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ASPECTS OF THE MORPHOLOGY OF THE SILK OF *BOMBYX MORI*

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ABSTRACT

Atomic force microscopy was used to examine the exterior surface of as-spun cocoon silk, raw silk, and degummed silk as well as the interior surface of peeled degummed silk of *Bombyx mori*. The images yielded a broad variety of features with a wide range of dimensions. They are qualitatively similar to those of the dragline silk of *Nephila clavipes*. Examination of the surface roughness, the surface profiles, and the Fourier transforms of the profiles showed the surface of the degummed silk to be quantitatively similar to the silk of *N. clavipes*. The average roughness is 4 nm. The *average* dimensions of the features are approximately 100 to 150 (>2000 in peeled samples) nm axially and 50 to 300 nm laterally. They appear to emerge from and to enter the surface, to vary in width, and to twist about one another in an irregular manner. In the peeled degummed silk they extend more regularly for long distances in approximately the axial direction. In some cases they appear to be oriented at different small angles with respect to the fiber axis in different layers. Taken together with other data, they suggest the presence of large fibrillar structures. The same suggestion also applies to similar structures in *N. clavipes*.

INTRODUCTION

We are investigating the structural/morphological bases of the properties of the dragline silk of *Nephila clavipes* [1–4]. Such an investigation is facilitated by related examinations of other silks including that of *Bombyx mori* which has been used for more than 5000 years [5]. There have been studies made by optical microscopy of spider silks [6–10] as well as by atomic force microscopy (AFM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) [1, 3, 10–14]. There also have been some microscopic investigations of shadowed or stained samples of the silk of *B. mori* obtained by digestion and/or mechanical fragmentation [15–18] of the fibers. Both types of fibers also have been examined by x-ray diffraction over many years [2, 4, 16, 18–22]. There has been little detailed, higher-resolution examination of the exterior surface of the silk of *B. mori*. In this note we report the initial results of our investigation of the exterior and interior surface morphology of *B. mori* using AFM.

EXPERIMENTAL METHODS AND MATERIALS

Samples of cocoon silk of *B. mori* were pulled from the outer surface of dry cocoons. Others were obtained from "raw" silk which had been reeled from cocoons held in water at about 45°C after first having been placed in boiling water for a few minutes. A third set was taken from degummed silk which had been obtained by "degumming" raw silk to remove the sericin (a term first used in 1865 [23]). This was done by washing the fibers for 30 minutes in a 1 wt% solution of marseille soap in water at about 96°C and then rinsing. The process was repeated several times with fresh solutions at successively lower temperatures. A few samples were prepared from degummed silk by peeling to expose areas below the surface. They were mounted on a glass holder with a 3% solution of collodion in amyl acetate and cut part way (ideally) through. The cut segment was then pulled away from the uncut part. (This was not always successful.) Both "halves" were examined. All materials were stored in vacuum at ambient temperature and away from ultraviolet light.

An optical lever type AFM, TopoMetrix 2000, was used in the repulsive mode at ambient conditions. It was mounted on a heavy stone plate hung from bungee cords in order to reduce unwanted motions of the tip relative to the sample. The samples were mounted on a holder with wax and examined without surface modification. Images were obtained with a constant force of approximately 10^{-10} N which was maintained by the appropriate vertical displacements of the scanner. A 7-micron scanner was used for the 1- and 2-micron images. A 1-micron scanner was used for the 0.5-micron images. Two types of silicon nitride tips were used. One had a pyramidal geometry with an aspect ratio of one-to-one and a tip radius of approximately 50 nm. The radius of the other tip was approximately 10 nm, but it had an extended geometry with an aspect ratio of 10-to-1. A line scanning frequency of 2 to 3 Hz was used. Unless noted otherwise, the images taken to be shown contain 200 by 200 data points and have the right-hand edge approximately parallel with the fiber axis.

RESULTS

Figure 1 is a typical image of the cocoon silk. There is a considerable amount of undulation of the sericin in both the axial and lateral directions. While there is some evidence of "extrusion" lines in the axial direction, they are not very pronounced. The curved surface is typical. An atypical image in Fig. 2 has a less curved and more irregular surface. It exhibits more defined "extrusion" lines. The somewhat periodic and larger axial and lateral surface undulations are also more clearly defined. Portions of other images of the cocoon silk exhibit features that appear to be the result of the sericin-coated fibers having been either pushed or rubbed against other fibers during the making of the cocoon. These also are not so typical.

