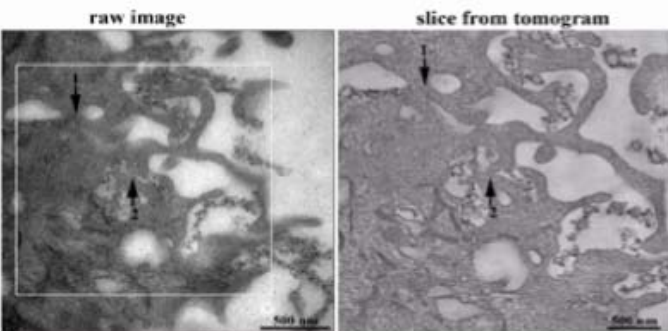


New York Structural Biology Center



User Highlight: Inna Grosheva from Cornell University. Macrophage Project

Inna Grosheva and colleagues from Fred Maxfeld's group at Weill-Cornell recently published a paper in *Molecular Biology of the Cell* (Haka et al, 2009. *Mol Biol Cell* 20,4932-40) which made the cover of the issue. They used a combination of fluorescence, light, and electron microscopy to study uptake of aggregated LDL by macrophages. The electron microscopy consisted of tomography of fixed cells in the process of engulfing gold-labeled LDL. The major reason for using tomography was for 3D reconstruction of aggregated LDL containing compartments, which cannot be achieved by regular TEM. In addition, slices from the tomograms show more detail than single images through sections, because they eliminate the superposition of material from the other levels in the section. In this paper, EM was used to investigate the morphology of SCC compartments, which are invaginations at the cell surface where LDL is sequestered. Sections from the tomograms demonstrated that SCC's are nearly sealed to the outside, which may explain how they are able to maintain a proton gradient despite being outside of the cell.



Cells were fixed, stained, embedded and sectioned by Lee Cohen-Gould at the Weill Medical College of Cornell University. Tomography of the sections was performed on the JEOL 2100F microscope at the NYSBC. SerialEM software, installed on all microscopes at the Center, was used for data collection. Maps were made of each grid and interesting points were marked for tomogram collection. Inna marked the regions of interest, and Bill Rice collected tilt series images at each point. The grid was rotated, and a second set of tilt images were collected. Image stacks were aligned on the computing

cluster at NYSBC using Protomo software, which does not need fiducial markers for this purpose. Orthogonal tomograms were then merged using IMOD, which is also set up at the center. Although segmentation of the tomograms was not necessary for this study, software for this (Amira, IMOD) is available at the Center, as well as at several of the affiliated institutions. This group is planning to use the NYSBC facility for high pressure freezing, which should preserve the samples better and will provide further information about compartment organization.

CCNY Construction Update:

As most of our users know, CCNY is building a new science complex next door to the NYSBC. As a result, we experienced continuous disruptions in the form of vibration during a good part of 2009. However, for the past few months we have not detected significant levels of vibration, and have returned to our normal operation schedule of 9-6. We have been informed that there might be a brief period of disruption later in the spring. The most current information on the construction is available at the corresponding link on the microscope schedule page.

Trivia Corner: A Little about Climate and EM

As irrelevant as the weather outside might seem, especially considering that most electron microscopy laboratories are located inside air conditioned buildings, it is helpful to keep in mind that environmental conditions can influence our work in several ways that are not entirely obvious at first glance.

The rooms assigned to house the electron microscopes have to meet certain specifications, such as a narrow temperature range and a maximum rate at which the temperature can change within this range; relative humidity, vibrations, etc. The temperature is usually targeted to be ~70F, and it can change no faster than about a degree per hour, while the relative humidity is usually specified to be below 60%. The reason for demanding thermal stability is to avoid expansion or contraction of mechanical components, which will lead to either specimen drift, or some other aspect of image degradation. The magnetic lenses carry significant electric currents, and therefore need to be cooled down. This is done most effectively with water circulating through channels in the lenses. In this process, the



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water comes into the microscope at ~65F, exits at ~73F, therefore keeping the room at ~70F is a good compromise to have both an adequate thermal stability of the instrument, and at the same time minimize user complaints about a hostile working environment (which typically occurs below 69F and above 75F). The relative humidity should be kept low mostly to insure that the circuit boards will be free of water condensation, and to achieve a better vacuum in the instrument.

