on the stub. Regardless of the origin of the polymer coating, a measurement of just the metal wire thickness is desired. The BSE-TLD image (not shown here) was similar to the SE-TLD image. Evidently, a large component of secondary electron signal is included in BSE-TLD images of these samples. Therefore, vCD was chosen for AgNW diameter characterization. For the FEI Nova NanoSEM field emission gun SEM used for this work, 5 keV accelerating voltage was chosen because it is low enough to give good spatial resolution, yet large enough to give good signal to noise for the vCD detector.

|  |  |
| --- | --- |
| (a)  1 µm | |
| (b) wire diameter measurements | 162.9  164.2  131.7  (c) wire diameter measurements |
| 1-3b_05kv_TLDSE-crop.jpg  (d) SE image | 1-3b_05kv_vCD-crop.jpg  (e) BSE image |

Figure S2: (a) Example SEM image. (b) and (c) Diameter measurements of the thick wire (indicated by the arrow) from SEM images collected at three accelerating voltages and three detectors. (d) SE image. (e) vCD BSE image.

The choice of a low accelerating voltage was also important so that the brightness of silver wires was not a function of their diameter. At high accelerating voltage and small wire diameter, the electron beam can pass through the wire and into the silicon substrate, resulting in a backscattered electron signal less than that of silver. This would result in thin wires being dimmer than thick wires in BSE images. Beam-sample interaction modeling using Casino software [S5] indicates that at 5 keV, 30 nm thick silver retains more than 80% of the brightness of infinitely thick silver (Figure S3). As mentioned earlier, at low accelerating voltage the efficiency of BSE detectors decreases. So, there is a practical limit to how low an accelerating voltage can be used for BSE image collection that is dependent on the make and model of electron microscope. For the AgNWs in this study, and the make and model of SEM available at the time, 5 keV was determined to be an adequate choice.

Figure S3. BSE coefficient as a function of silver thickness over silicon substrate.

In order to measure wires down to 30 nm diameter the pixel size for the image needs to be some small fraction of that dimension. For practical purposes, pixel size also needs to be balanced against the number of pixels per image, dwell time per pixel, and the number of images per sample needed. We decided on images that were 6 µm and 2048 pixels across, resulting in pixels about 3 nm in size. A dwell time of 30 µsec/pixel was adequate for imaging purposes, leading to ~2 min per image acquisition time. As explained in the “Reproducibility and Repeatability” section of this paper, 18 images per sample were collected for diameter/yield characterization. Given some overhead time for stage movement and focus, the result was about one hour per sample for image collection. The images were collected in two sets of nine images, 20 µm apart (Figure S4).



Figure S4. Diagram illustrating SEM image location determination. Yellow Xs are the stage locations chosen by the SEM operator. Black boxes represent the location of the 18 images collected. Not to scale.

## Image Analysis

Image analysis was performed using in-house written macros for ImageJ software [S6] to identify and report dimensions of silver fibers and non-fibers – referred to as globs. Images were of sufficient contrast, low noise and uniform illumination that the silver objects could be segregated from the smooth silicon background with a simple threshold operation. The general procedure was as follows:

1. Threshold to highlight silver, both wires and equidimensional particles (globs). Choice of threshold is explained in a section below.
2. Automated check of image to confirm silver area fraction was in a useful range (<25 area % silver)
3. Use gray-level watershed segmentation (ImageJ’s Process=>Find Maxima… command) to create binary features in the image that represent separated objects commonly referred to as blobs. This step isolated individual fibers, fiber segments and non-fibers by removing pixels at locations where the objects intersected such as crossing fibers or globs on top of fibers. The number of pixels lost at these intersections was small compared to the overall pixels representing the blobs.
4. Analyze and report blobs as “fibers” and “non-fibers (globs)” in separate populations:
   1. Fibers: aspect ratios > 3, regardless of absolute size. See below for description of the diameter measurement technique.
   2. If aspect ratio < 3, then additional tests are used. If both of the following conditions are true, then the blob is reported as a fiber
      1. Ratio of Fiber Length to Circle-Equivalent-Diameter > 2
      2. Solidity ≤ 0.5
5. Color-code blobs in the image as fibers (black) and globs (gray). See Figure 1(a) and (b).

Definitions:

1. Aspect Ratio is calculated from the Major and Minor axes of a best-fit ellipse for the blob
2. Fiber Length is calculated as Fiber Length = 0.5\*Perimeter - 2\*Area/Perimeter
3. Circle equivalent diameter = sqrt (4\*Area/pi)
4. Solidity is defined as Area/(Convex Area) where the Convex Area is best described as the area that a rubber band would surround when stretched over the blob. For a circle and other solid convex shapes, Solidity is exactly 1. For blobs that have indents, such as the letters C and S, the solidity is less than 1.

The gray-coded blobs are presented to the user as a feature map where “black” objects are fibers and “gray” objects are non-fibers (globs) based on the software’s calculations (Figure 1(b)). If these automatic classifications were not correct, the user could swap the assignment. In practice, the amount of manual reassignment of fiber versus glob for these AgNW images was minimal.

