Computer-Integrated Systems for Microscopy and Manipulation

R. M. Taylor II*, W. Robinett, V. L. Chi, F. P. Brooks Jr., W. V. Wright, R. S. Williams, E. J. Snyder, J. Chen, S. Okimoto, N. Llopis-Artime, M. Falvo, S. Paulson, P. Thiansathaporn, D. Glick, S. Washburn, R. Superfine, D. Jen, P. Parente, J. Robbins, C. Weigle, A. Burette, R. Weinberg, G. Varadhan, D. Erie

UNC Chapel Hill: Computer Science, Physics & Astronomy, Applied Science, Cell Biology, Chemistry; UCLA: Chemistry

ABSTRACT

This article presents scientific results obtained through the use of custom visualization and control systems designed to support materials and biomedical nanoscale science. We have selected the two highest-impact tools from the dozen released by CISMM.

KEYWORDS: Scientific Visualization, Haptic display, microscopy.

1 INTRODUCTION

Computer-Integrated Systems for Microscopy and Manipulation is an NIH/NIBIB National Research Resource at UNC Chapel Hill that uses interactive 3D computer graphics, haptics, and image analysis techniques to map the data and control from scientific instruments' native coordinate systems into the 3D space of the specimen, where the scientist most directly perceives and acts.

We here present two of our tools, along with the scientific insights gained using each.

2 NANOMANIPULATOR

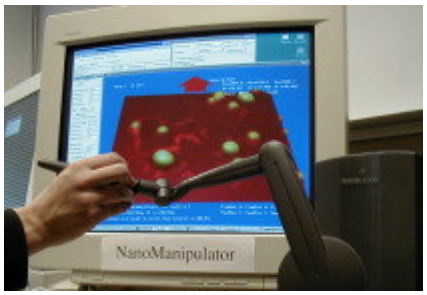

Figure 1. Commercialized version of the nanoManipulator interactive graphics + haptic interface to AFM.

The nanoManipulator (nM) system shown in figure 1 provides a scientist with the ability to interact with objects as small as single molecules while quantitatively measuring both the surface shape and forces applied. The nM uses the ultra-sharp tip of an atomic-force microscope (AFM) as a tool both to scan and to modify samples. It uses advanced computer graphics to display the scanned surface to the user while a robot arm enables the user to feel and modify the surface [1]. The following features of the system enabled new scientific insights [2]:

Graphics: Augmenting standard real-time 2-D views with user-controlled, real-time, publication-quality 3-D views enabled scientist collaborators to gain insight during experimentation that otherwise would be missed. Subtleties of shape and interactions between 3D objects become clearer when viewed in their natural 3-D context and from changing viewpoints.

CB #3175, Sitterson Hall
University of North Carolina
Chapel Hill, NC 27599-3175
taylorr@cs.unc.edu
919-962-1701

A scientist's ability to recognize specific molecular structures within the noisy, sampled data is improved by using stereoscopic, shaded 3D color graphics with specular highlights. This improved perception of 3D structures, compared with 2D gray-scale images with brightness coding height, was evident from the first month of the collaboration; the Williams team at UCLA recognized the up-tilted graphite planes on the first viewing of their SPM data rendered as a fly-through with shaded 3D color graphics. They had puzzled over the data for months previously [1].

Virtual tips: We developed several virtual-tip technologies to support particular experiments. Switching between oscillating mode for imaging and contact mode for modification has enabled imaging of fragile samples (adenovirus, tobacco-mosaic virus, fibrin clots, and DNA), which were then modified with known force. A sewing-machine mode enabled finer lines to be formed in thin metal films without tearing. A virtual whiskbroom enabled extended structures (tobacco-mosaic virus, fibrin) to be moved.

