An Improved Experimental Deconvolution Technique for 3-Dimensional Laser Confocal Microscopy of Particles in Aerogel

#1630
LPSC 2013

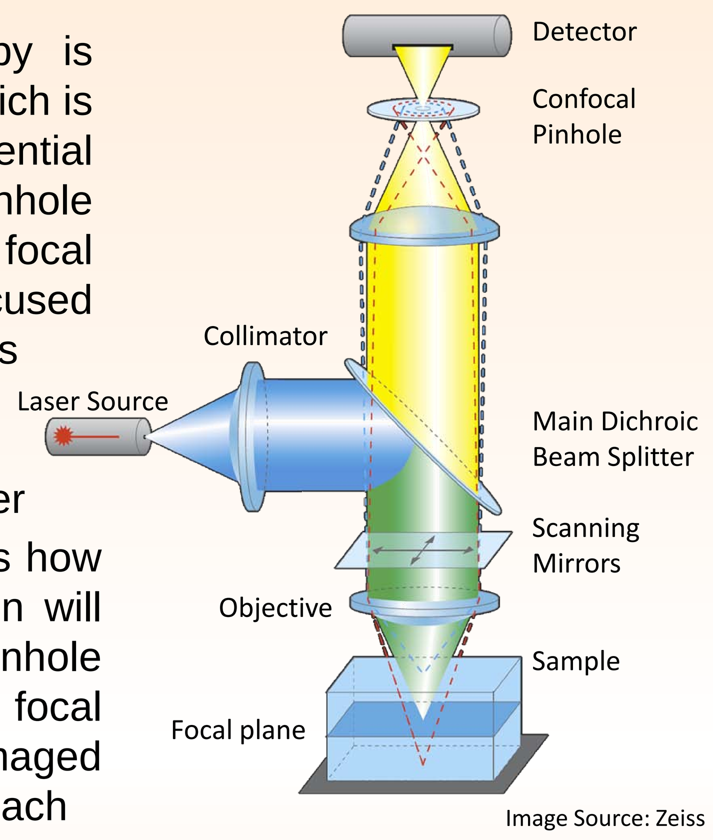
Amanda J. White[1], Denton S. Ebel[1], Michael Greenberg[2,1]
[1]Dept. of Earth and Planetary Sciences, American Museum of Natural History, New York, NY 10024. (awhite@amnh.org) [2]Northwestern University, Evanston, IL 60208.

Aerogel

Aerogel is an effective capture medium for hypervelocity particles. An ultra-low-density solid, the molecular structure and transparency of aerogel allow it to capture particles impacting at hypervelocity intact and for researchers to locate the captured particles [1, 2]. Aerogel has been used successfully in past particle capture missions, including the NASA Stardust mission which returned material from comet Wild2 in 2006 [3]. It will potentially be used again in future sample return missions. Particles captured in aerogel through hypervelocity impacts create three-dimensional (3D) tracks representing unique impact events. The nature of each track-forming event, including the original state of the impactor, is recorded in 3D track morphology and material distribution. Locating single grain particle fragments and identifying true track and particle size and shape are necessary to understanding original impactor properties.

Confocal Microscopy and 3D Images

Confocal microscopy is an imaging technique which is capable of imaging sequential optical sections. A pinhole placed in a conjugate focal plane allows only focused light to hit the instrument's detector and blocks stray light from all other focal planes. The diameter of the pinhole determines how thick each optical section will be. By using a small pinhole diameter and varying the focal plane, samples can be imaged layer by layer. Because each optical section is imaged separately, the three-dimensional distribution of particles is preserved in the resulting image stack without disrupting or destroying the sample.



