

# The Fourth International Congress on Electron Tomography

November 5 - 8, 2006



Paradise Point Resort and Conference Center  
San Diego, California



Program Schedule  
Abstracts  
List of Participants  
Credits

## History of Paradise Point Resort and Mission Bay Aquatic Park

Occupying the western half of Vacation Island, the secluded Paradise Point Resort was selected by the ICET organizers to create a Gordon Conference-like atmosphere where participants would be encouraged to engage in discussions that will generate the kinds of ideas needed to propel the field of electron tomography forward.

The lushly landscaped Paradise Point Resort is nestled in the center of Mission Bay Aquatic Park -- a 4,600 acre City of San Diego Recreation area. Just 50 years earlier, this location was a tidal mud flat inhabited by algae, snails, and wading birds. Originally named 'False Bay' by Portuguese explorer João Rodrigues Cabrilho in 1542, the estuary was not navigable. City planners submitted a Preliminary Plan for Mission Bay State Park in 1930 to realize the dream of a public river park for San Diego residents. The state of California began securing tidal property along Mission Bay, but the Depression and World War II effectively halted progress for the next 15 years.

In 1945, the State ceded its interest in the lands to the City of San Diego under the condition that the city improve the lands with parks, playgrounds, bathhouses, piers and facilities for use by the public. Over the next 14 years and after more than seven revised planning submissions, the Park plan was finally approved in 1958. Voters approved bond measures for \$10.5 million to fund construction over the next 13 years and over 1959-1961, Vacation Island and other elevated lands were created from mud and sand dredged to create navigable bays and harbors. Parking lots, roads, bridges, and other improvements quickly followed over the next four years. Originally named Vacation Village, Paradise Point Resort's original structures and tropical landscaping were constructed in 1962. A major renovation was completed in Fall 2006.



# FOURTH INTERNATIONAL CONGRESS ON ELECTRON TOMOGRAPHY

## Sunday, November 5, 2006

- 4:00PM Check-In (*Hotel Lobby*)  
5:00PM Meeting Registration (*Paradise Ballroom Foyer*)  
6:00PM Dinner (*Mission Bay Room*)  
7:30PM **Welcoming and Conference Opening Event** (*Paradise Ballroom*)  
Introduction, Mark Ellisman (UC San Diego)  
Keynote, Roger Y. Tsien (UC San Diego)  
8:30PM Reception (*Paradise Lawn*)

## Monday, November 6, 2006

- 7:30AM - 9:00AM Breakfast (*Paradise Lawn*)  
9:00AM - 11:45AM **Session I: INSTRUMENTATION** (*Paradise Ballroom*)  
Mark Ellisman\* (UC San Diego) – 30 min.  
Michael Marko (Wadsworth Center) – 30 min.  
— *BREAK* — 30 min.  
Nguyen-Huu Xuong (UC San Diego) – 30 min.  
Abraham Koster\* (Leiden University Medical Center) – 30 min.  
(\* co-chairs)  
12:00PM - 1:30PM Lunch (*Paradise Lawn*)  
2:00PM - 4:00PM **Poster Session A** (*Island and Sun Rooms*)  
5:30PM - 6:30PM Dinner (*Mission Bay Room*)  
7:00PM-9:30PM **Session II: IMAGING OF DYNAMIC STRUCTURES  
(CORRELATIVE MICROSCOPY)** (*Paradise Ballroom*)  
Ohad Medalia\* (The Ben Gurion University) – 30 min.  
Andrew Leis (Max-Planck-Institut für Biochemie) – 30 min.  
— *BREAK* — 30 min.  
Thomas Deerinck (UC San Diego) – 30 min.  
Jack Johnson\* (The Scripps Research Institute) – 30 min.  
(\* session co-chairs)

## Tuesday, November 7, 2006

- 7:30AM - 9:00AM Breakfast (*Paradise Lawn*)  
9:00AM - 11:45AM **Session III: 3-D RECONSTRUCTION ALGORITHMS**  
(*Paradise Ballroom*)  
Michael Radermacher\* (University of Vermont) – 30 min.  
Achilleas Frangakis (EMBL Heidelberg) – 30 min.  
Salvatore Lanzavecchia (University of Milano) – 30 min.  
— *BREAK* — 30 min.

Friedrich Foerster (UC San Francisco) – 30 min.  
Niels Volkmann\* (The Burnham Institute) – 30 min.  
(\* session co-chairs)

**12:00PM - 1:30PM** Group Picture  
Lunch (*Paradise Lawn*)

**2:00PM - 4:00PM** **Poster Session B** (*Island and Sun Rooms*)

**5:30PM - 6:30PM** Dinner (*Mission Bay Room*)

**7:00PM - 9:30PM** **Session IV: VISUALIZATION AND QUANTITATIVE ANALYSIS**  
(*Paradise Ballroom*)  
Alasdair Steven (National Institutes of Health) – 30 min.  
Brad Marsh (University of Queensland) – 30 min.  
— *BREAK* — 30 min.  
Ross Whitaker\* (University of Utah) – 30 min.  
David Mastronarde\* (University of Colorado, Boulder)–30 min.  
(\* session co-chairs)

### Wednesday, November 8, 2006

**7:30AM-9:00AM** Breakfast (*Paradise Lawn*)

**9:00AM-11:45AM** **Session V: MOVING TOMOGRAPHY TO THE MAINSTREAM**  
(*Paradise Ballroom*)  
Jose-Maria Carazo\* (Universidad Autónoma de Madrid)–30 min.  
Maryann Martone\* (UC San Diego) – 30 min.  
— *BREAK* — 30 min.  
Endre Majorovits (University of Oxford) – 30 min.  
Terrence Sejnowski (The Salk Institute) – 30 min.  
(\* session co-chairs)

**12:00PM-1:00PM** Lunch (*Paradise Lawn*)

**1:00PM-1:45PM** Check-Out (Hotel Lobby)

**1:45PM-4:30PM** **Session V: EMERGING TECHNOLOGIES FOR MULTISCALE**  
(*Paradise Ballroom*)  
Carolyn Larabel\* (UC San Francisco, LBNL) – 30 min.  
James Sharpe (El Centro de Regulación Genómica,  
Barcelona)– 30 min.  
Guive Balooch (UC San Francisco) – 30 min.  
— *BREAK* — 30 min.  
Grant Jenson (California Institute of Technology) – 30 min.  
Stephan Nickell (Max-Planck-Institut für Biochemie) – 30 min.  
Dorit Hanein\* (The Burnham Institute) – 30 min.  
(\* session co-chairs)

**4:30PM** Congress Adjourns

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## MAPPING ELEMENTAL DISTRIBUTIONS IN THREE DIMENSIONS BY EFTEM TOMOGRAPHY

Presenting Author: MARIA. A. ARONOVA, Postdoc

R. Harmon Gatan Research and Development, Pleasanton, CA A. Sousa, G. Zhang, and R.D. Leapman Division of Bioengineering and Physical Science, NIH, Bethesda, MD

We have used automated electron tomography in the energy filtering transmission electron microscope to map the three-dimensional distributions of chemical elements in unstained, freeze-substituted and embedded cells. A group of images is collected at each angle in a tomographic tilt series: zero-loss and unfiltered images are used to track specimen drift and focus, after which post-edge and pre-edge images are acquired. Using the zero-loss and the unfiltered intensities we calculate the  $t/\lambda$  map of the specimen which enables us to correct for change of spectral shape due to plural inelastic scattering when the specimen thickness increases at high angles. The core edges together with the zero-loss intensities allow us to determine the absolute number of atoms of a specific element per unit specimen area. By normalizing the projection of the reconstructed tomogram to the quantitative 2D elemental map, we can estimate the number of atoms of a specific chemical element in each voxel. By using the phosphorus signal as an intrinsic label for nucleic acid, we have investigated the distribution of DNA in cell nuclei. We show how quantitative analysis of the 3D phosphorus distribution might provide information about DNA packing density in chromatin.

