ARE CURRENT TEM TECHNIQUES ADEQUATE TO RESOLVE THE ALH84001 LIFE-ON-MARS CONTROVERSY? P. R. Buseck[1], M. Weyland[2], P. A. Midgley[2], R. E. Dunin-Borkowski[2], and R. B. Frankel[3], [1]Departments of Geological Sciences and Chemistry/Biochemistry, Arizona State University, Tempe, Arizona 85287-1404, USA, [2]Department of Materials Science and Metallurgy, University of Cambridge, Pembroke Street, Cambridge CB2 3QZ, UK, [3]Department of Physics, California Polytechnic State University, San Luis Obispo, California 93407, USA.

Introduction: Great significance has been ascribed to the supposed similarity or identity (both terms have been used) between the shapes of some magnetite crystals from the Martian meteorite ALH84001 and terrestrial magnetotactic bacteria, and specifically those from strain MV-1 [1-3]. An implied presupposition is that those shapes are sufficiently well known for measurements of such similarities or identities to be reliable. Bright-field imaging using transmission electron microscopy (TEM) is the major instrumental method that has been used to determine the shapes. We recently introduced the use of high-angle annular dark-field (HAADF) electron tomography to determine the three-dimensional shapes of magnetite nanocrystals with greater accuracy [4], and we showed that there is sufficient uncertainty to be skeptical about morphological conclusions.

Electron tomography: A response was provided by [5] that independent electron tomography validated the prior results of Thomas-Keprta and colleagues [1-3]. There are, however, serious problems in that work, and thus in the supposed validation. The methods used were those of biological electron tomography, and these are not suitable for crystalline materials such as the magnetite crystals in question. In addition, the results were acquired over a limited range of tilt angles. Another component of the controversy appears in the results of [6, 7], who concluded on the basis of bright-field transmission electron microscopy that their synthetic magnetite crystals are identical to those in ALH84001. Thus, the dispute remains unresolved.

It is critical that the morphologies of such magnetite crystals, whether they be meteoritic from ALH84001, biogenic from magnetotactic bacteria, or synthetic from laboratory experiments, are known with adequate specificity and precision to determine differences or similarities free of ambiguities.

Electron tomography is ideal for determining three-dimensional shapes of small objects, but it is only valid for images that have been recorded using a parameter that varies monotonically with thickness in order to provide an accurate representation of morphology. Biological electron tomography has a longer history than that used in materials science. Most biological materials have low contrast, and bright-field images can be used. However, bright-field tilt series of crystalline materials, such as magnetosomes, are dominated by contrast generated from diffraction, and such contrast is not a monotonic projection of any property of the specimen through its thickness. There are also other problems, but the bottom line is that there is insufficient information content in such images to permit reliable conclusions to be drawn about the shapes of nanocrystals that have complex morphologies.

Results from bacterial strain MV-1: We have been using bacteria strain MV-1 to test and to develop electron tomography as a method that can be used to determine the morphologies of nanocrystals reliably and with high precision. Figures 1 and 2 were obtained using a Philips CM300 transmission electron microscope equipped with a field-emission gun and a HAADF detector. Thirty-nine 1024×1024 pixel images of a chain of magnetite crystals from bacterial strain MV-1 were acquired over a sample tilt range of ±76° using a dwell time of 60 micro seconds, an extraction voltage of 4.34 keV, a gun lens setting of 5 and spot size 5 (probe size ~0.6 nm). The results show several features that lead to shape uncertainties. These include shape variations within a chain and rounding of facets, so that faces are not nearly as well developed or flat as some published interpretations show. These effects are best displayed in animations as the crystals are tilted.

Discussion: The presumed biogenic origin of some of the magnetite crystals in ALH84001 is based on a set of properties, the most distinctive of which is morphology, and that is the one we are addressing. Our conclusion is not that the meteorite crystals are necessarily non-biogenic, but rather that further work is required to make the case for a conclusion as significant as their indicating extraterrestrial life.

Electron tomography and electron holography hold the potential to provide the necessary information, but it will be necessary to refine these methods to increase their resolution and to improve the algorithms required for reconstruction of the many images required, even for a single data-set. Automation of the reconstructions will be essential to allow the study of enough crystals from each source to permit statistically reliable conclusions to be reached.

References: [1] Thomas-Keprta K. L. et al. (2000) *GCA* 64, 4049–4081. [2] Thomas-Keprta K. L. et al. (2001) *PNAS* 98, 2164–2169. [3] Thomas-Keprta K. L. et al. (2002) *Appl. Environ. Microbio.* 68, 3663-3672. [4] Buseck P. R. et al. (2001) *PNAS 98*, 13490-13495. [5] Clemett S. J. et al. (2002) *Am. Min.* 87, 1727–1730. [6] Golden D. C. et al. (2001) *Am. Min.* 86, 370-375. [7] Golden D. C. et al. (2002) *MAPS* 37, Suppl., A53.