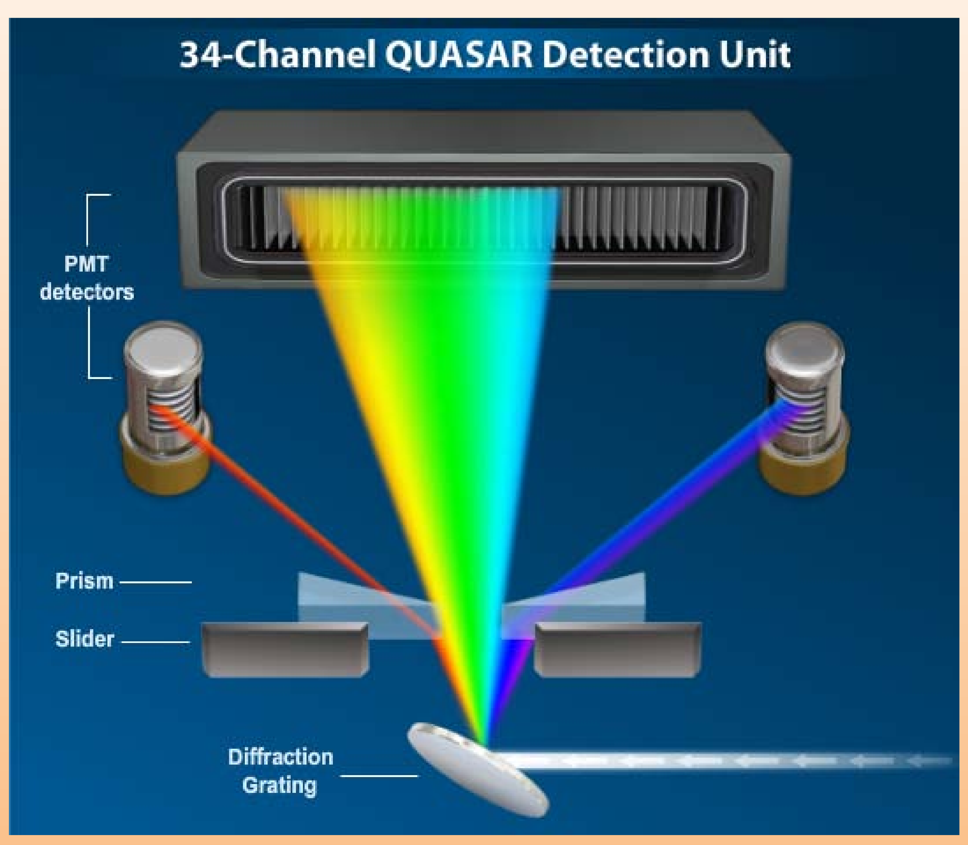


Figure 1: The spectral detector of a Zeiss LSM 710 is capable of imaging in 34 channels simultaneously to obtain fluorescent and reflection spectra.

Imaging Parameters Quick Look:

Scaling in X and Y:	74 nm/pixel
Scaling in Z:	360 nm/pixel
Laser wavelengths:	488 nm and 561nm
Pixel dwell:	0.79 μs
Pinhole diameter:	28 μm

Imaging Keystones at AMNH

All confocal images were taken at the Microscopy and Imaging Facility located at the American Museum of Natural History using a Zeiss LSM 710 laser scanning confocal microscope (LSCM). The 710 is equipped with lasers at five wavelengths and a 34 channel detector which is capable of recording spectral information (Figure 1). Images of keystones were obtained at a resolution of 74 nm/pixel in the x-y plane and at 360 nm/pixel along the z-axis.