Surfaces similar to the latter type were found more often in the fibers of raw silk. A typical image is shown in Fig. 3. It is relatively flat but exhibits some irregularity in the lateral direction and lines with less interruption in the axial direction. These surfaces may be the result of the interaction between fibers from the interior of the reeled cocoon. They also might be enhanced by the sericin-coated fibers having been immersed in heated water. Features somewhat similar to others

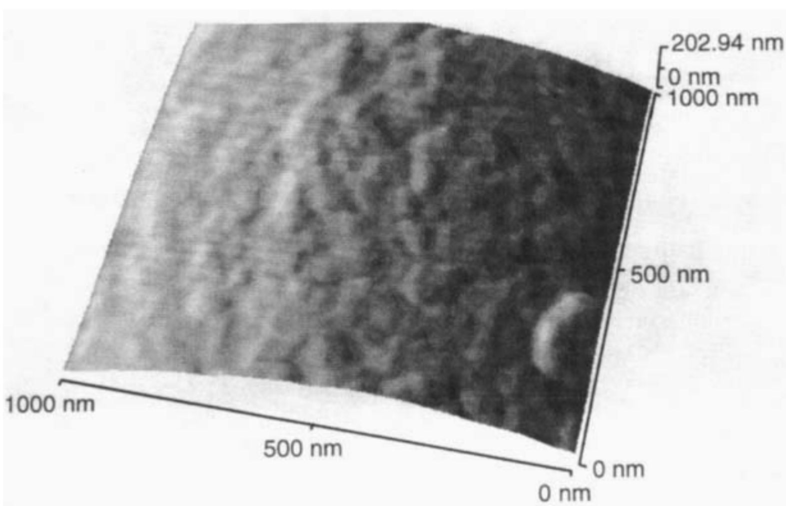

FIG. 1. A typical AFM constant force image of the surface of cocoon silk. Note the undulations in the lateral and axial directions.

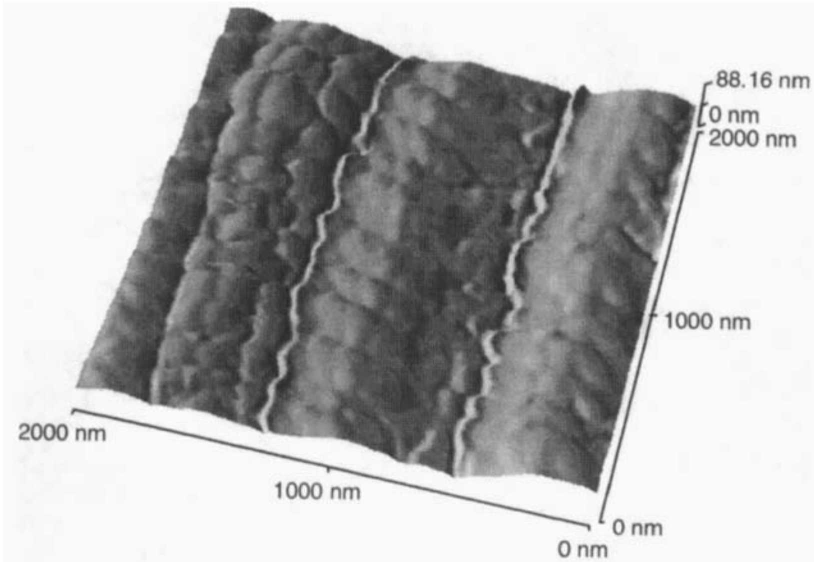

FIG. 2. An atypical AFM constant force image of the surface of cocoon silk. Note the "extrusion" lines and the more prominent, somewhat periodic and larger undulations.