## HIGH RESOLUTION SINGLE AND DUAL AXIS TOMOGRAPHY TO SOLVE MATERIALS PROBLEMS IN THREE DIMENSIONS

Presenting Author: ILKE ARSLAN, P.I.

I. Arslan<sup>1</sup>, J. R. Tong<sup>2</sup>, J. Walmsley<sup>3</sup>, E. Rytter<sup>3</sup>, E. Bergene<sup>3,4</sup>, P.A. Midgley<sup>2</sup> 1. Sandia National Laboratories, 7011 East Avenue, Livermore, CA 94550, USA. 2. Department of Materials Science and Metallurgy, University of Cambridge, Pembroke Street, Cambridge, CB2 3QZ, UK. 3. Statoil Research Centre, Trondheim, N-7005 Norway. 4. SINTEF Materials and Chemistry, Trondheim, N-7491 Norway.

Nanotechnology now plays a key role across many fields of science, and especially materials science in furthering current environmental and semiconductor technology. Rather than analyzing and determining the properties of bulk single or poly-crystals where the third dimension is assumed to be uniform, we must now analyze materials that have a finite size and shape in three dimensions, and not necessarily uniform in any of the directions. This new demand on materials characterization has led to the development of electron tomography for inorganic materials using Z-contrast imaging in the scanning transmission electron microscope (STEM). Here we present high resolution single and dual axis tilt tomography results of Co-based Fischer Tropsch (FT) catalyst systems and CdTe tetrapods, respectively. We studied two Co-based catalyst systems with different supports to elucidate the size, shape, and distribution of the catalyst in its support in three dimensions. This nanoscale characterization gives us insight as to why one catalyst is more selective than the other. To study CdTe tetrapod structures, we must turn to dual axis tomography due to the geometry of the nanostructures. Their four thin, long legs in tetrahedral symmetry make it almost impossible to reconstruct all four legs with single axis tomography since one of the legs usually fall victim to the missing wedge. This new demonstration of dual axis tomography in materials science allows us to understand the structural properties of the tetrapods by studying their size, shape, and interaction with their support in three dimensions.

## DEFINING A POTENTIAL DUAL ROLE FOR SYNAPTOTAGMIN IV USING ELECTRON TOMOGRAPHY

Presenting Author: CHRISTOPHER ARTHUR, Student

Christopher P. Arthur (University of Colorado) Michael H.B. Stowell (University of Colorado)

The synaptotagmins are thought to be essential for the docking and fusion of synaptic vesicles with the pre-synaptic active zone following depolarization and influx of calcium in presynaptic terminals. Synaptotagmin IV (SytIV) is an immediate early gene which is induced by membrane depolarization in the hippocampal neurons of the brain. Little is known about the localization and function of SytIV at the hippocampal synapse. Fluorescence studies have shown it to be localized at the Golgi as well as neurite tips during synaptogenesis and following depolarization events. Using electron tomography on cultured hippocampal neurons from SytIV knockout mice we have shown that SytIV may potentially be involved in vesicle trafficking from the Golgi to the neurite tips. Tomograms of synapses from mice lacking SytIV show a greater than six-fold decrease in the number of vesicles present at the synapse as compared to wild-type mice. Mice which lack SytIV have been shown to have a decreased memory capacity which is associated with the hippocampus. These results suggest that this decrease in hippocampal function may in fact be due to a lack of neurotransmitter containing vesicles at the hippocampal synapse. We have also examined the Golgi in these SytIV knockout mice and found a significant increase in the size and number of vesicles surrounding the Golgi as compared to the wild type neurons. These results seem to imply that SytIV is in fact involved in vesicle trafficking to the presynaptic active zone.

# CRYO-ELECTRON TOMOGRAPHY OF MOUSE HEPATITIS VIRUS: NEW INSIGHTS INTO THE STRUCTURE OF CORONAVIRUSES

Presenting Author: ABRAHAM J. KOSTER, P.I.

Montserrat Barcena(1,2), Berend Jan Bosch(3), Willem Bartelink(3), Peter J. M. Rottier(3), Abraham J. Koster(1) (1) Molecular Cell Biology (MCB-EM), Leiden University Medical Center, Einthovenweg 20, 2300 RC Leiden, The Netherlands. (2) Molecular Cell Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands (3) Virology Division, Department of Infectious Diseases and Immunology, Faculty of Veterinary, Utrecht University, 3584 CL Utrecht, The Netherlands

Coronaviruses are enveloped plus-stranded RNA-viruses that have attracted considerable attention since the outbreak of SARS (severe acute respiratory syndrome), caused by a member of this group. These viruses are also of great veterinary importance, since they infect a wide range of mammals and avians. The current structural model for coronaviruses depicts a particle of 80-120 nm size, consisting of a nucleoproteic core that is surrounded by a lipid envelope. From this envelope arises a layer of spikes of about 20 nm which is the hallmark of coronaviruses [1]. In spite of multiple biochemical and morphological studies, a complete structural characterization of coronaviruses is missing. A major reason for this is the pleiomorphic nature of these viruses that traditional methods seem to indicate. Thus, Electron Tomography becomes the methodology of choice to characterize the structure of these viruses. In the present work, we have applied cryo-electron tomography to isolated samples of mouse hepatitis virus (MHV), obtaining for the first time three-dimensional reconstructions of an unstained coronavirus. Several tilt series were collected in a 300 kV microscope equipped with a post-column energy filter covering an angular range of  $\sim 130^\circ$ . A total of about 60 viruses were reconstructed and analyzed from the resulting tomograms. In cryo-preparations, MHV showed a globular shape and a relatively homogeneous size of about 85 nm (standard deviation: 7 nm). Most remarkably, the reconstructions show the presence of an inner shelled structure or core. Traditionally, the inner part of the virus has been believed to consist only of a helical assembly of the nucleocapsid protein and the genomic RNA, a model based on studies of disrupted viruses. The only challenge to this model was a two-dimensional microscopy analysis that showed evidence of the existence of a core structure, possibly icosahedral, in MHV and, more clearly, for transmissible gastroenteritis virus (TGEV) [2]. Our preliminary results confirm the existence of this new substructure. Furthermore, the results indicate the existence of a very close interaction between the envelope and the core. The three-dimensional character of our data allows the *in silico* isolation of the cores and a subsequent analysis to definitely establish their structure. These results, together with the analysis of other viral subcomponent, will set the basis for a complete model of the architecture of coronaviruses. [1] de Haan, C.A.M. and Rottier, P. (2005). *Adv. Vir. Res.* 64: 165. [2] Risco, C., Antón, I.M., Enjuanes, L, Carrascosa, J.L. (1996). *J. Virol.* 70: 4773. Acknowledgments. This work was supported by a Marie Curie Intra-European fellowship to M.B. (MEIF-CT-2004-501540). We would specially like to acknowledge Gert Oostergetel for the help with data collection on the FEI Polara at the University of Groningen.

