NONDESTRUCTIVE 3D CONFOCAL LASER IMAGING OF STARDUST TRACKS IN AEROGEL AND DECONVOLUTION TECHNIQUES.

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Introduction: The Stardust mission to comet Wild 2 returned many cometary particles trapped at a relative velocity of 6.1km/sec in aerogel, leaving 'tracks' of melted silica aerogel. Particles of size 40-300μm reached track terminal regions, leaving myriad smaller particulate fragments behind along tracks [1,2]. A small number of < 1 μm interstellar dust particles was collected on separate aerogel collectors [3]. It has been our goal to perform non-destructive 3D textural analysis on *both* types of tracks. We see this as a necessary, high-value initial step in track analysis, before flattening, cutting, and other destructive methods. We have turned to Laser Scanning Confocal Microscopy (LCSM) as an accessible alternative to synchrotron-based x-ray tomography [4].

Cometary tracks have been isolated, extracted, and distributed in triangular 'keystones' [5]. We have also worked on many analog keystones, from aerogel shot with various particles in labs [4], and a single Wild 2 track (#82). Here, we demonstrate greatly improved LCSM images of track #82 at 0.07μm/voxel edge and analogous images of aerogel shot with basaltic glass, imaged at 0.023μm/voxel edge. Axial distortion of 3D images is a serious issue in LCSM, resulting from optical effects along the optic axis of the instrument [4]. Here we describe the correction of axial distortion using a 3-dimensional deconvolution method, based on knowledge of the point-spread function (PSF) for aerogel.

Samples: Stardust track #82 (C2092,1,82,00) is a single 898μm long track in a keystone mounted on a standard 'forklift' and 25mm rod. An analog aerogel track shot with crushed basalt glass particles (1-100 μm) at 5.88 km/sec by M. Burchell (05-Dec-06 at University of Kent) was prepared by C. Snead at U.C. Berkeley. This keystone was analyzed on its extraction forklift [4], fell off, and was housed within a closed slide and coverslip box. This housing enabled the testing of high magnification oil immersion lenses. The LCSM instrument is optimized for use of particular coverslips (#1.5 type; ~150μm thick) to minimize refraction effects at interfaces along the light path.

Technique: LCSM Images were taken using a Zeiss Axiovert 100 at the Microscopy and Imaging Facility in the American Museum of Natural History. The Axiovert 100 is equipped with 4 separate laser wavelengths for analysis: 458nm Ar, 488nm Ar, 543nm HeNe, and 633nm HeNe. All data was acquired using the 488nm Ar laser to achieve optimal resolution [4]. Laser intensity was varied for each lens to preserve a strong reflection signal. For 10x and 20x images, the laser was kept at 20.9% and 18.9% power, respectively. Data was acquired using the Zeiss LSM 510 v.3.2 software package on a 64bit XP Intel machine. Data is collected in a 3-dimensional array format X by Y by *n*, where X and Y are horizontal coordinates, and *n* is the number of vertical optical slices (normal to optical axis). All scans are at 2048x2048 resolution, and *n* was varied according to the desired thickness of analyzed region in the sample. An 8bit grayscale depth was chosen because 12bit depth yielded little difference in results, and to minimize data size.

Since the point of best focus in a LCSM scan is in the midplane of each optical slice, all stacks of slices were overlapped 1/2 or greater thickness, in order to obtain optimal clarity. Higher resolution images were enabled with an additional digital zoom. Unlike certain types of digital zoom, the Axiovert 100 changes its laser scanning area, yet keeps the same scan resolution. This allows for scans of greater magnification without any loss of image quality. In LCSM, a pinhole is used to limit the slice thickness and limit the photons entering the detector. Our pinhole size was optimized for each magnification, but it is important to keep the pinhole size to 1.0 Airy units or below. Furthermore, a Detector Gain value of ≤ 500 allows for sufficiently clean images that can be easily enhanced using other software packages.

The LSM 510 software allows for scans of variable speeds. A scan speed of 3 or 4 was used, giving each pixel a scan time of 12.80μs or 6.40μs per pixel respectively. Lower scan speeds create higher quality images, yet below speed 4 little difference is seen. Furthermore, each line of pixels was scanned twice and the values for each pixel averaged. This "line-mean-2" scan method greatly improved the clarity of our images. Datasets were saved in Zeiss' proprietary .lsm file format (a variation on regular .tiff stacks), and were subsequently processed using Huygens Professional 3.0 (SVI) for 3D deconvolution, and Imaris 4.5.2 64bit for display and animation.

