Project Name: Analysis of Cryo-TEM Images of LDL

Team Members:	Lewis Collier, CSC592, Lewis-Collier@Cox.net, 401-864-3175	Will design and develop software to analyze the TEM images.
	Mila Tsikhotskiy, A student of Dr. Martin's, vickaluda@yahoo.com	Is being trained to run the TEM equipment. Will take images for the project.
	Dr. Martin, CMB Dept., martin@uri.edu	Will provide biological background for the project.
	Dr. Bose, Chair CHE Dept, bosea@egr.uri.edu	Will provide TEM background for the project.
	Paul Johnson, CMB Dept. pwj419@mail.uri.edu	Will provide TEM support for the project.

Outside Meeting Times: To Be Determined

Resources Needed: JEOL JEM-1200EX Transmission Electron Microscope
Serum Samples and Preparation

Abstract: Cryogenic transmission electron microscopy (Cryo-TEM) previously has been applied to blood samples[1],[2] in order to analyze the sizes and shapes of lipids. More recently, attempts to replicate these studies at URI have been made (see Figure 1). These recent attempts studies were unable to determine the shapes and sizes due to constraints of the Cryo-TEM process and the need for better processing of the images (see Figure 2). This project will extend the previous results by applying computer vision technology in order to improve the image acquisition process and by automating the analysis of the Cryo-TEM images.

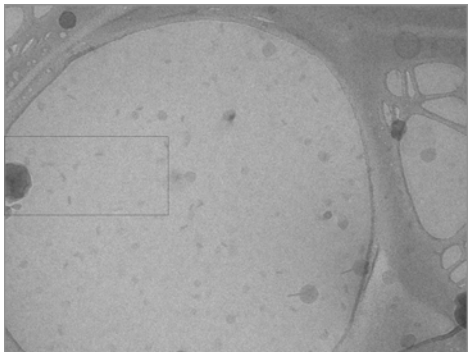

Figure 1. Cryo-TEM sample image (with enlargement area shown)

[1] Rik van Antwerpen and John C. Gilkey, "Cryo-electron microscopy reveals human low density lipoprotein substructure", Journal of Lipid Research Volume 35, 1994 pp:2223-2231.
[2] Rik van Antwerpen, Michael La Belle, Edita Navratilova, and Ronald M. Krauss, "Structural hetrogenity of apoB-containing lipoproteins visualized using cryo-electron microscopy", Journal of Lipid Research Volume 40, 1999 pp:1827-1836.