The NM allows a second new type of interactive exploration of the sample: the user can interactively modify the scanning parameters of the SPM. Current practice at most sites is to collect data first, and then to view and analyze it later, off-line. In such an arrangement, if a feature of interest lies halfway off the sample grid, or if the grid is too coarse to get a good look at the feature, there is not much to be done. But with the ability to scan different areas and at different scales as the exploration progresses, the scientist is empowered to explore more effectively. For example, when a feature of interest on a carbon nanotube was seen in a wide-area coarse scan, Falvo could interactively focus the scan on the end of the tube to get a high-resolution view. Having an expert human observer in the control loop makes this sort of interactive exploration very powerful [2].

Haptics: Touch enables the scientist to find the correct location to measure or start an experiment, even in the presence of drift and positioner nonlinearities. During manipulation, the probe is busy, so no new scanned images can be produced. The user works blind. But forces are continually measured. When these forces are displayed, this feedback during manipulation enables understanding and controlling the path of delicate modification. This enabled Falvo to move a colloidal gold particle across a field of debris into a gap that had been hand-formed in a thin gold wire (this was described as akin to "pushing a bag of Jell-O across a table in the dark with a screwdriver without breaking it"). Slow, deliberate feeling can find the location of objects that scanning knocks aside.

Using the nM, the experimenter can directly, immediately and naturally control the parameters of an experiment and can directly and immediately observe its results. This allows a mode of experimentation consisting of a sequence of mini-experiments with immediate feedback. The scientist can direct the exploration continuously and make impromptu changes to the viewing parameters and experimental plan between each mini-experiment.

This mode of operation enabled a series of experiments that led to the discovery of a process by which voltage pulses from an SPM tip modify the surface. The tip welds itself to the sample after a pulse and is then drawn back until breaking free. Around

300 mini-experiments were performed and analyzed in four 5-hour blocks to determine the range and frequency of results possible; the time taken for this many mini-experiments using conventional methods would have been prohibitive.

Touch also enabled the creation of a sub-micron ring of gold by scraping the inner ring and then scraping along the "snow plow" ridge on the outside to form the outer gap around the ring [2].

2.1 Impact

Use of the nM has enabled our science collaborators to produce more than two dozen publications, including high-profile papers in materials science ([3, 4], etc.) and biomedical science ([5, 6], etc.).

The nM was commercialized by 3rdTech and has been sold to laboratories across the U.S., Europe, and Asia, where it is used for research in materials and medicine (www.nanomanipulator.com).

The multidisciplinary author lists (on both the visualization and science publications) indicate the level of intellectual involvement of the entire team. Our collaborators report that it has changed the way they do science:

> "The ability to rapidly explore hypotheses with immediate visual analysis of results led to fundamental new understanding in nanoscale bending and buckling and to the demonstration of atoms acting as gear teeth, atomic-lattice interlocking controlling how electrons flow between nanoscale parts, and nanoscale torsional coupling. Coupling the visualization into a direct-manipulation control system lets us perform pilot experiments in minutes that used to take days." – R. Superfine

3 ImageSurfer

The *ImageSurfer* program was designed to display relationships between data sets in multi-fluorophore 3D confocal microscopy images [7]. It includes several standard 3D visualization techniques: isosurface, direct volume rendering (DVR), maximum-intensity projection (MIP) rendering. It also includes a custom colored isosurface technique to show correlation among two data sets, and dimensional-reduction tools (slice plane and spline) to enable quantitative analysis in user-selected subspaces of the data.

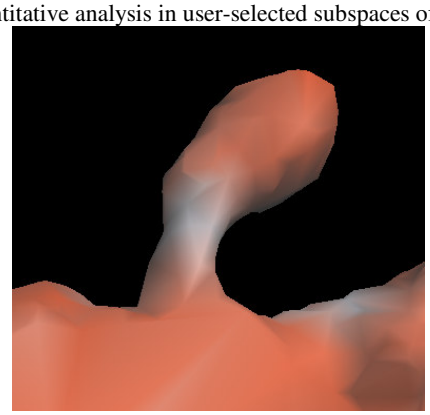

Figure 2. ImageSurfer displaying surface of dendritic spine membrane (DiO) colored by calcium (PMCA) concentration.