## TOM GOES 2D - HIGH-THROUGHPUT SINGLE PARTICLE ANALYSIS WITH THE TOM TOOLBOX

Presenting Author: FLORIAN BECK, Student

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High-throughput single particle analysis (HT-SP) offers tremendously new perspectives in structural investigations of macromolecular machines. Especially for protein complexes with a high amount of conformational changes this technique can lead to structural insights at a reasonable resolution. The 'TOM software toolbox' designed for the acquisition and analysis in electron tomography was therefore extended over the last two years to deal with the special needs of high-throughput single particle analysis, especially in cryo-applications. It provides a easy-to-use unified platform for automated acquisition, particle picking, alignment, classification, image-correction and the reconstruction of particles. All these steps can be done for 2 dimensional (2D) or 3 dimensional (3D) single particle analysis. To address the poor signal-to-noise ratio (SNR) in electron microscopy (EM) micrographs of vitrified specimens all critical measurements and procedures can be done additionally in a semi-automatic way. The 3D SP-analysis workflow can also be used for in vivo (cellular) examinations. Designed as a collection of computational procedures it is a complete software solution within a highly flexible framework for dealing with large data amounts. This way TOM for 2D represents not only a transparent but also a very user-friendly platform to acquire and process huge quantities of 2D EM micrographs.

# ARCHITECTURE OF AN HIV NEUTRALIZING ANTIBODY CONSTRUCT BY CRYO-ELECTRON TOMOGRAPHY

Presenting Author: ADAM E. BENNETT, Student

Adam E. Bennett (1,2), Jun Liu (1), and Sriram Subramaniam (1) 1 Laboratory of Cell Biology, National Cancer Institute, NIH, Bethesda, MD, USA 20892 2 Department of Pharmacology, University of Cambridge, Cambridge CB2 1PD, UK

We are using electron tomography to understand the three-dimensional structures of the HIV-1 envelope glycoproteins and antibodies against them that neutralize viral infectivity. We visualize the individual three-dimensional structures of a potent CD4-based antibody fusion protein and of IgM using cryo-electron tomography. An extreme case of neutralizing capability, this antibody has a heterogeneous structure that contrasts with the relatively uniform structure of IgM, despite similar primary structures. We propose a mechanism of HIV neutralization by this antibody, based on its unique structure, and on the current model of the gp120-gp41 trimer.

# SOFT CLUSTERING FOR IMAGE CLASSIFICATION AND RECONSTRUCTION

Presenting Author: CHRISTOPH BEST, Postdoc

Wolfgang Baumeister (MPI of Biochemistry)

Soft clustering methods such as self-organizing maps and multidimensional scaling are designed to offer more flexibility and richer information about similarities and dissimilarities in sets of objects. They are based on assigning each object in a set a position in a low-dimensional space--such as a plane---to most closely reproduce the similarities between the objects. The problem of performing this assignment can be cast in a conventional minimization problem, while solving it can be difficult due to local minima. These problems can be overcome by using Monte Carlo algorithms combined with successive dimensional reduction of the problem. Once a map has been found, it can be further analyzed to reveal the classification patterns that the algorithm uncovered and further boost the sensitivity of the algorithm. These algorithms are very well suited to classifying large number of objects with many degrees of freedom. They can also be given a Bayesian interpretation by associating the potential in the two-dimensional map with a probability density, and similar to kernel methods they can be fine-tuned by using different mapping kernels for the similarity function. We apply these methods to identifying and discriminating macromolecules in cryo-electron tomographic images. We identify subvolumes of interest either by correlation with molecular templates or by denoising/segmentation-based method using anisotropic diffusion or the scaling index. Sub-volumes are clustered by a self-organizing point map both to discriminate between molecules and artifacts as well as between different macromolecules. In analyzing the resulting clusters and their relation, we attempt to iteratively boost the effectiveness of classification and derive a classifier that is focused on the molecules of interest. We also aim to tune the classifier so that it will, given sufficient data, be able to account for conformational variability. We use these methods both on two-dimensional projection images before tomographic reconstruction and on three-dimensional volumes reconstructed with different algorithms. By applying it to the two-dimensional images, we avoid the missing-wedge problem at least in part. We can also apply the algorithm to single particle analysis and to random conical tilt situations. The additional information available in tomography, i.e. the fact that different images are related by a given angular displacement, can be incorporated directly into the classification algorithm by adding a suitable a priori probability distribution between related images.

## CRYO-ELECTRON TOMOGRAPHY OF A PREDATORY BACTERIUM

Presenting Author: MARIO J. BORGNIA, Staff Scientist

Mario J. Borgnia, Sriram Subramaniam and Jacqueline L. S. Milne / Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD.

*Bdellovibrio*-and-like organisms (BALOs) are Gram-negative bacteria of potential interest as antibiotic agents as they prey on many Gram-negative human pathogens. Propelled by a single sheathed flagellum, BALOs are small, *vibrio*-like, fast-swimming bacteria. These predators find prey, attach to its outer membrane and digest their way into the periplasmic space. The outer membrane is sealed and reshaped into a globular “bdelloplast”. The invading bacterium then digests the prey's cytosol and grows inside the periplasm into a long spiral cell which finally divides by multiple segmentation. Each of the emerging cells develops a flagellum and swims inside the bdelloplast until the latter breaks and the new generation of predator is released. We are using cryo electron tomography to investigate the spatial arrangement of key molecular assemblies in *Bdellovibrio bacteriovorus* cells. Tomograms from plunge-frozen cells show defined densities corresponding to ribosomal assemblies, the nucleoid and membrane-associated structures. We anticipate that these results will provide a foundation to explore structural aspects of phenomena such as chemotaxis, bacterial cell flexibility and cell-cell interactions.

## THE ARCHITECTURE OF THE PRESYNAPTIC ACTIVE ZONE

Presenting Author: ALAIN BURETTE, Postdoc

Alain Burette<sup>1</sup>, John Crum<sup>3,4</sup>, Maryann E Martone<sup>3,4</sup>, Mark H Ellisman<sup>3,4</sup>, and Richard J Weinberg<sup>1,2</sup> 1. Cell & Developmental Biology, 2. Neuroscience Center, Univ. of North Carolina, Chapel Hill, NC, USA 3. Department of Neurosciences, 4. National Center for Microscopy and Imaging Research, Univ. of California, San Diego, La Jolla, CA, USA

Neurons are uniquely complex cells; they communicate with each other at the synapse. Synaptic regulation in the brain plays a special role in learning and memory, motivating intense study of the underlying mechanisms. Recent work has learned a great deal about the biochemistry of synaptic transmission, but little progress has been made on its underlying structural basis in the past 20 years. Active zones (AZs), specialized sites for release of neurotransmitter from presynaptic terminals, are characterized by distinctive electron-dense aggregates of proteins facing the synaptic cleft. These aggregates represent machinery that mediates and regulates transmitter release. Pioneering tomographic analysis of the frog neuromuscular junction, a convenient peripheral model of synaptic transmission, finds that the AZ is organized into an array of 'beams' and 'ribs' that connect docked presynaptic synaptic vesicles with putative Ca<sup>2+</sup> channels, thus optimizing reliability of transmitter release. The limited structural data available for the mammalian CNS synapse suggests a different architecture. Early ultrastructural studies reported that presynaptic dense projections were organized into a hexagonal grid, interspersed with small depressions. The dense projections were postulated to represent the machinery that guides synaptic vesicles to the plasma membrane, while the concavities might represent docking/fusion sites for the vesicles. We have combined specialized techniques of specimen preparation with dual-tilt EM tomography to study the arrangement and association of structural components of the AZ in tissue sections from rat forebrain. We are generating realistic 3D models of the AZ to determine the physical characteristics of the dense projections and their relationship to the presynaptic vesicles, revealing in situ structural features that have not been previously visualized. Support Contributed By: NIH P41 RR-O4050 (MHE) NS-35527 & NS-39444 (RJW)

# QUANTITATIVE ASSESSMENT OF APPLICATION OF HILBERT CONTRAST CRYO EM TOMOGRAPHY

Presenting Author: WEI-HAU CHANG, P.I.