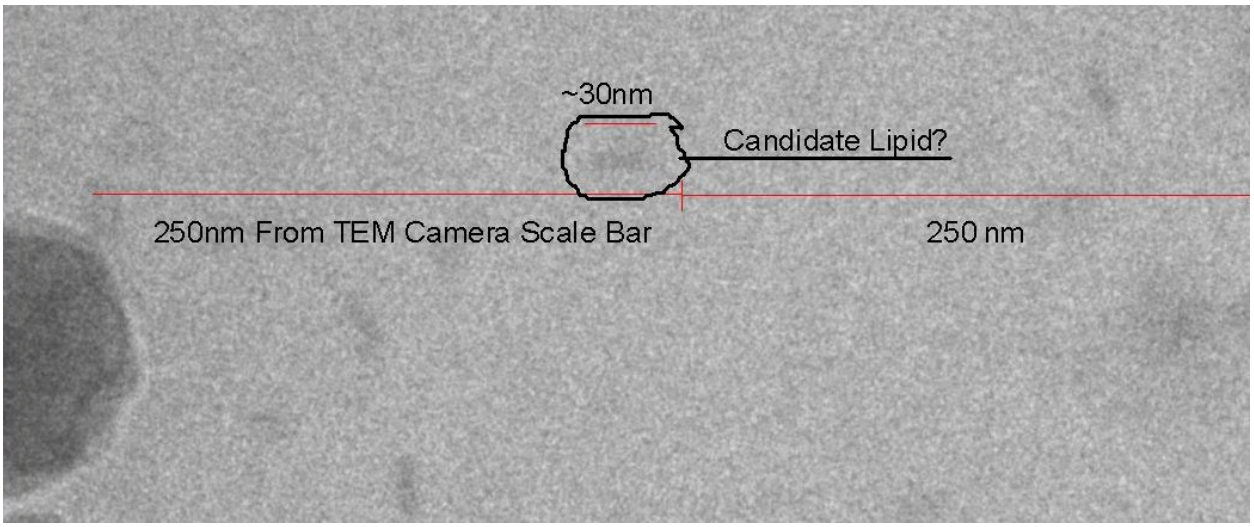

Figure 2. Enlargement area of Cryo-TEM sample image

Project Impact: The shape of the lipids to be studied is currently unknown. Knowing the shape will help better understand how these lipids attach themselves within the body. While most researchers consider the lipids to be spheres, there is some belief that they may actually be disks surrounded by a protein. A successful completion of this project may help better ascertain the shape of these lipids, thus allowing further research into the geometric properties and interactions of these compounds.

Background: The Cryo-TEM images are acquired via a transmission electron microscope (TEM) that is used with a “cold station”. The liquid samples are placed in a thin film of “holey” or “lacey” carbon such that a very thin layer of the sample fluid is left in each of the holes in the carbon film. By quickly cooling the samples in ethane and then transferring the cooled samples into liquid nitrogen, the sample liquid is frozen but is not crystallized. Thus, the elements of the sample liquid can be imaged via the TEM.

Imaging and analysis of the frozen liquid samples is made difficult by two issues. First, the TEM beam can warm the frozen sample liquid, thus making the material unstable for imaging. This makes the acquisition of multiple images difficult since the added exposure times can melt the sample liquid. Second, multiple perspective angles are currently required to allow for the size and shape analysis through 3-D reconstruction of the images at a range of exposure angles. The current motion stages are manual in nature, and as such, require human interaction to acquire images from multiple angles. The stages can be moved manually but this requires the TEM beam to be on continuously in order to move and refocus the image on the region of interest (ROI).

When combined, the two above issues significantly reduce the ability to acquire and perform analysis on cryo-TEM images in this manner.

Project Summary: This project will provide assistance to the two above mentioned image acquisition problems as well as provide computer vision assistance with the image analysis.

The continuous exposure will be addressed by using computer vision to analyze the images rather than requiring human vision. Since only single exposures are required for computer vision, the TEM beam can be blanked or "turned off" while the prior image is analyzed. Once an ROI and reference points within the ROI are defined, the computer can provide the required stage movements and TEM settings required to achieve focus or centering of the reference items. Once the manual stage has been repositioned, a new image can then be acquired for analysis, thus creating a feedback loop that does not require continuous exposure of the sample.

The multiple perspective angles will be addressed by applying geometry and computer vision to the manual stages. Once a perspective has been identified, a next location can be determined and the required manual stage movements can be provided to the human operator. Once the operator has performed this movement, a new image can be acquired and analyzed. This fine-tuning process can be repeated as necessary in order to get a well-defined image at each perspective angle. If sufficient images can be acquired, a movie of the rotation of a lipid of interest can be generated.

The final stage of this project is to provide image analysis of specific images in order to measure the shapes and sizes of lipids in the sample liquid. As noted above, there is some evidence that the lipids of interest are actually disks surrounded by a protein band. When viewed from the flat side, the disks appear as a dark line with a length determined from the diameter of the disk. When viewed from the top or bottom, the disks may appear invisible or they may appear as a circle. This appearance will depend upon the density of the protein belt. In any case, as the disk is rotated through perspective angles, the relative shape and darkness will change. Computer vision techniques will be applied in order to help model the expected changes in order to determine the shape of the lipid.

Project Deliverables: The overall goal of this project is to determine the requirements for the necessary image acquisition assistance software and the image analysis software, to develop prototype software to assist in these tasks, and to use this software to help determine the feasibility of the use of computer vision to aid in Cryo-TEM studies of biological systems. The ultimate goal of this project is to obtain practical evidence of the usefulness of computer vision in this research area to obtain additional funding in this area.

The deliverables of this project will include a design report (requirements analysis), prototype software, software documentation, and assistance (as applicable) with analysis of results obtained from images to be analyzed during this project. Testing of the prototype software will be performed with manually generated images for the analysis code and through analysis of the acquired images for the acquisition assistance code.