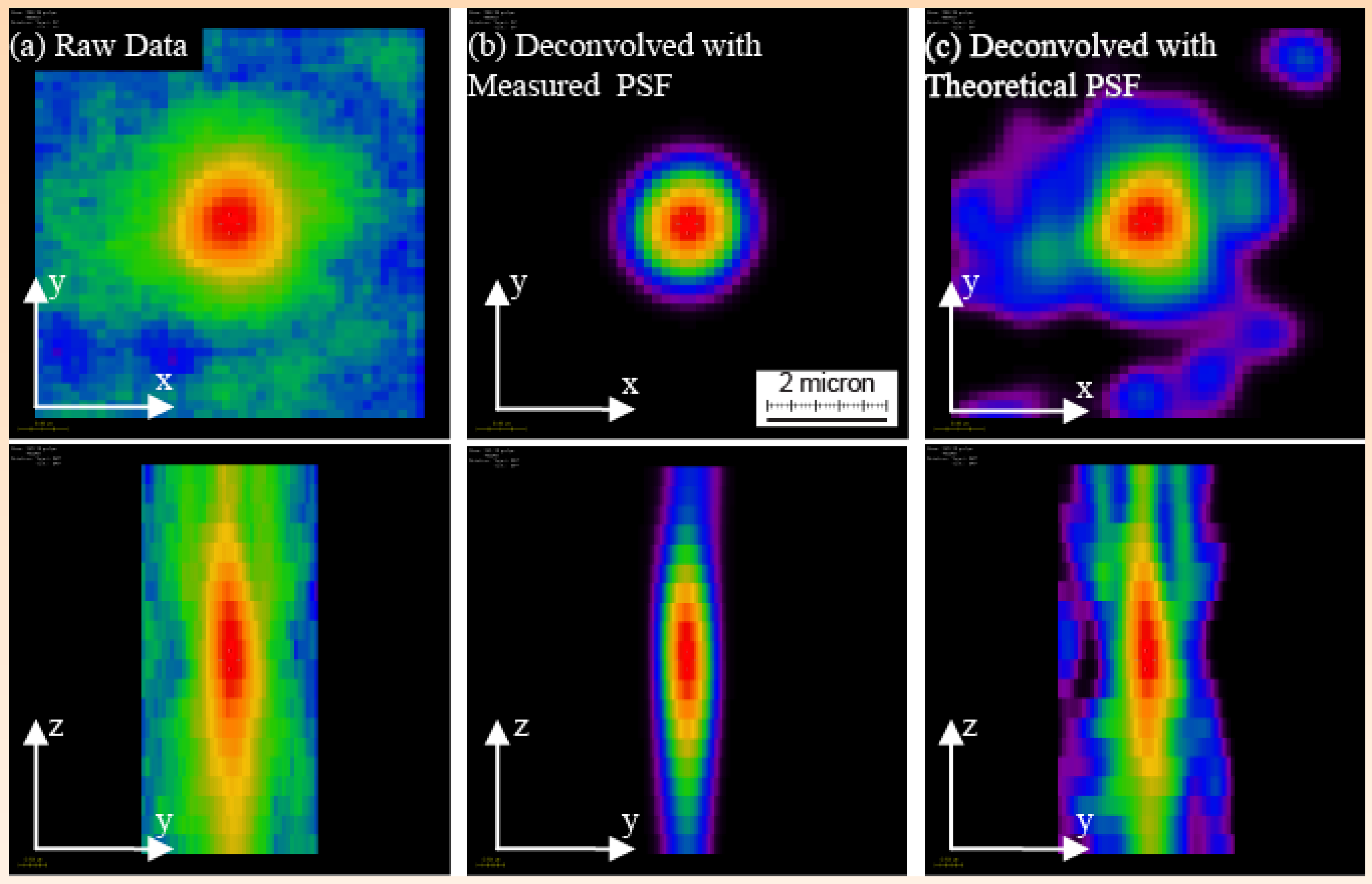


Figure 2: A side by side, same scale comparison of a 500nm fluorescent bead image before it has been processed (a), and after being deconvolved using a measured PSF (b) and a theoretical PSF (c). Both deconvolutions improve image contrast. The measured PSF (b) removes more structure from the image which is believed to be unreal and caused by reflections off background aerogel.

Why Deconvolve?

