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Exploring Ultrastructure With Quantitative 3-D Intermediate Voltage Electron Microscopy

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The ability to view thick material using accelerating voltages higher than 100 KeV has greatly expanded the usefulness of electron microscopy. At or above 300 KeV, intact cultured cells and tissue sections as thick as 5 μm can be visualized. However, under normal viewing conditions this increased depth produces confusing superposition of structures located at different planes along the Z axis. In order to separate spatial information in different planes, specialized procedures such as stereo pairs or animated tilt series have been employed[1]. Although rapid, these procedures do not allow quantitation of parameters such as size, volume, or circumference. Computed Tomography produces a computer generated 3-D reconstruction from which quantitative data can be easily extracted[2]. Computed Tomography, though, as implemented for electron microscopy is a laborious process. To provide more rapid quantitation we are developing a set of algorithms based on a simplification of tomographic principles. We call these procedures Tuned Aperture Computed Tomography (TACT). The TACT program was developed in conjunction with Dr. Richard Webber of the Department of Dentistry, Wake Forest University.

TACT is rapid, allowing a complete reconstruction to be computed in a matter of hours and can be implemented on inexpensive PC's (either MSDOS or Macintosh). Although TACT is not capable of producing quite the resolution of a full computed tomogram, the resolution is more than adequate for most quantitative microscopic needs. Like Computed Tomography, TACT produces a reconstruction from a series of images of the same specimen taken at different tilt angles. However, one of the major strengths of TACT is that the resolution of the procedure can be tuned in a task dependent manner. Thus, as few as 3 tilt angles are required to produce a relatively low resolution reconstruction suitable for quantitating changes in very large structures. Sampling the specimen more extensively improves the resolution so the researcher can determine in advance the resolution required and then expend only the effort required for the task. In particular, the ability to use a limited number of tilt angles allows TACT to readily explore beam sensitive material which can not be probed by Computed Tomography.

Among the studies significantly aided by TACT reconstruction are our investigations on lysis of blood clots. "Clot Busting" drugs such as urokinase or tissue-plasminogen activator are now routinely given to heart attack victims. The enzyme activity of these drugs dissolve the obstructing clot and returns blood flow to the heart. However, in 20% of patients these drugs are ineffective. In addition, there is a critical time dependency for lysis drugs to be efficacious. Clots older than 6 hours are significantly more difficult to lyse. The reasons for these failures is not understood. To explore this question my colleagues and I have developed a model artery which allows us to control the various parameters at work during the initial formation of an occlusive thrombus (clot) and during its lysis after thrombolytic drugs are administered. The model consists of a glass tube having a similar diameter to the arteries of the heart. The tubes are lined with endothelial cells, the same cells which normally line the inside of a blood vessel. A clot is initiated in the vessel using the same plasma proteins that form a clot in vivo. The formation and lysis of the clot can be dynamically monitored using laser light scattering. As drugs dissolve the clot the density of the clot diminishes and light is scattered less. Using light scattering we show that our model system exhibits the same time dependency as an in vivo clot. The longer a clot is aged prior to administering lysing drugs the harder it is to dissolve the clot. Using antibodies to a major protein secreted by endothelial cells (Plasminogen activator inhibitor-1; PA1-1) which inhibits the clot busting drugs, we determined that PA1-1 is found within the clot bound to the clot fibers. Using TACT to quantify the distribution of PA1-1 within the clot we found that PA1-1 is threefold more concentrated at the periphery of the vessel within the vicinity of endothelial cells than in the more central areas away from the endothelium. This information would have been harder to obtain using alternative quantitative methods and suggests that regions of clot closer to endothelial cells are more difficult to lyse. This finding was confirmed by scanning electron microscopic analysis of clot fiber breakdown. Using a biophysical model of diffusion, the PA1-1 concentrations determined by TACT fit the distribution predicted by simple diffusion. This suggests that the time dependent increases in lysis resistance are the result of increased diffusion of PA1-1 into the center of the clot.

In these studies TACT provided an efficient, cost effective method for rapid quantitation of 3-D information obtainable from thick samples. This quantitation facilitated comparison of microscopic studies with quantitative data obtained by biochemical and physiiological approaches. In addition, since the TACT data set is similar to that obtainable by confocal microscopy, in future studies high resolution 3-D electron microscopic images could be easily correlated with images from dynamic confocal studies. ■

1. Rieder CL, Rupp G, Bowser SS: Electron microscopy of semithick sections, advantages for biomedical research. J Electron Microsc Tech 1985, 2:11-28.
2. Frank J, McEwen BF, Radermacher M, Turner JN, Rieder CL: Three-dimensional tomographic reconstruction in high voltage electron microscopy. J Elect Microsc Tech 1987, 6:193-205.