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Recent advance of cryo electron microscopy (cryo-EM ) and automated tomography collection allow rapid 3-D reconstruction of unique objects such as organelles and cells in their native state. Because of the constraint imposed by radiation damage, the total doses have to be fractionated among views in a tomography series, resulting in very noisy images of views and reconstructions with resolution much inferior than that of macromolecules reconstructed from single particle analysis. With the introduction of the half phase plate, it has been shown tomography series with so called Hilbert contrast, analogous to DIC contrast in optical microscopy, can be obtained. Intuitively, from Rose visibility criteria, such improved contrast implies more details can be observed in raw images of views. Moreover, gain of signal at low resolution regime would be helpful to the alignment of very noisy tilt series. Here, we challenge these hypotheses with a numerical study to assess the application of half phase plate to cryo-EM tomography.

## ELECTRON CRYO-TOMOGRAPHY OF HSV-1 REVEALS THE PORTAL

Presenting Author: JUAN CHANG, Student

Juan Chang [1,2] Michael F. Schmid [1,2] Frazer Rixon [3] Wah Chiu [1,2] [1] Graduate Program in Structural and Computational Biology and Molecular Biophysics, [2] National Center for Macromolecular Imaging, Baylor College of Medicine, Houston, Texas 77030, USA. [3] MRC Virology Unit, Institute of Virology, Church Street, Glasgow G11 5JR, United Kingdom.

Increasing evidence points to common biological elements that are shared among dsDNA viruses. The structures of the capsid proteins of P22, Epsilon 15, and Herpes Simplex Virus 1 (HSV-1) reveal a common fold. Their assembly processes are also similar. A procapsid is pre-assembled in the host cell. Subsequently, viral dsDNA is packaged through a portal complex located at one of the 5-fold vertices of the procapsid. Further maturation of the virus results in acquisition of a tail or envelope, and/or other elements. In both phages and animal viruses, the portal serves a similar and important function: packaging and releasing viral DNA. Studies of various portals in biochemical isolation reveal that they can adopt different oligomeric states. However, there is a consensus that the portal complex is a dodecamer in the intact virion. The portals of HSV-1 and other viruses are located at one of the 12 vertices, and, as a result, their 12-fold nature breaks the 5-fold symmetry of the icosahedral vertex. This mismatch has been proposed as necessary to permit the portal to rotate and package viral DNA. Portals from different viruses are different sizes, and the extra domains may serve other functions, in addition to the essential DNA packaging role, which are specific to each particular virus. Because it does not match the icosahedral symmetry of the capsid, it is not possible to reconstruct the portal using the traditional single-particle icosahedral reconstruction approach. In addition, the portal complex of HSV-1 capsid has a similar mass as the capsid protein (VP5) in the other 11 vertices making it hard to differentiate. However, electron cryo-tomography is well-suited for the purpose of direct visualization of the complex. To facilitate the identification of this unique vertex, HSV-1 capsids were subjected to chemical treatment that retains the portal while preferentially removing the pentons from the other vertices, resulting in pentonless capsids. Electron cryotomograms were collected in a JEM2010F electron cryomicroscope using the Mr.T data collection software. Tomograms were reconstructed with the IMOD software. Because the capsid has 11 holes in the 5-fold locations but has density due to the portal complex at one of the 12 pentameric positions, we are able to align them with reasonably high level of reliability. With the aligned tomograms, we are able to average 11 pentonless capsids to enhance the contrast of the map. The averaged capsid exhibits all the icosahedral components including hexons and triplexes as seen in the icosahedral reconstructed map. In addition, the portal complex, at only one of the vertices, is clearly visible and its location relative to the capsid shell protein is similar to those seen in dsDNA phages such as Epsilon15 and P22. This research has been supported by NIH grants (P41RR02250 and R01AI38469) and Robert Welch Foundation.

## QUANTITATIVE 3D IMAGING BY CRYO-EM (SHAPES, MEMBRANES, AND MAPPING OF SUBCELLULAR STRUCTURES IN WHOLE BACTERIA)

Presenting Author: LUIS R. COMOLLI, Staff Scientist

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The shape of the bacteria cell wall during division and the deformation at the division plane are a function of the material properties of the cell wall, the growth rate, and the force due to the FtsZ ring. We have obtained 23 cryo-EM tomographic reconstructions through a time course following the cell division process in *Caulobacter crescentus*. We have also acquired over 300 images of the process by high resolution cryo-EM. The cell membranes have been segmented by an in-house developed utility, and represented on a canonical triangular mesh. These cell membrane surfaces provide us local curvatures; together with independently determined elastic parameters for the membrane, these data allow us to estimate the local forces exerted during the process. Our current models support the conclusion that cell growth and cell contraction occur on similar timescales, and that the contractile force at the division midplane is minimal, serving to direct the growth of the peptidoglycan mesh very near the division plane so as to eventually pinch and isolate the two halves of the dividing cell. The potential energy stored in the peptidoglycan mesh under the strain induced by the FtsZ ring is always minimal while insertion of new cell wall material near the division midplane at the point of inflection in the dividing cell is essential. During the late stages of the *Caulobacter crescentus* cell division process, the inner membrane (IM) separates from the cell wall and proceeds to compartmentalize the cytoplasmic spaces of mother and daughter cells. The outer membrane (OM) and S-layer lag behind forming a narrow tubular structure that keeps both cells connected until the IM process is completed. The shape of the IM surface evolves in a seemingly independent process. We compare the modeled membrane surfaces obtained from our data with simulated evolutions of close membrane surfaces, with realistic elastic parameters, to try to provide a first quantitative estimate of the work involved in this process. We also illustrate the combined use of cryo electron tomography and spectroscopic difference imaging in the study of sub-cellular structure and subcellular bodies in whole bacteria. We limit our goal and focus to bodies with a distinct elemental composition that is in sufficiently high concentration to provide the necessary signal-to-noise level at the relatively large sample thicknesses of the intact cell. This combination proves very powerful, as demonstrated by the characterization of a phosphorus-rich body in *Caulobacter crescentus*. We also confirm the presence of a body rich in carbon, demonstrate that these two types of bodies are readily recognized and distinguished from each other, and provide for the first time to our knowledge structural information about them in their intact state. In addition we also show the presence of a similar type of phosphorus-rich body in *Deinococcus grandis*, a member of a completely unrelated bacteria genus. Cryo electron microscopy and tomography allow the study of the biogenesis and morphology of these bodies at resolutions better than 10 nm, while spectroscopic difference imaging provides a direct identification of their chemical composition.