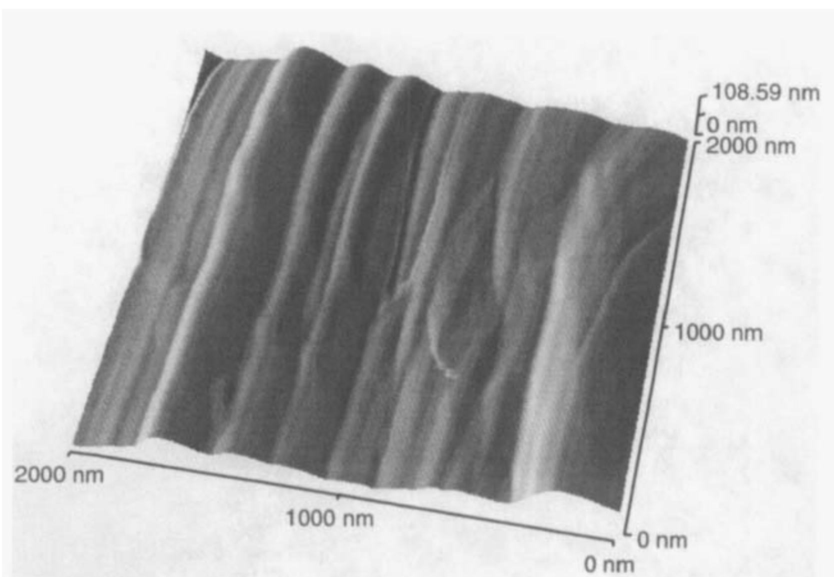

FIG. 3. A typical AFM constant force image (300 by 300 data points) of the surface of raw silk. Note the relatively "smooth" nature in the axial direction.

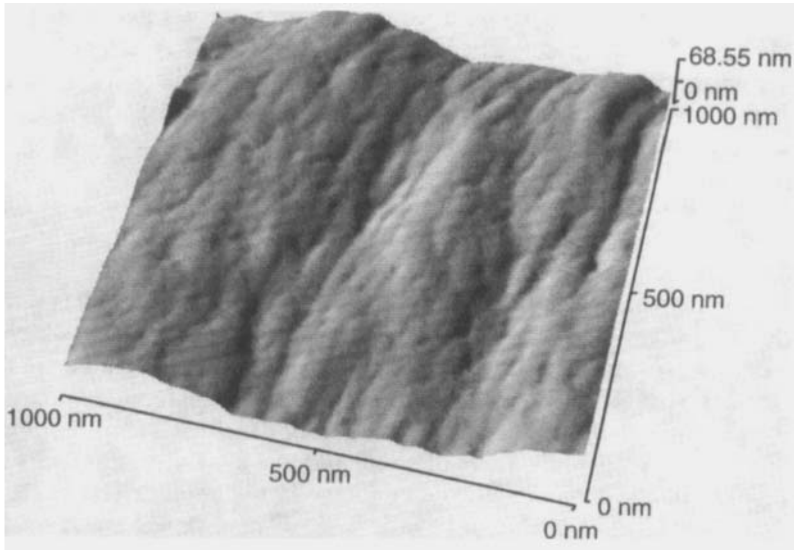

FIG. 4. AFM constant force image of the surface of raw silk. The features are somewhat similar to those found on exterior cocoon silk.

found on the exterior cocoon silk were also observed (Fig. 4). In general, fewer curved surfaces were found.

The surface of the degummed silk is more consistently uniform in texture. It is also qualitatively similar to that of the dragline silk of *N. clavipes* [1]. The curvature in Fig. 5 is typical, but other flatter and more irregular surfaces were observed also.

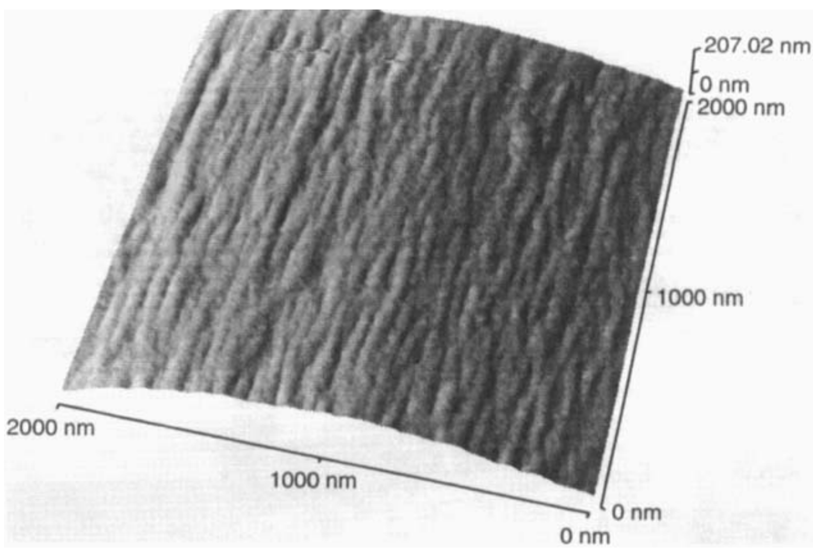

FIG. 5. A typical AFM constant force image (300 by 300 data points) of the surface of degummed silk. The features are qualitatively similar to those found on the dragline silk of *N. clavipes*.