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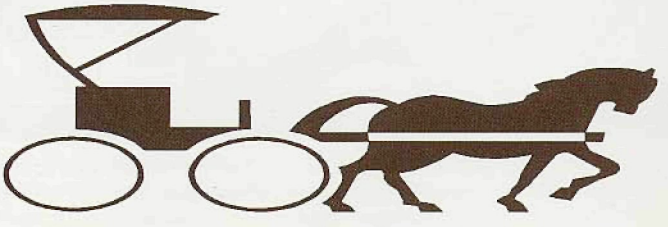

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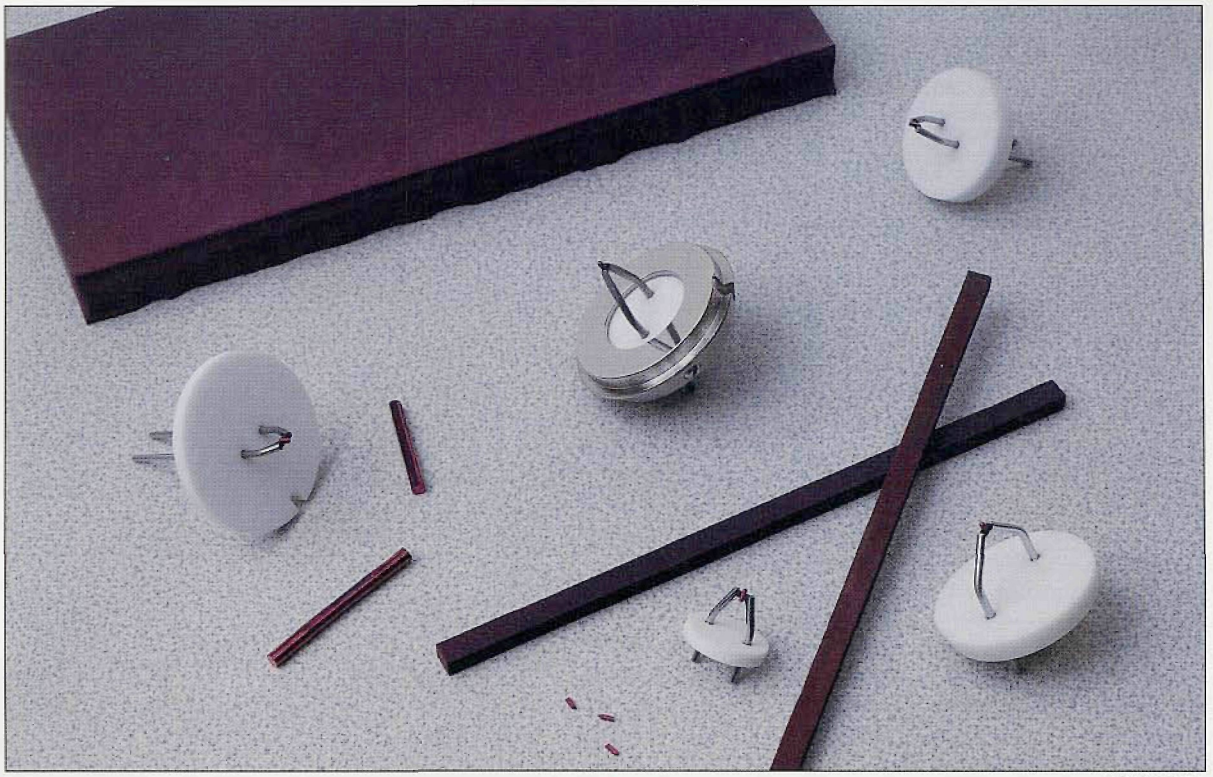

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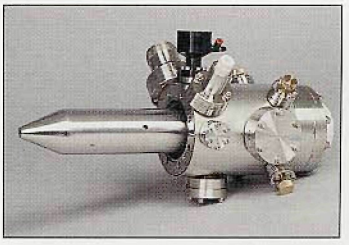

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