TEM TECHNIQUES AND THE ALH84001 LIFE-ON-MARS CONTROVERSY: P. R. Buseck, M. Weyland, P. A. Midgley, R. E. Dunin-Borkowski, and R. B. Frankel

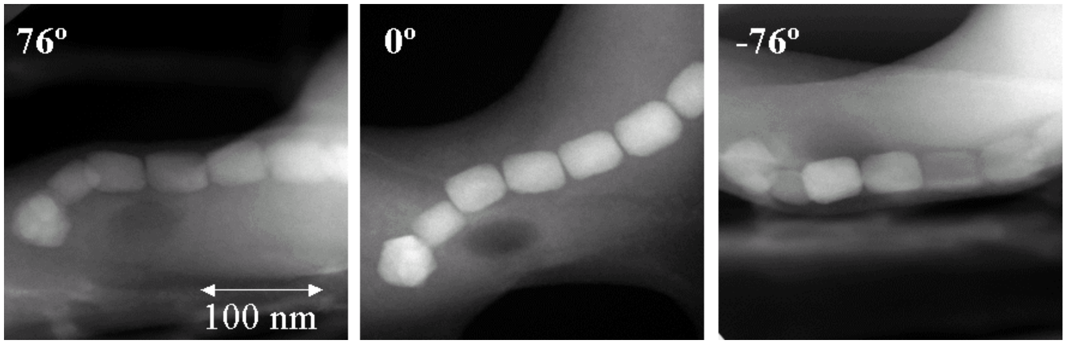

Figure 1. Three images taken from a tilt series of 39 high-angle annular dark field images of a chain of magnetite crystals from bacterial strain MV-1.

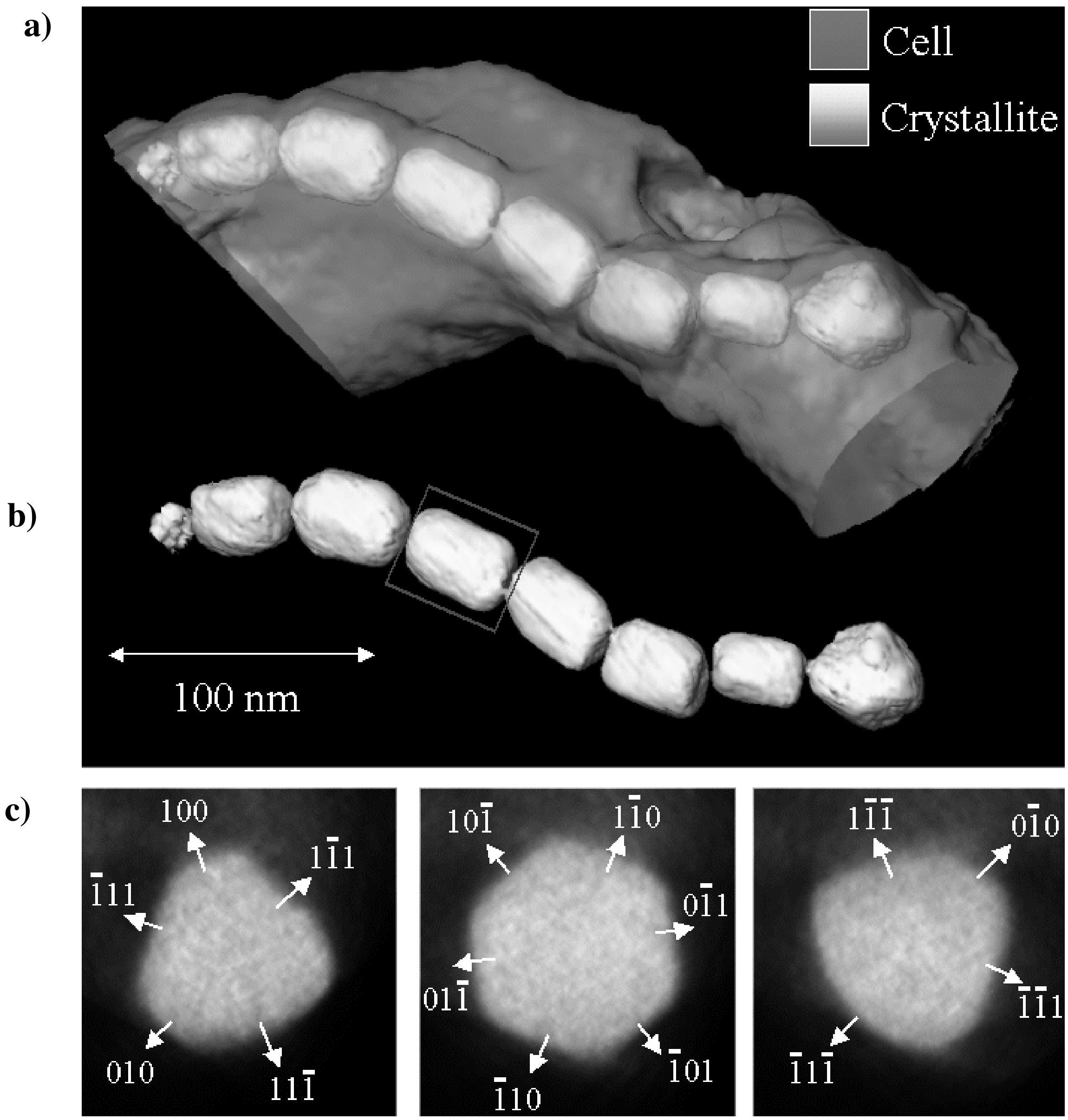

Figure 2. a) Three-dimensional reconstruction of both the chain of magnetite crystals and the bacterial cell wall. b) The chain of magnetite crystals alone. c) Sections through the reconstruction of the crystal marked in b).