The features running parallel to the fiber axis are also typical. Perspective views of these are shown at higher magnification in Figs. 6 and 7. A top view at higher magnification is shown in Fig. 8. The last three exhibit similar elongated features that appear to emerge from and to enter the surface at somewhat irregularly located different places on the image. In some areas they vary in width and appear to twist irregularly about one another. Figure 7 is rather similar to the image of the surface of dragline silk of *N. clavipes* given in Fig. 7 of Ref. 1. In general, the features in Figs. 5–8 bear some resemblance to those in some of the images of the dragline silk of *N. clavipes* [1, 11].

The surface of the peeled silk was quite variable. Figure 9, which is rather flat, exhibits features with a quite large lateral extent and flattened surfaces which extend the full length of the image in the axial direction. The flatness might be the result of peeling. Images akin to this were not seen on the exterior surface of the degummed silk. Another image of a type not seen on the exterior surface is shown in Fig. 10. It is flat and exhibits fibril-like features of about 100 to 400 nm in lateral extent. These too extend the full 2000 nm length of the image in the axial direction. The small features which cross some of the fibrils in a "helical" manner are about 60 nm wide and 3 nm high. Figure 11 also shows fibril-like features extending the full 2000 nm length of the image in the axial direction. They are about 100 to 200 nm in the lateral direction. It also shows fibrils on a lower level (A) and at about 30° to those on the upper. Some of these can be "traced" from one side to the other of the approximately axial fiber at the left. They appear to have some influence on the surface shape of the upper fiber. Possibly this image represents a layer near the exterior surface with only a few fibrils removed. Many images akin to Figs. 9–11 were seen as well as others that were relatively featureless.

Since the surface contour information is digitized, it is possible to perform various types of image analysis. The mean value of the absolute vertical displace-

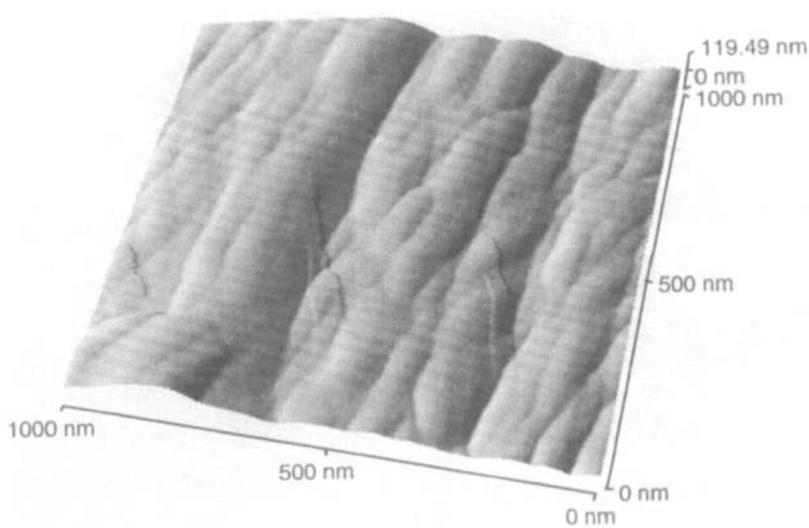

FIG. 6. AFM constant force image (300 by 300 data points) of the surface of degummed silk. Note the apparent twisting of the elongated features.