Results: We performed several LCSM scans of varying magnification on regions of track #82 in its keystone. Two field scans of the overall region (A and B), a slightly more magnified scan of the terminal region (C), followed by an extremely detailed scan of the aerogel entry point (D). Finally, scan E samples a particularly interesting region in the middle of the particle track. The table below reports effective magnification (mag), scan speed/pixel dwell time (*t*, µs), pixel size resolution (*r*, µm/pixel edge), and number of slices (*n*):

Scan Label	mag	*t* (µs)	*r*	*n*
A - Field	10x	4 / 6.4	0.45	113
B - Field	20x	3 / 12.80	0.22	158
C - Terminal	40x	4 / 6.4	0.11	116
D - Entry	60x	3 / 12.80	0.07	199
E - Middle	40x	4 / 6.4	0.11	96

The structure of the tracks is best observed via 3D projections, and in movies of these 3D projections. The pixel resolution obtained here is significantly better than reported previously [4].

Scans were also taken on the analog keystone with the 40x lens to test the feasibility of the coverslip box to protect keystones from the immersion oil. The coverslip box apparatus worked flawlessly, producing no more axial distortion than the open air scanning with lower power zoom lenses. Since this method proved to be effective, we will further explore oil immersion lenses for analysis in the near future. Resolution of at or better than 0.04 µm/pixel edge should be possible.

We made extensive use of a deconvolution method involving calculation of a theoretical PSF, followed by iterative deconvolution. The Huygens software uses a classic maximum likelihood estimation (CMLE) method to deconvolve blocks of the image stack, one at a time. This method, while efficient, is still computationally heavy, and memory intensive, and thus all deconvolution was done on an XP 64bit machine with 8GB of RAM.

Even with our theoretical (estimated) PSF for aerogel, the results of deconvolution are a marked improvement in the data quality. We are currently working to further improve these results by experimentally determining a PSF for aerogel. It is important that this correction, and, indeed, any further improvements in deconvolution, can be applied at any time after data collection, since we have already returned track #82 to the sample curation facility.

Complementary Work: We have also performed synchrotron x-ray fluorescence (XRF) 2D mapping the entire track #82 at 2 µm/pixel resolution. Particles observed in LCSM and tomography also appear in XRF. A complete spectral analysis of this data has not been completed yet; however it appears that a large particle (5 µm) rich in Ca, Al, and Ti is present in the upper regions, and that the terminal particle(s) is a metal alloy. Attempts to obtain diffraction patterns on these grains at the Brookhaven synchrotron were not successful.

Discussion: There are many interesting aspects of our images to be investigated further. These include, but are not limited to, the apparent rifling effects on the entry scan [5], and the abrupt kink in the middle of the track. We have also observed 'rifling' in tomographic images of analog tracks, in successive z-axis slices [6]. After we have determined the PSF experimentally, we expect to be able to perform quantitative analysis of the particles in track #82, and report particle size distributions, size concentrations, and total amounts of material. In theory, it should be possible to constrain the nature of particles (e.g., opaque sulfide versus translucent silicate) by careful analysis of their reflectance properties in LCSM. Other groups are also working on this problem using related confocal Raman spectroscopic technique [7].

Conclusions: We have demonstrated technical improvements in using LCSM for non-destructive sub-micron 3D analysis of grains and tracks in aerogel returned by the Stardust mission. Most importantly, we have now developed the ability to reduce distortion inherent in the raw LCSM results through 3D deconvolution methods. We are firmly confident that we can do a total nondestructive analysis on any stardust track, given our suite of tools. We have also shown the possibility of using oil immersion lenses as a means of getting higher magnification images of tracks or portions of tracks, if they are enclosed in the proper container.

The pico-keystones containing interstellar dust are excellent candidates for LCSM analysis [3]. Nondestructive 3D analysis is a natural preliminary technique before invasive work, to recover the maximum possible textural information about each track and particle [7].

References: [1] Burchell M.J. *et al.* (2006) *Ann. Rev. Earth Planet. Sci*. 34: 385-418. [2] Westphal A.J. et al. (2004) *Meteor. Planet Sci., 39*, 1375-1386. [3] Westphal et al. (2006) LPSC XXXVII, #2225. [4] Ebel D.S., Mey J.L., Rivers M.L. (2007) LPSC *XXXVIII*, #1977. See f. http://research.amnh.org/~debel/tomo-aps/aerogel1.html [5] Nakamura-Messenger K. et al. (2007) *LPSC XXXVIII*, Abs. #2191. [6] Ebel D.S., Rivers M.L. (2007) *MaPS* 42: 1627-1646. [7] Kearsley A. T. et al. (2007) *LPSC XXXVIII* #1690.

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