### 3-DIMENSIONAL RECONSTRUCTION OF CYANOPHAGE P-SS2 USING CRYO-ELECTRON TOMOGRAPHY

Presenting Author: ANGELA CRUCIANO, research technician (staff)

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*Prochlorococcus* and *Synechococcus* are two genera in the marine photosynthetic bacteria, picocyanobacteria. These cyanobacteria play a significant role in the oxygen production in the oceans. Like terrestrial bacteria, cyanobacteria have viruses that infect them called cyanophages. We are looking at a cyanophage that infects *Prochlorococcus* which belongs to the *Siphoviridae* family called P-SS2. We performed cryo-electron tomography and multi-particle averaging to examine the structure of both the head and the tail. Using Mr T, multiple tilt-series were acquired at 200keV on a JEM2010F with a Gatan 4k CCD camera and reconstructed using the software package IMOD.

Like other siphoviruses, P-SS2 has a DNA-packed capsid head and a long flexible tail. Multiple capsid heads were extracted from tomograms and merged to generate an average model. That model shows a non-isometric head, similar to *E. coli* phage T4 and *B. subtilis* phage f-29. The head is 1200 Å long and 620Å in diameter, with 5-fold symmetry. Both the top and bottom of the capsid appear to be comprised of five equilateral facets. In an icosahedral capsid, the top five facets are staggered from the bottom five by 36°. However, the P-SS2 reconstruction shows a different staggering angle.

The total length of the long flexible tail of P-SS2 is ~3000Å. The main portion of the tail appears to be made of a long stack of disks each with a diameter of ~106Å and center-to-center spacing between the disks is ~42Å. At the end of the tail, the density appears to be wider than the tail body. The tail terminus has multiple protrusions of varying lengths and shapes. We call the longest protrusion the whip. Approximately fifty tail termini were isolated out of the tomogram, merged and averaged. The whip is flexible with respect to the tail, so the averaged density of the whip fades out at a few hundred angstroms away from the tail termini.

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# ELECTRON TOMOGRAPHY OF HUMAN CYTOMEGALOVIRUS AND ITS INFECTION

Presenting Author: WEI DAI, Student

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Human cytomegalovirus (HCMV) is among the most complex animal viruses and causes severe morbidity or mortality in newborns and immunocompromised individuals. Infectious HCMV virion shares similar architecture with other herpesviruses, consisting of a pleomorphic glycoprotein containing envelope, a poorly understood tegument layer, and an icosahedral capsid enclosing a dsDNA genome. Previous structural studies of HCMV only revealed the icosahedral capsid and structures closely associated, the inner tegument layer, due to the lack of symmetry and structural uniformity in the bulk of the tegument or the entire membrane. To define the outer layers of the HCMV and their structural roles during infection, we have used electron tomography (ET) to reconstruct frozen-hydrated HCMV virions and thin-sections of HCMV-infected cells. CryoET 3D reconstruction of purified HCMV virions showed that the inner layer tegument closely attached to the top of the capsid, and the outer layer tegument fills the space between the inner layer and the envelope, conforming to the shape of the viral membrane. 3D reconstruction of tegumented particles labeled with anti-pp150 antibodies confirmed that pp150 is one of the inner tegument proteins, localized at the tip of the capsomer. CryoET reconstruction of pp65-minus mutant showed empty space in the outer layer compared to the wild type, suggesting that pp65 is an abundant outer-tegument protein. 3D tomograms of thin sections of the infected cells reveal both enveloped viral particles inside the cellular vesicles and de-enveloped particles, suggesting the use of both endocytosis and membrane fusion pathways for virus entry. Integration of our structural data with biological experiments provides insight into the organization of this most complex herpesvirus and its entry mechanisms.

## THREE-DIMENSIONAL STRUCTURE OF LENTIVIRUS BY ELECTRON TOMOGRAPHY

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The general structure of viruses from the Lentivirinae subfamily is well-known, with the models hypothesized from TEM (transmission electron microscope) tilt images. However, recent advances in electron microscope automation and integration as well as computer technology have allowed rapid digital imaging and image analysis directly from the raw images. Thus, electron tomography (the computed acquisition of tomograms, or slices through an object, via electron microscopy) has become easier and much more feasible. Post-processing involves alignment of the areas of interest and 3D (three-dimensional) reconstruction of the tomogram from tilt images. 3D modeling involves particle isolation/segmentation from the entire image volume and combination of the resulting data to form the 3D structure. Several different algorithms exist for computing 3D structure from raw tilt images (3D reconstruction), and there are many applications which can render 3D models. This experiment aims to produce a general structure of the lentivirus SIV (simian immunodeficiency virus) from the actual images, focusing on a 3D reconstruction algorithm from the IMOD software package and involving two different software tools for 3D segmentation (MIPAV) and model generation (IMARIS).

# ELECTRON CRYOTOMOGRAPHY OF KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS CAPSIDS REVEAL STRUCTURES ESSENTIAL TO VIRAL ASSEMBLY AND DNA PACKAGING

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Kaposi's sarcoma-associated herpesvirus (KSHV), a recently discovered DNA tumorigenic virus, is the etiologic agent of Kaposi's sarcoma and several AIDS-associated lymphomas, including primary effusion lymphoma and multicentric Castleman's disease. Like other herpesviruses, KSHV has a multilayered architecture, which consists of an icosahedrally ordered nucleocapsid surrounding the double-stranded DNA genome, a poorly defined tegument protein layer, and a glycoprotein-containing pleomorphic envelope. KSHV viral particles undergo drastic morphological changes during the various stages of viral infection, capsid assembly and viral maturation, producing different intermediate particles in addition to the final infectious virions. The processes of capsid assembly are essential for continued spread of the virus and lead to the formation of three types of stable capsids in the host cell nucleus: the empty A capsid, the scaffolding-containing B capsid, and the DNA-containing C capsid. Although these capsids share the same basic icosahedral shape, they are distinguished by their contents. In addition, genetic and biochemical studies have suggested the existence of a bacteriophage-like, DNA-packaging/ejection portal, presumably located at one of the 12 vertices. However, the putative herpesvirus portal complex and the three-dimensional (3D) structural differences among the different capsid forms have not been discernable by single particle reconstruction with or without symmetry. This is likely due to various reasons including the relative small size of the portal complex and the flexibility of the relative orientation of the internal core with respect to the outer capsid shell. We have used cryo-electron tomography (cryoET) to reconstruct these capsid forms in order to gain insights into the 3D organization of the portal and its internal structures. We found that the 'empty' A capsids contain a cylindrical shaped protein complex attached to the inner capsid shell surrounding one of the 12 vertices. This complex is most likely associated with the KSHV capsid portal. In addition to this density, B capsids also contain a scaffolding core, which is closely associated with the putative portal complex and is organized as a sphere of 750 Å in diameter with a 250-Å diameter hollow cavity. The scaffolding core and the outer capsid shell are non-concentric, consistent with the observation that icosahedral reconstruction failed to resolve this core structure. The DNA density inside C capsids is less well resolved but appears to distribute evenly throughout the capsid shell. Taken together, our 3D structural observations provide a structural basis for understanding the essential steps involved in capsid assembly, as well as DNA packaging and ejection during KSHV infection.