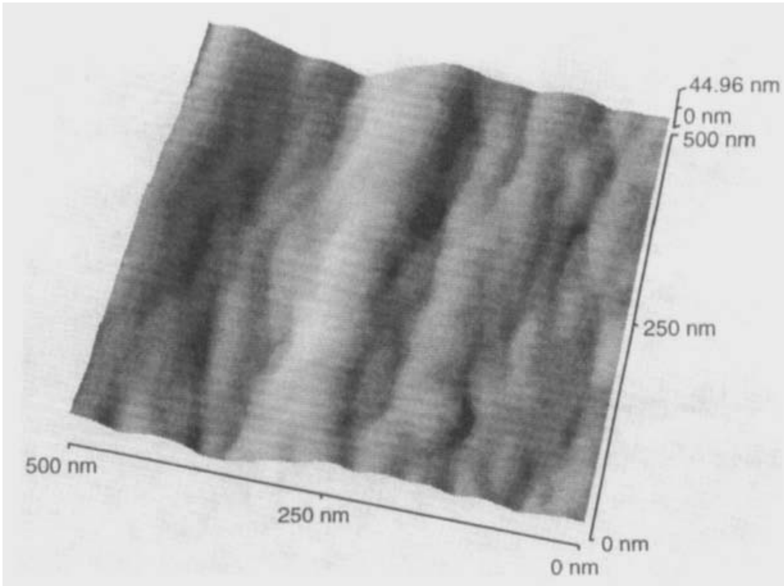

FIG. 7. AFM constant force image (300 by 300 data points) of the surface of degummed silk. Note the irregular nature of the elongated features that are parallel to the fiber axis. This image is similar to the image of *N. clavipes* given in Fig. 7 of Ref. 1.

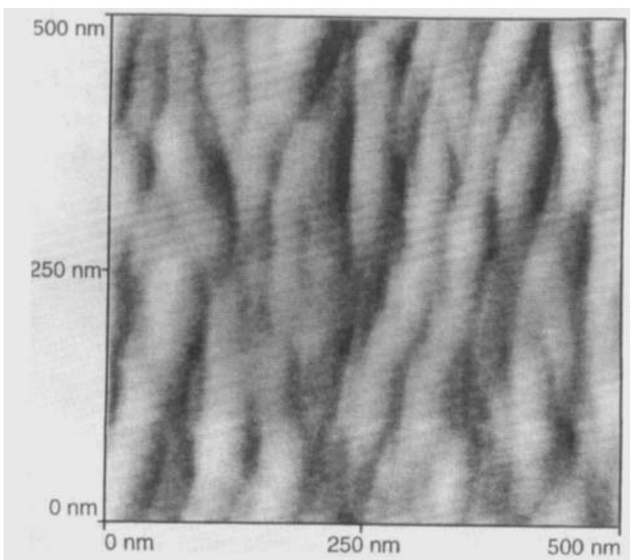

FIG. 8. A top view AFM constant force image of the surface of degummed silk. Note the variations in width of the elongated features and their apparent entering and leaving the surface.

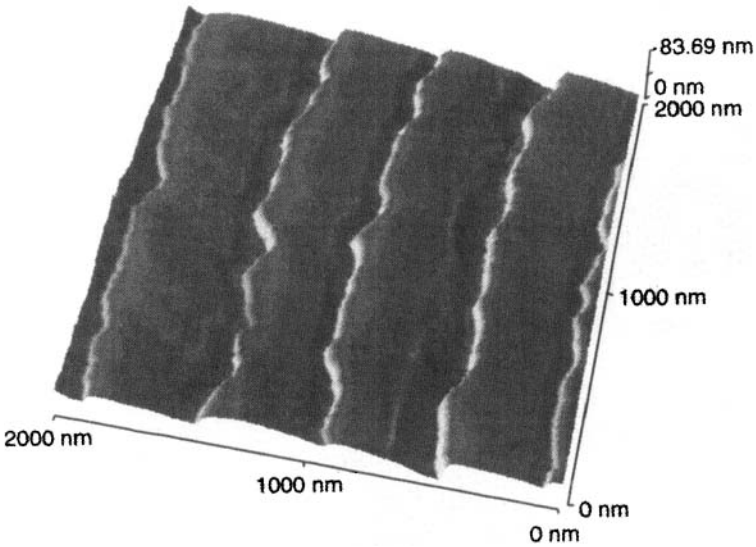

FIG. 9. A typical AFM constant force image of a peeled surface. Note the very wide, flat features extending the full axial length.