The colored isosurface enabled Burette and Weinberg to immediately identify spines with PMCA accumulations. In their original data exploration method, they would alternate between an image of DiO and an image of PMCA, tediously comparing positions of PMCA and spines. They found that the current technique of coloring the dendrite based on the concentration of PMCA permits more rapid understanding of PMCA organization.

Displaying PMCA density throughout the volume obscured the view of the dendritic spines, whether it was done using volume rendering or isosurface rendering as shown in Figure 2. This led us to add a *slice* view of the data sampled in 2D slices from dendrites from any angle. In their original method of alternating between images to explore spines, only 4-5 dendritic spines were aligned in the z plane; our custom-built slice extractor allows the scientists to explore spines at any orientation, drastically increasing the number of useable dendrites in each data set.

The use of height to display concentration values also proved very useful. Traditionally, they relied on comparing color values to determine relative concentrations of proteins. The new display enabled them to compare heights, an easier task for the human visual system. More accurate qualitative conclusions about the concentration and location of PMCA are possible using this alternative technique than using color alone.

3.1 Impact

Due to its usefulness to our collaborators, ImageSurfer has been widely disseminated by them to the neuroscience community through publication [8], distributing CDs at annual conferences, and at www.imagesurfer.org.

ImageSurfer has been downloaded by more than 4000 users since 2004, when it was first released as a free tool for biomedical researchers. It has become known among biomedical researchers as a useful tool for insights.

As scientists around the world have used ImageSurfer to transition from 2D slice-based viewing to 3D viewing, they have discovered new insights into the structure of the Golgi apparatus of cortical pyramidal neurons [9], human breast cancer cells, actin/myosin molecular motors, and nano-fabricated devices.

4 Conclusion

By following Fred Brooks' toolsmith approach (working closely with collaborators to support their science), we have been able to develop applications that not only push forward visualization but also ones that are widely adopted and useful.

We've described two of the tools we have developed. These and many others are available for scientists to use for free from our software download page at http://www.cismm.org/downloads.

The work presented here was made possible by funding from NSF (ASC-9527192), NIH/NCRR and NIBIB (5-P41-RR02170).

References

1. Taylor II, R.M., et al. *The Nanomanipulator: A Virtual-Reality Interface for a Scanning Tunneling Microscope*. in *SIGGRAPH 93*. 1993. Anaheim, California: ACM SIGGRAPH.
2. Taylor II, R.M., et al. *Pearls Found on the way to the Ideal Interface for Scanned-probe Microscopes*. in *Visualization '97*. 1997. Phoenix, AZ: IEEE Computer Society Press.
3. Paulson, S., et al., *Tunable resistance of a carbon nanotube-graphite interface.* Science, 2000. **290**: p. 1742-1744.
4. Falvo, M.R., et al., *Bending and buckling of carbon nanotubes under large strain.* Nature, 1997. **389**(October 9): p. 582-584.
5. Guthold, M., et al., *Controlled Manipulation of Individual Fibrin Molecules.* Biophys. J., 2000. **78**: p. A53.
6. O'Brien, E.T., 3rd, et al., *Ultrathin self-assembled fibrin sheets.* Proc Natl Acad Sci U S A, 2008. **105**(49): p. 19438-43.
7. Jen, D., et al. *ImageSurfer: A Tool for Visualizing Correlations between Two Volume Scalar Fields*. in *IEEE Visualization 2004*. 2004. Austin, Texas.
8. Burette, A., et al., *ToolBox: Stepping into the third dimension.* Journal of Neuroscience, 2007. **27**: p. 12757 - 12760.
9. AC, H., et al., *Polarized secretory trafficking directs cargo for asymmetric dendrite growth and morphogenesis.* Neuron, 2005. **48**(5): p. 757-71.