## MULTIPLE LARGE FILAMENT BUNDLES OBSERVED IN *CAULOBACTER CRESCENTUS* BY ELECTRON CRYOTOMOGRAPHY

Presenting Author: PRABHA DIAS, Staff Scientist

Ariane Briegel, Caltech Prabha Dias, Caltech Zhuo Li, Caltech Achilleas Frangakis, EMBL Heidelberg Rasmus B. Jensen, Roskilde University, Denmark Grant Jensen, Caltech

While the absence of any cytoskeleton was once recognized as a distinguishing feature of prokaryotes, it is now clear that a number of different bacterial proteins do form filaments *in vivo*. Despite the critical roles these proteins play in cell shape, genome segregation, and cell division, molecular mechanisms have remained obscure in part for lack of EM-resolution images where these filaments can be seen acting within their cellular context. Here, electron cryotomography was used to image the widely-studied model prokaryote *Caulobacter crescentus* in an intact, near-native state, producing three-dimensional reconstructions of these cells with unprecedented clarity and fidelity. We observed many instances of large filament bundles in various locations throughout the cell and at different stages of the cell cycle. As shown in the figure below, the bundles appear to fall into four major classes based on shape and location: a) 'inner curvature,' b) 'cytoplasmic,' c)'polar,' and d) 'ring-like.' In an attempt to identify at least some of the filaments, we imaged cells where crescentin and MreB filaments would not be present. The inner curvature and cytoplasmic bundles persisted, which together with their localization patterns, suggest that they are composed of as-yet unidentified cytoskeletal proteins. Thus bacterial filaments are frequently found as bundles, and their variety and abundance is greater than previously suspected.

# MORPHOLOGICAL CHANGES IN CYANOBACTERIUM *CYANOTHECE* 51135 DURING THE CIRCADIAN RHYTHM VISUALIZED BY ELECTRON TOMOGRAPHY

Presenting Author: ALICE DOHNALKOVA, Sr. Research Scientist

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Photosynthetic bacterium *Cyanothece* sp. undergoes dramatic morphological changes during circadian cycle related to respiration, carbon storage and nitrogen fixation. Electron tomography allowed us to investigate structural changes on a much finer scale than the traditional TEM imaging approach.

Cells were collected in 4-hour intervals during the 24-hour light and dark cycle, high pressure frozen, and freeze substituted. Tilt series of plastic sections were acquired, and tomographic slices were obtained from the reconstructed volume. Thylakoid membranes and other ultrastructural features showed improved resolution, and provided a better insight into cellular processes during the dynamic reactions during photosynthesis.

Imaging was done at FEI's Tecnai T-12, using Inspect3D software for reconstruction.

The capability of electron tomography and cryostage applications was recently established at Pacific Northwest National Laboratory in the Environmental Molecular Sciences Laboratory (EMSL), a national user facility. This effort is a part of DOE's multidisciplinary Grand Challenges in Membrane Biology project, which is focused on assembling a blueprint of reactions that occur in *Cyanothece* during the circadian cycle.

Authors would like to seek input on the single particle imaging, cryo electron tomography and post-reconstruction visualization techniques at the meeting, as well as the new technology in the tomography area.

## FOCUSED ION BEAM (FIB) SAMPLE PREPARATION FOR TOMOGRAPHY OF BIOLOGICAL SAMPLES

Presenting Author: DAMJANA DROBNE, Professor

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Electron tomography is an important tool for investigating cellular ultrastructure. It is applied on conventional plastic sections. With the resolution of 4-5 nm one can address structure of macromolecules complexes inside the cell. At millimeter scale, the 3D structure analyses of biological samples is based on 3D reconstruction of serial histological sections ([http://www.fgg.uni-lj.si/~sdrobne/DDrobne/Project\\_Toxicity/](http://www.fgg.uni-lj.si/~sdrobne/DDrobne/Project_Toxicity/)). In order to fulfill the gap in 3D observation at micrometers scale, FIB/scanning electron microscopy (SEM) was used for imaging and sample preparation. The FIB / SEM allows to study a features with dimensions from tens of nanometers to tens of microns. The FIB/SEM system is a combination of a focused ion beam, an electron beam and secondary ion and / or secondary electron detectors. The beam of gallium (Ga<sup>+</sup>) ions operated at low beam currents is used for imaging, and high beam currents are used for site specific in situ manipulation. The application of FIB/SEM system has advantages over classical microscopy techniques because of (1) in situ site-specific 2D-sectioning and imaging of subsurface microstructures; (2) no need/the absence of embed the sample prior to sectioning; (3) a wide range of magnifications while imaging the same sample. The FIB tomography technique is a powerful way of performing 3D reconstructions of samples in materials sciences. The FIB tomography of biological samples is almost unknown. Recently some successful applications of FIB milling and imaging of biological samples were presented (Drobne D, Milani M, Zrimec A, Leser V, Berden Zrimec M. 2005. Electron and ion imaging of gland cells using the FIB/SEM system. *J Microsc* 219:29-35). The aim of our work presented here was to assess the applicability of FIB system in biological sample preparation for tomography. The prime question was whether the sample surface sustain successive bombardment during FIB sectioning with energetic Ga<sup>+</sup> ions without observable damage. We studied digestive gland cells of a terrestrial isopod crustacean *Porcellio scaber* (Isopoda, Crustacea). Samples were prepared for conventional scanning electron microscopy. The FIB/SEM was used for in situ site-specific 2D sectioning (milling). The thickness of the milled layers was 50 nm. The time needed for a total removal of 750  $\mu\text{m}^3$  sample volume was 4 min. The results show that after successive milling operations no structural deformations of the exposed cell interior were observed. Successive FIB milling revealed a lamellar body and a lipid droplet being connected to each other. The FIB/SEM images of investigated structures are comparable to those we obtain by transmission electron microscopy (TEM). The limitation of FIB sample preparation for tomography is the resolution in the z axis that is 50 nm. We discuss the perspectives of FIB tomography as a new tool in life sciences for structural research at micrometer scale.