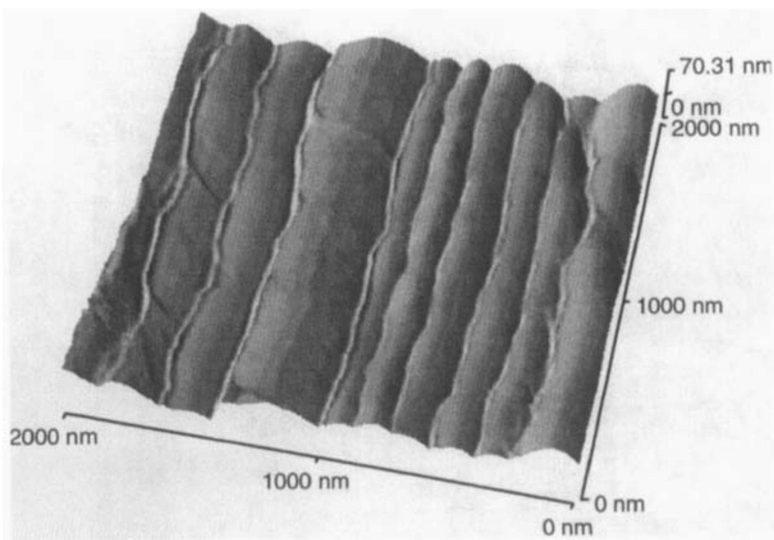

FIG. 10. A typical AFM constant force image of a peeled surface. Note the wide range of lateral widths of the fibril-like features extending the full axial length.

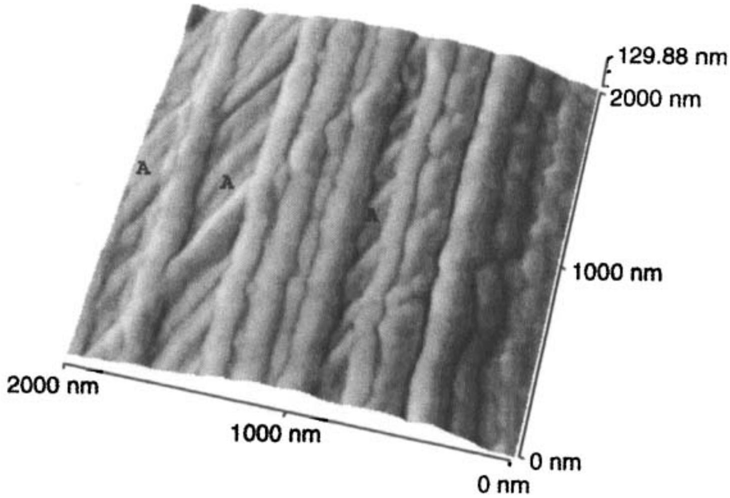

FIG. 11. A typical constant force image of the peeled surface. Note the fibrils (A) which are at about 30° to the upper layer of fibrils and which run under the single axial fiber on the left.

ment of the surface from the mean height of the surface over the area of an image was calculated for a large number of surfaces as a measure of the roughness. (Note that the average difference between measurements made with the two tips described above was less than the standard deviations of the measurements and, therefore, the results were averaged together.) This number is affected by both the curvature and the large-scale irregularities described above as well as the morphological features of interest. Therefore, the surface was "flattened" by fitting a second-order function to the surface contour and subtracting the function from it. The procedure does not perfectly remove the unwanted effects. The apparent roughness was, therefore, extrapolated as a function of scanned area to zero scanned area. This yielded average roughnesses of 4 nm for the cocoon silk, raw silk, and degummed silk. (The peeled silk was not analyzed since the surface reflects the effects of peeling as well as the inherent morphology.) A standard deviation of ± 1 nm for each reflects the considerable variability of the roughness even on the same fiber. Examples can be seen in the images given above. Further, the nature of the surface of the raw silk (discussed above) limited the number of samples which could be measured. Nevertheless, the three values of roughness are effectively the same. It is not clear whether this equivalence results from different morphological entities in the fibroin and sericin coincidentally having the same dimension or from another origin. An equally plausible explanation is that the relatively thin layer of sericin is simply replicating the underlying features of the fibroin. However, the idea should be considered in light of the fact that degumming can cause an increase in the lateral dimensions of the crystals [24]. This could have an effect on the comparison of roughness. Finally, it can be noted that the roughnesses above are of the same order as that for the outer surface of the dragline silk of *N. clavipes*, 9 ± 6 nm [1].