Due to the configuration of the optical path in any LSCM, axial distortion occurs along the z-axis of three-dimensional images (Figure 3, b and c)[11]. Removing this aberration is a necessary step in order to accurately quantify track dimensions and particle locations [7]. For 3D deconvolution of raw image data, we use SVI's Huygens Professional v4.1 software. The Huygens's Software [6] is designed to restore convoluted images using an instrumental point spread function (PSF). The PSF can be obtained from either a theoretical calculation, or by experimental means through a series of measurements. A theoretical PSF provides a good approximation, however only takes into account the best alignment and imaging conditions on a given microscope and does not account for any deviations from an ideal setup. An experimental PSF uses images obtained under the same conditions as the sample in question accurately accounts for more variables of the experimental setup than the theoretical PSF. Using aerogel keystones with 100nm fluorescent latex beads embedded in the aerogel during its manufacture, we have created an experimental PSF from images obtained under the same conditions as images of Stardust cometary tracks.

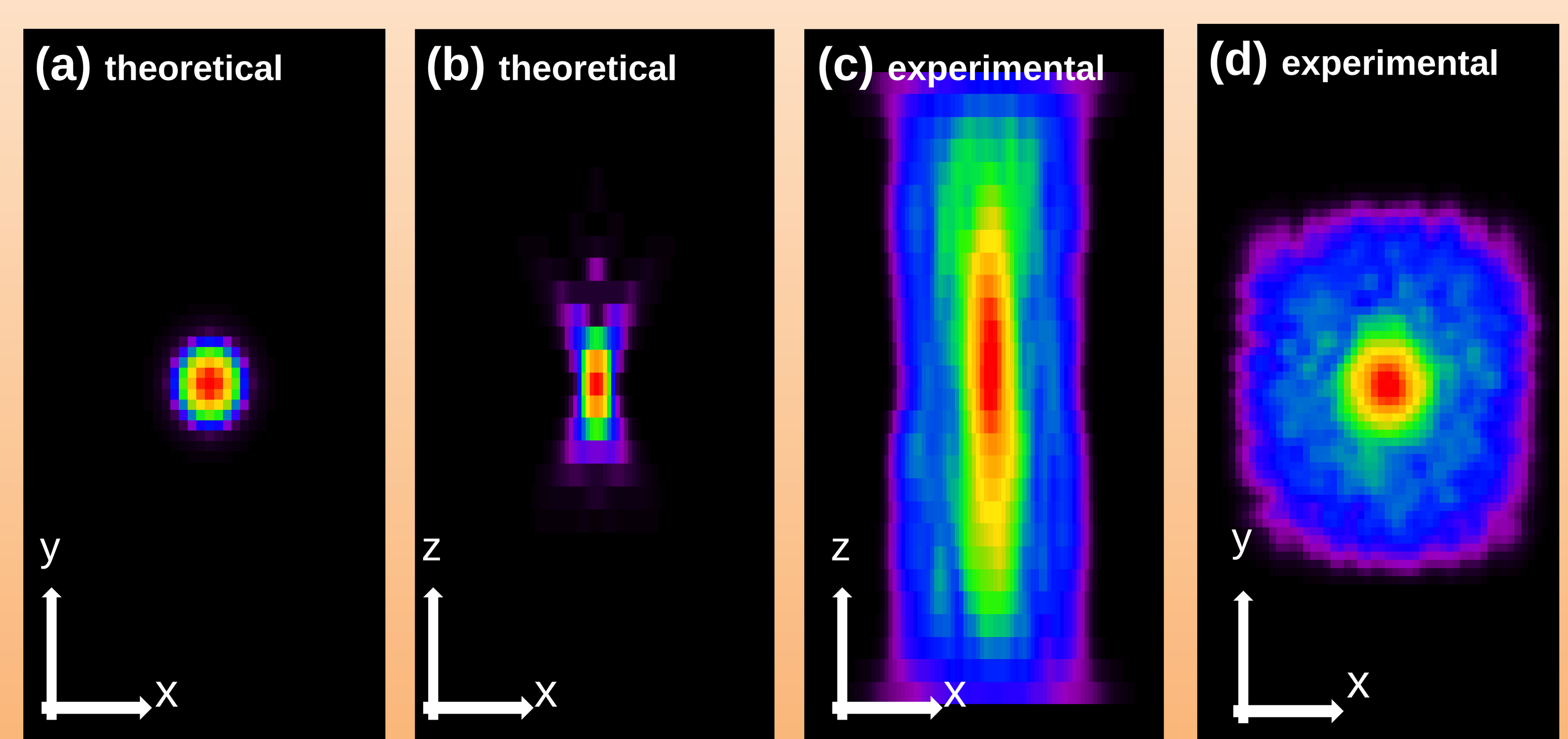


Figure 3: A side-by-side, same scale view of the theoretical PSF in the x-y plane(a), the experimental PSF in the x-y plane (d), and the theoretical PSF (b) and experimental PSF (c) in the x-z plane. The experimental PSF is noticeably larger and more elongated along the optical axis (z-direction) of the instrument. The measured PSF is also less symmetrical than its theoretical counterpart. Pixels measure 74 nm x 74nm x 360 nm (xyz).

Comparing PSFs