## IMAGE ANNOTATION FOR ELECTRON TOMOGRAPHIC DATA

Presenting Author: MARYANN E. MARTONE, Associate Adjunct Professor

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Segmentation tools for electron tomographic data have been available to researchers for a number of years, providing a feature for users to extract the structures of interest for their particular study. As an increasing number of objects are segmented, it becomes difficult to manage the data; all of the structures essentially become disjoint from one another. There is no way to record whether a segmented structure is located next to another structure, or whether a structure is part of a larger structure. In addition, there is no way to compare structures annotated by different researchers. To address this challenge, we have developed Ovoxtrace, a segmentation tool that allows microscopic image data to be annotated in a highly structured manner. The prototype for Ovoxtrace was developed as an extension of Xvoxtrace, a widely used tool developed at the National Center for Microscopy and Imaging Research (NCMIR) for segmenting EM tomograms. A web based version, Jinx, is currently under development. During segmentation in Ovoxtrace, as objects of interest are identified in each slice throughout a tomographic volume, rather than supplying their own object name identifiers, users select entities that have already been defined in the Ontology for the Subcellular Anatomy of the Nervous System (Fong et al., submitted). An ontology consists of a set of concepts linked by relationships such as 'is a' and 'has part,' e.g., 'neuron is a cell; cell has part plasma membrane.' It is an important means by which knowledge in a field is formalized for classifying and describing scientific observations. The Subcellular Anatomy Ontology (SAO) is an ontology for describing the subcellular anatomy of the nervous system. It includes neurons, glia, multicellular microdomains and their associated functional compartments, cellular components, and molecular constituents. Cell components are drawn from the Gene Ontology (<http://www.geneontology.org/>). The SAO includes general characteristics of neuronal and glial morphology and illustrates specialized views of the properties of individual neuronal classes, e.g., Purkinje cells and Pyramidal cells. During the segmentation process, users create instances of the structures contained in the tomographic volumes. A given instance can either be independent or defined as part of another entity, e.g., mitochondria\_0000 is part of dendrite\_0001, and additional relationships such as 'associated with' and 'synapses with' are also supported. . The system provides user feedback by showing users a complete description of their object relative to other existing elements within the SAO. We have also incorporated a feature to enable users to add new terms not currently present in the ontology during the segmentation process for immediate use in annotating their data. The output of Ovoxtrace includes a list of entities and their parts, along with other relationships. As objects are annotated and segmented based on the concepts and relationships in the SAO, they are stored as instances in OntoQuest, a knowledge base designed to capture structural and molecular properties of nerve cells, their parts, and supracellular domains (Chen et al., 2006). In addition to storing all of the annotated information, OntoQuest generates statistical and aggregate properties of cells, assembling information of associating compartments, components, and morphometrics from these instances. Many of the datasets contained in the CCDB have been annotated with the SAO and stored as instances in OntoQuest (Martone et al., 2003). Because the SAO is publicly available and is cross referenced to other public ontologies, data annotated with the SAO can be compared and aggregated with data accruing from other sites and at other scales. The advantage of Ovoxtrace is that annotation occurs concurrently with segmentation rather than after the fact. The image annotation function of Ovoxtrace thus becomes an important piece in streamlining the development and validation of novel hypotheses of neuroscience data based on these aggregate properties. We are implementing the ontology extensions with automatic segmentation routines to be offered through the web-based version, Jinx. In addition to the standard manual tracing tools available in Xvoxtrace, Jinx will implement fuzzy segmentation (Garduno and Wong, 2006) and level set segmentation (Sethian 1996, Whitaker et al) methods. In addition to the Nuages (Geiger, 1995) surface tiling method, we will include the Power Crust method (Amenta, Choi, Kolluri, 2001) method.

# ELECTRON CRYOTOMOGRAPHY OF THE MINIMAL EUKARYOTE, *OSTREOCOCCUS TAURI*

Presenting Author: LU GAN, Postdoc

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The picoplankton *Ostreococcus tauri* is the smallest known eukaryote. The 12.6 MB genome of *O. tauri* has just 5,441 nuclear genes, but includes a minimal-yet-complete set of cell-cycle control genes. This photoautotrophic unicellular organism, which measures 1 - 2  $\mu\text{m}$  in diameter, is also the simplest of eukaryotes: it has just one nucleus, one chloroplast, and one mitochondrion. These properties make *O. tauri*, to our knowledge, one of the few eukaryotes that can be imaged *in toto* by electron cryomicroscopy. Here, we present the first tomograms of whole, vitrified *O. tauri* cells, determined by electron cryotomography. We confirm earlier findings that each cell has one copy of each the organelles listed above, and we also find that each cell has a number of storage vesicles and a golgi body that is adjacent to the endoplasmic reticulum (ER). The remaining volume within the cytosol is packed with dense, ribosome-like particles. The chloroplasts, mitochondria, and storage vesicles have consistent shapes in most of the cells we have analyzed. By contrast, the nuclei are pleiomorphic. Most of the cells that we have examined have an 'open' nucleus, indicating that *O. tauri* undergo open mitosis, which is characterized by either partial or full nuclear-envelope breakdown. The nucleus is simultaneously adjacent to the ER, the chloroplast, the mitochondrion, and the plasma membrane - a consequence of the small cell-size. The lumen that separates the inner- and outer-nuclear envelope has uniform width when the outer envelope is in contact with another organelle, but variable width when it faces the cytosol. Our tomograms of *O. tauri* provide authentic 3-D views of eukaryotic ultrastructure.

## IMMUNOGOLD LABELING COMBINED WITH 3D ELECTRON TOMOGRAPHY

Presenting Author: W.J.C. GEERTS, P.I.

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The early biosynthetic pathway starts at the endoplasmic reticulum (ER) where cargo becomes synthesized and arranged. The cargo leaves the ER at specialized exit sites and is delivered to the Golgi. An outstanding characteristic of this area is the presence of COP I and II coated membranes, which are involved in cargo concentration and vesicle formation. On ultra-thin cryosections COP coats can be distinguished by using specific antibodies against the different coat-subunits and subsequent detection with protein-A-gold. However due to the restriction of conventional 2D-EM postulated membrane continuities and interconnections are difficult to verify and the question has been raised whether transport is mediated solely by vesicles or more complex pleiomorphic membranes. To address this issue we established a method to combine 3D-electron tomography with immunogold labeling techniques on thick cryosections (400 nm). On independent reconstructions of ER-exit sites in HepG2 cells labeled for COPII we traced membranes in three dimensions. The resulting modeled objects expose vesicular and tubular shaped COP II coated membranes loose from the ER. These data unequivocally show the existence of COP II vesicles and implicate a role in transport in addition to cargo recruitment. Similar vesicles were resolved in high-pressure frozen material, confirming their existence. We show the double labeling of COP II and the SNARE protein Sec 22 by employing silver enhancement and gold labeling and subsequent detection by Energy dispersive Xray analysis (EDX).

## QUANTUM DOTS FOR PROTEIN IDENTIFICATION IN LIGHT MICROSCOPY AND EM-TOMOGRAPHY

Presenting Author: BEN N. G. GIEPMANS, Research Biologist

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The importance of locating proteins as part of functional systems in their context within cells has been heightened recently by the accomplishment in molecular structure and systems biology. New methods are required to map the ultrastructural localizations of proteins in situ, particularly in spatially complex tissues such as the nervous system. Here we report the application of small nanocrystals (Quantum dots) to specifically and efficiently immunolabel multiple distinct endogenous proteins in cells and tissues simultaneously for light and electron microscopy analysis. Focus is on cytoskeletal elements and identification of Connexin43-based gap junctions in cerebellum. The pre-embedding immunolabeling procedure is straightforward and allows optimization at the light level, before continuing with electron microscopy preparation and analysis. We show correlated microscopy and double and triple labeling at the light and ultrastructural levels. Because QDs allow for labeling with several microns penetrations, as opposed to immunogold, they can be successfully used to label proteins in thick EM-sections for immuno-tomography as will be shown in cerebellar gap junctions. We conclude that Quantum dots may aid in higher-throughput analysis of protein-protein interactions in EM tomograms.