## Data Processing

Data from the Image Analysis step were analyzed and graphed using JMP software [S7] by SAS.

### Wires

As explained in the main article, wire-crossings are removed during image analysis, leaving original wires represented as a sequence of segments. Therefore, some consideration regarding appropriate summary statistics, such as average wire diameter, was necessary. A simple number average based on segments in the image can give an erroneous representation of the image. Figure S5a shows an illustration of one thin wire and ten thick wires, each about 4 µm long. The desired statistic should reflect this ~10:1 ratio of thick to thin wires. In the case illustrated in Figure S5b, image analysis results in 14 segments of thick wires and 5 segments of thin wires. A simple tabulation of the number of segments returns this erroneous 3:1 ratio of wire thickness (Figure S5c). However, if the diameter of each segment is tabulated based on the length of the segment, the correct 10:1 thickness ratio is returned (Figure S5d). This is implemented in JMP software by selecting segment length as the frequency for the diameter measurement (Figure S5e). The frequency scale then becomes the length of wires measured, about 40 µm of thick wires and ~4 µm of thin wires.

|  |  |  |  |
| --- | --- | --- | --- |
| cartoon4.jpg  (a) | | | (b) |
| (c) | (d) | ScreenShot056.jpg  (e) | |

Figure S5: Data processing of image analysis results. (a) Original image; one thin wire and ten thick wires, each ~4 microns long. (b) During image analysis, wire crossings are removed. (c) Number-based tabulation of wire diameters, incorrect. (d) Segment-length-based tabulation of wire diameters, correct. (e) User interface in JMP to accomplish correct wire diameter statistics.

### Particles

Data were also collected for the particles. As described earlier, for each object identified as a particle, its equivalent diameter (diameter of a circle that has the same imaged area as the object) was tabulated. The volume of each particle was estimated by the volume of a prolate ellipsoid (assume axial symmetry about the observed long axis), defined as (EqDiam)3/(6\*sqrt(AspectRatio))

### Wire bundles

In most samples, wires were well dispersed and separate from one another. However, in some cases instances of two or more wires touching along their length was not uncommon. In order to assess how much such an occurrence would bias wire diameter results, a relatively bundle-rich sample (Figure S6a) was measured for wire diameter. The same set of 18 images were analyzed in two ways: (1) Counting any bundled wires as single thick wires (Figure S6c); (2) Excluding any bundled wires from diameter measurement (Figure S6b). Histograms of wire diameter show that a small peak is present at about twice the most common diameter when bundles are included (Figure S6c). However, the overall impact on summary diameter statistics is very small. The average diameter increased only 3 nm when bundles were included. But in order to minimize even this small bias toward erroneously thick wire measurement, each image was visually examined before image analysis; if many wire bundles were present, the image was not measured.

|  |  |
| --- | --- |
| (a) | ScreenShot028.jpg  (c)  (b) |

Figure S6: (a) SEM image from a sample with many wire bundles. Wire diameter statistics (for full set of 18 images) as a function of (b) exclusion or (c) inclusion of wire bundles.

# References

[S1] J. Lunn, A. Malek (2015) Methods of Manufacturing High Aspect Ratio Silver Nanowires. Patent US9034075 B2.

[S2] G.L. Athens, P.T. McGough, J.D. Lunn, W. Wang, T.C. Kuo, C. Todd, J.M. Goss, R.M. Collins, A. Malek, E.M. Calverley (2015) Hydrothermal Synthesis of Silver Nanowires and Application as Transparent Conductive Materials. *Advanced Materials: TechConnect Briefs* **1,** 211-214. <https://briefs.techconnect.org/papers/hydrothermal-synthesis-of-silver-nanowires-and-application-as-transparent-conductive-materials/>

[S3] C.S. Todd, J. Blackson, G. Bar, E. Garcia-Meitin, D. Reuschle, M. Janus, M. Darus, A. Nickles, (2008) Automated image acquisition of polymer blend morphology in an SEM. *Microscopy Today* **16** 24-27.

[S4] J. Goldstein, D. Newbury, D. Joy, C. Lyman, P. Echlin, E. Lifshin, L. Sawyer, J. Michael (2003) Scanning Electron Microscopy and X-Ray Microanalysis, 3rd Ed. Springer, New York.

[S5] D. Drouin, A.R. Couture, D. Joly, X. Tastet, V. Aimez, R. Gauvin (2007) CASINO V2.42—A Fast and Easy-to-use Modeling Tool for Scanning Electron Microscopy and Microanalysis Users. *Scanning* **29**, 92-101.

[S6] W. Rasband, ImageJ Software. National Institutes of Health, USA. http://imagej.nih.gov/ij/, 2018 (accessed 5 March 2018).

[S7] JMP statistical software. SAS. <http://www.jmp.com>, 2018 (accessed 5 March 2018).