The surfaces also can be characterized with respect to the axial and lateral extent of the features described above. Simple direct measurements of the images

TABLE 1. Average Dimensions of Surface Features[a]

	Simple measurements		Fast Fourier transform	
Silk	Axial, nm	Lateral, nm	Axial, nm	Lateral, nm
B. mori:				
Cocoon	169 ± 17	501 ± 137	240 ± 21	573 ± 97
Raw	–	330 ± 71	–	518 ± 100
Degummed (lower magnification)	119 ± 16	168 ± 21	148 ± 7	284 ± 71
Degummed (higher magnification)	91 ± 14	66 ± 11	–	–
N. clavipes [1]	98 ± 13	113 ± 21	342 ± 40	220 ± 24

[a]There is a broad range of features ranging down to tens of nm in size.

yielded the average results and standard deviations shown in columns two and three of rows one to three in Table 1. Fast Fourier transforms also can be used to characterize the surface dimensions. One-dimensional transforms were made perpendicular and parallel to the axial direction. The power spectra of these exhibited a continuous distribution with a peak at longer wavelengths and a tail extending to quite short ones. The minimum number of the highest components required to reproduce the prominent features of the original image in the inverse transform ranged from four to six. The weighted average and standard deviation of these are given in columns four and five of rows one to three of Table 1. Again, the nature of the raw silk limited the possibility of making both types of meaurements, and the standard deviations of all the data reflect the wide range of dimensions of surface features present. There is an apparent tendency for the cocoon and raw silk to exhibit larger features than the degummed silk. In any event, all are of the same order, and for the degummed fibers are similar to the corresponding ones for the outer surface of the dragline silk of *N. clavipes* [1] which are given in the last row of Table 1. Finally, it should be noted that the overall axial lengths of the features on the degummed silk are greater than the numbers in columns two and four. The emergence, reentry, twisting, and variation in width of the features tend to limit the measured length.

In the lower magnification images analyzed just above, the larger features could be measured readily. However, the finer ones, which are observed especially on the surface of the degummed silk of *B. mori*, could not be measured satisfactorily. In the higher magnification images such as Figs. 7 and 8, the situation was reversed and the simple direct measurements could be made on the finer features. These yielded the numbers in the fourth row of data in Table 1. As expected, the finer features are smaller in lateral extent. Again, the overall axial lengths of the features are greater than the number in column two.

DISCUSSION

In addition to the similarities noted above, dimensions in the same range have been reported by other workers using a variety of techniques. Thus, AFM, small-angle x-ray scattering (SAXS), TEM, and SEM have been used to investigate the degummed silk of *B. mori* [15–18, 25] and the dragline silk of *N. clavipes* [1, 11, 14, 25]. For both fibers the results from all the techniques taken together indicate elongated features of average lateral extent 50 to >250 nm and average axial extent of 100 to >2000 nm (somewhat shorter laterally and longer axially in the case of most of the electron microscopic observations which examined the interior of the fiber). Thus, it seems certain that there are fairly large structures suggestive of fibrils present in these silks. (The features observed are somewhat similar to fibrils observed in both flexible and "rigid rod" synthetic polymers [26, 27].) Further, many details have been observed. However, the finer scale features such as the reported pleating, twisting, variation in width, the apparent emerging from and entering the surface, as well as the orientation at different angles to the fiber axis, are not completely resolved and reconciled. The twisted/helical structures in Fig. 6 as well as in some others of the degummed and peeled samples might be related to the variety of such structures calculated for β-sheets [28–30] and grown from a solution of the c_p fraction of *B. mori* silk [15]. The orientation at different angles to the fiber axis bears a resemblance to that observed for spider silks [10, 31]. The location of the crystals with respect to the structures is not so well established. Nor is the crystal size and shape fully known. Finally, the overall arrangement of the fibrils within the fibers is subject to some uncertainty.

CONCLUSION

The surface morphology of the cocoon, raw, degummed, and peeled degummed silk of *B. mori* exhibit a broad range of features with a wide range of dimensions. They are qualitatively similar to those of the dragline silk of *N. clavipes*. In the case of the degummed silk, the similarity is quantitative. It has an average surface roughness of 4 nm. It exhibits features that are approximately 100 to 150 nm (>2000 nm in peeled samples) in average axial extent and 50 to 300 nm in average lateral extent. They appear to emerge from and enter the surface, to vary in width, to twist about one another in an irregular manner, and to be oriented at different small angles to the fiber axis. Taken together with SAXS, SEM, TEM, and other AFM results, they suggest the presence of large structures akin to fibrils. The same types of data also suggest the presence of such structures in the dragline silk of *N. clavipes*. Questions remain on many aspects of such fibrils and the crystals.

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