To quantitatively compare the functionality of both the measured and theoretical PSFs, we imaged an aerogel keystone containing 500nm beads. Deconvolutions using both measured and theoretical PSFs were then performed on this image set. Deconvolution is expected to increase an image's contrast and to bring out small spatial features by removing larger, out of focus features. This can be seen in Figure 2, which shows the 3D center of the initial raw data (a) alongside the results of both deconvolution tests (b and c). Comparing the raw data (a) with the results of a deconvolution using a theoretically determined PSF (c), one can observe how the deconvolution enhances some features from the original image; however, these features are not necessarily real. The extra structure could be due to a deviation from ideality in our optical setup, the aerogel that the beads are embedded in causing a mismatch of refractive indices, or a combination of the two. A reconstructed 500nm bead is represented best by the results of the deconvolution performed using a measured PSF (b). *Both the optical setup of our LSCM and the effects of the aerogel are better accounted for by the experimental PSF than by the theoretical PSF.* This is further shown in Figure 4 which displays the intensity profile along the Z and X planes for the raw data and the results of both deconvolution tests. In both tests, the intensity peaks were sharper than in the raw data and a decrease in the FWHM is observed. The measured PSF restored much more of the image's intensity than the theoretical PSF did. As FWHM is a good estimation of spatial resolution [6], the sharper peak also indicates a greater positional accuracy for resolved grains.