However, there are some additional environmental considerations for the type of work that we do. The use of cryogenic specimens introduces a number of problems related to water condensation. Water molecules will condense on any cold surface exposed to the air in the room, such as the specimen grid, forming ice chunks that interfere with imaging (for some mysterious reason, the ice tends to be attracted to the best areas of the specimen!) This condensation problem is more pronounced as the relative humidity increases, therefore it becomes imperative to minimize the relative humidity as much as possible if we want to be able to produce clean specimens for cryogenic work. Obviously, in facilities with less than optimal climate control, the best time to work with very cold specimens will be the winter months, when the relative humidity dips to the single digits on some days.

On the other hand, the preparation of frozen hydrated specimens is a case in where we want an impossible combination of very high humidity in the region where we will load the specimen onto the grid, and low humidity once frozen, to avoid the previously mentioned contamination problem. Being more specific, the idea is to make an extremely thin film of water (~100nm), that will be vitrified on contact with liquid ethane. This is accomplished by placing a drop of sample on the specimen grid, blotting most of the sample away, and plunging the tweezers with the grid into a cup with liquid ethane. During this last step, the grid has to travel through a region (albeit small) of the lab atmosphere before reaching the ethane cup. Since the evaporation rate is a function of the surface area, a thin film of water will have an extremely high evaporation rate in comparison to the volume of sample. This can lead to either a nonexistent film of sample, or an unacceptable increase in the salt concentration, thus the desire to keep the sample moist, while also wishing to keep the moisture away from the whole area where the frozen grid will be handled. Other situations in which a high humidity environment is desired, is when preparing negatively stained samples or when making holey film to be used as a substrate for frozen hydrated specimens.

Negative stained samples tend to dry a little too fast in environments with low humidity, leaving behind particles that look severely collapsed, or, in the worst cases, precipitated salts. In a similar way, the "Japanese" method to produce holey plastic film relies on the formation of dew-like water drops to make the holes in the plastic film. On dry days, the miniscule water drops evaporate before the plastic (dissolved ethyl acetate) dries into a film, and instead of a film with holes, a continuous plastic film is formed. Of course, nowadays we can rely on the existence of commercial grids such as Quantifoil, and few of us have to go through the tedium of making our own holey grids. However, for some specimens this is still a necessity, and in those rare occasions, a rainy day is certainly welcome.

tWiki Tips: User Logbook

Although the intranet (tWiki) site can feel intimidating for several of our users, we have and continue to make a significant effort to make it more useful to the community. We have created pages to maintain a record of current projects, accessible only to the staff and the owner of the project. We also maintain an inventory of available equipment with instructions, and sample preparation protocols. One of the most significant updates in the past year has been the way we track the progress of the different projects. The new project proposal page creates three tWiki pages: a project page, a user log, and a staff log. Additional users can be given access to these pages, but only upon request of the originator. These pages are especially useful for NYSBC staff when preparing for grants and board meetings. Not to mention the fact that they can be a very valuable tool to log problems arising during the course of the project. The NYSBC staff log is used to keep track of publications and interactions with the affiliate scientists. Keeping a good log book will help us to serve your needs to the best of our ability. You can find your User Logbook on your tWiki home page. A link to your homepage is available on the left panel under My Links: in tWiki.

Announcements:

We are happy to announce the award of a grant to purchase a dual beam instrument. These are instruments in which a gallium beam can be used to mill surfaces, to be imaged by an SEM. More information in the next issue.