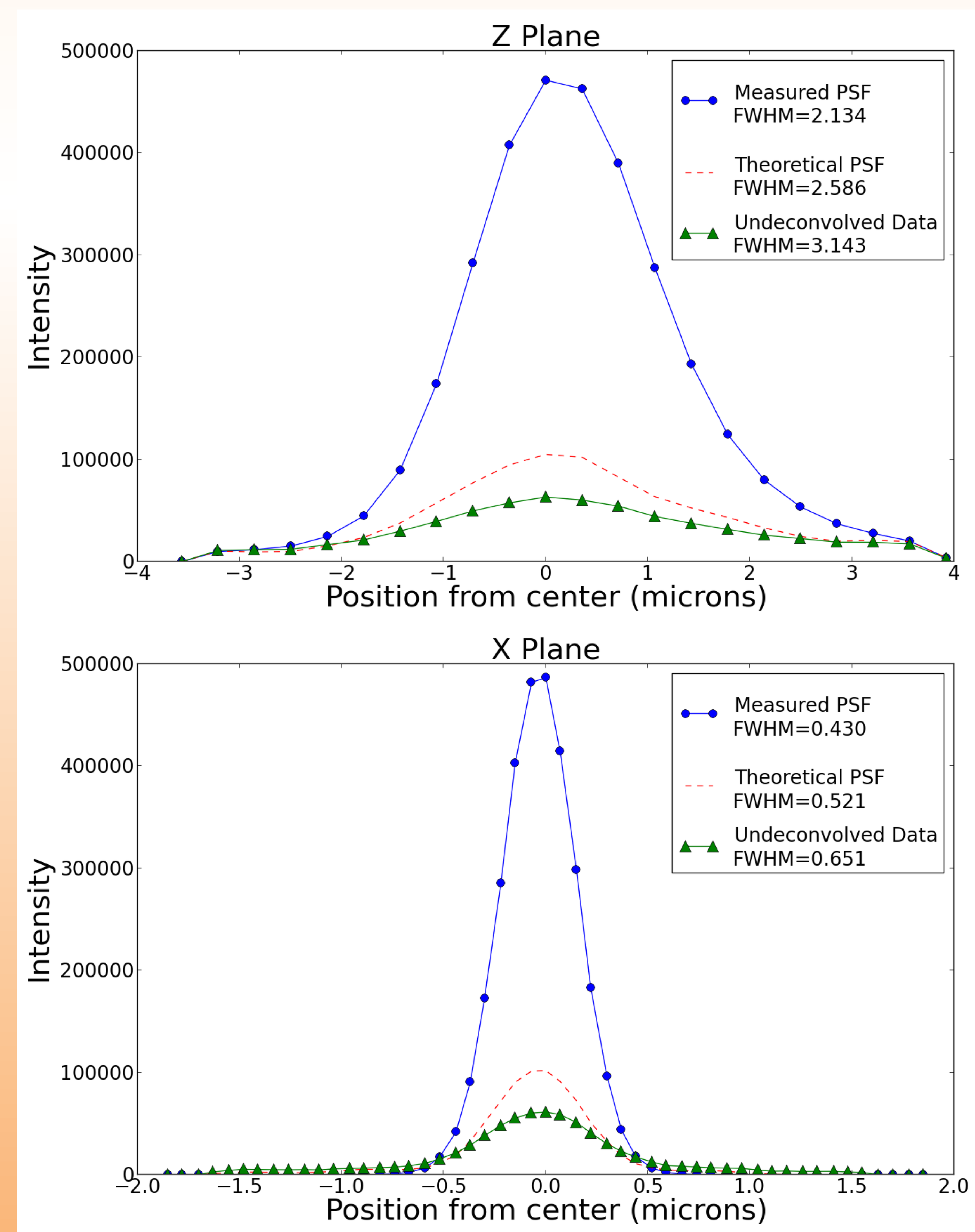


Figure 4: Intensity profiles of the deconvolved and raw data in the Z plane (top) and X Plane (bottom). Y plane data is nearly identical to the X plane. Both deconvolution techniques resulted in a sharper peak and smaller FWHM over the raw data. The measured PSF restored much more intensity and positional accuracy to the image than the theoretical PSF did.

References

[1] Tsou P. (1995) *J. Non-Crystal. Solids,* 186:415–27. [2] Westphal et al. (2002) *Meteoritics & Planet. Sci., 37*, 855-865. [3] Brownlee D. et al. (2006) *Science, 314*, 1711-1716. [4] Greenberg M. et al. (2011) *LPSC XLII* #2640. [5] Greenberg M. et al. (2010) *LPSC XLI* #2346. [6] http://www.svi.nl/HuygensProfessional [7] Greenberg M. et al. (2008) *LPSC XXXIX* #1800. [8] Greenberg M. et al. (2010) *Geosphere, 6*, 515-523 [9] Ebel D. S. et al. (2009) *Meteoritics & Planet. Sci., 44*, 1445-1463. [10] Greenberg M, and Ebel D. S. (2012) *Meteoritics & Planet Sci., 47*, 634-648. [11] White A.J. et al. (2012) *LPSC XLIII* #1542.

Acknowledgements

We thank S. Jones (JPL) for making Stardust grade aerogels with embedded beads; D. Frank (JSC) for cutting the aerogel with beads into keystones; and J. Thostenson (AMNH) for LSCM assistance. This research was funded by NASA SRLDAP (NNX09AC31G, DSE) and NASA LARS (NNX12AF28G, DSE). The LSM 710 was acquired by AMNH with partial support from NASA LARS (NNXIOAH06G, DSE).

American Museum of Natural History

NASA