# THREE-DIMENSIONAL PATHWAYS OF TRANSPORT MATERNAL ANTIBODY ACROSS THE EPITHELIAL BARRIERS IN SUCKLING RAT SMALL INTESTINE REVEALED BY ELECTRON TOMOGRAPHY

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Before its own immune system is fully developed, the suckling rat is dependent upon the neonatal Fc receptor (FcRn) to transport maternal IgG from ingested milk into its blood circulation. FcRn also functions to protect IgG from lysosomal degradation. In both its transport and protection roles, FcRn binds to IgG at acidic pH (6.0) inside endosomes and releases IgG at the pH (7.4) of blood. Early conventional electron microscopy studies using IgG tracers provided direct evidence that the proximal small intestine of neonatal rat selectively transports antibody into the circulation. However, due to the large dimension of the tracers, like ferritin, which may bind to multiple antibodies, and the nonspecific conjugating sites may block the receptor binding sites, so it may not reveal the true pathways of IgG uptake in intestinal epithelial cells. In order to trace the precise three-dimensional (3D) pathways during dynamic endocytic processes and to examine possible configurations of FcRn-Fc complexes within different types of intracellular vesicles in the intact neonatal rat small intestine, we need technique that permits us to trace the full functional antibody conjugates in the full physiological state *in vivo* system with high resolution. We are using FcRn affinity column purified 1.4 nm nanogold clusters which are small chemically-defined particles covalently attached to the hinge region of rat Fc, as a more reliable tracer to explore functional endocytic processes. However, it is really a challenge work to visualize 1.4nm gold particles within the thin section of the tissue sample. Therefore, we are combining techniques, improved gold-enhancement method for chemical fixed samples, new developed freeze-substitution (FS) based silver-enhancement method for high pressure frozen samples, and electron tomography to visualize the gold particles and trace the precise pathways of antibodies in 3D. Our results clearly demonstrate that the proximal small intestine uptake the antibodies through FcRn protection mechanisms, most gold particles are located in the tubular vesicles and coated vesicles, and discharge the conjugates to the basolateral extracellular space, only a small portion of conjugates found in the lysosomes. While in the distal small intestine without FcRn receptor protection, all the gold particles are delivered to lysosomal degradation pathway. Hence, these techniques are confirmed very useful to trace the 3D transport pathways and analyze the dynamical events in the process of transcytosis.

## CRYOELECTRON TOMOGRAPHY STUDY OF FCRN COUPLED LIPOSOMES BOUND WITH FC FRAGMENTS

Presenting Author: YONGNING HE, Postdoc

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FcRn is a cell surface IgG receptor that binds the Fc portion of IgG under acidic pH at the apical side of intestine epithelial cells and transport IgG to the basolateral side of the cells. However, the mechanism of how FcRn escorts IgG through cells remains unclear. Here we coupled FcRn molecules on the surface of liposomes, and modeled the transcytotic vesicles by adding Fc fragments and IgG and to the liposomes. Cryoelectron tomography is then utilized to investigate the Fc bridged liposomes three-dimensionally, hence providing an inside-out version of the possible structures formed in the transcytotic vesicles containing FcRn-ligand complexes inside epithelial cells. The 1.4nm gold-labeled Fc fragments have also been used as probes to detect the distribution of Fc between the adjacent liposome membranes. The direct visualization of 1.4nm gold particles by cryoelectron tomography can provide a useful labeling tool for many biological systems.

## STRUCTURE OF MYCOPLASMA PNEUMONIAE'S ATTACHMENT ORGANELLE AND A MODEL FOR ITS ROLE IN MOTILITY

Presenting Author: GREGORY HENDERSON, Student

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While most motile bacteria propel themselves with flagella, other mechanisms have been described including retraction of surface-attached pili, secretion of polysaccharides, or movement of motors along surface protein tracks. These have been referred to collectively as forms of 'gliding' motility. Despite being simultaneously one of the smallest and simplest of all known cells, *Mycoplasma pneumoniae* builds a surprisingly large and complex cell extension known as the attachment organelle that enables it to glide. Here, three-dimensional images of the attachment organelle were produced with unprecedented clarity and authenticity using state-of-the-art electron cryotomography. The attachment organelle was seen to contain a multi-subunit, jointed, dynamic motor much larger than a flagellar basal body and comparable in complexity. A new model for its function is proposed wherein inchworm-like conformational changes of its electron-dense core are leveraged against a cytoplasmic anchor and transmitted to the surface through layered adhesion proteins.

## STRUCTURE AND POLYMORPHISM OF CLATHRIN-COATED VESICLES

Presenting Author: BERNARD HEYMANN,

J. Bernard Heymann<sup>1</sup>, Dennis C. Winkler<sup>1</sup>, Yang-In Yim<sup>2</sup>, Evan Eisenberg<sup>2</sup>, Lois E. Greene<sup>2</sup>, Alasdair C. Steven<sup>1</sup> <sup>1</sup>Laboratory of Structural Biology, NIAMS, and <sup>2</sup>Laboratory of Cell Biology, NHLBI, NIH, Bethesda, MD 20892

Several major trafficking pathways in eukaryotic cells employ the protein, clathrin, to deform membranes into coated pits and pinch them off into coated vesicles (CVs). During endocytosis, the formation and subsequent dissociation of CVs are key steps in the ingestion of nutrients, recycling of receptors and neurotransmitters, and invasion of parasites such as viruses. Clathrin is a highly versatile coating agent, being capable of assembling into coats of diverse size, shape, and regularity, depending on the cargo. The building-block of each coat, the clathrin triskelion, has three arms which interlock with other triskelions to form a network around the lipid bilayer. To better appreciate the interactions of clathrin lattices with enclosed vesicles and the distributions of regulatory and adaptor proteins on individual CVs, we did cryo-electron tomography of CVs isolated from bovine brain. Tilt series of images were collected of frozen CVs using the package, SerialEM [1], with a Tecnai T12 microscope with a LaB6 source at a nominal under-focus of 5  $\mu\text{m}$ . The images were processed with programs from the Bsoft package [2] and visualized with Chimera [3]. The CVs range in size from  $\sim 660$   $\text{\AA}$  to 1200  $\text{\AA}$ , with the larger particles ( $>800$   $\text{\AA}$ ) containing well-formed internal membrane vesicles of 230  $\text{\AA}$  to 570  $\text{\AA}$  in diameter, whose surface is separated from the clathrin legs by 160  $\text{\AA}$  to 340  $\text{\AA}$ . Simplified models were built into the clathrin coats of many CVs extracted from the tomograms, showing the large variation in the networks and the distribution of the various symmetric forms. The smallest form found is the tetrahedron, as well as one with D2 symmetry (the tennis ball) and the well-known D6 barrel. The larger particles with internal membranes have mostly C1 or C2 symmetry. The simplified models were used to extract and average the hubs and spars of the clathrin network, followed by reassembly to produce a representation of the full coat. Compared to spars extracted from a single particle reconstruction, the resolution was estimated as 51  $\text{\AA}$  (FSC 0.3 cutoff). This was then used to subtract the clathrin part of the CV to better identify the underlying adaptor proteins (APs). Several of the densities between the clathrin coat and the vesicle membrane could be identified as AP1 or AP2. The vesicle membrane was also modeled as a flexible sphere and rebuilt from the average to complete it across the parts affected by the missing wedge. This provides a clear picture of the proteins inside and outside the membrane. In some places the clathrin N-termini are close enough to the membrane to make direct contact, while in other places the contact is through APs. [1] Mastrorarde, D.N. (2005) *J. Struct. Biol.* 152, 36-51. [2] Heymann, J.B. and Belnap, D.M. (2006) *J. Struct. Biol.* (accepted). [3] Pettersen, E.F. et al. (2004) *J. Comput. Chem.* 25, 1605-1612.

## STRUCTURE OF CYSTOVIRUS PHI12 BY ELECTRON CRYO-TOMOGRAPHY

Presenting Author: GUOBIN HU, Postdoc

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The cystoviruses (phi6 to phi14) are a unique group of viruses that have proven very useful for elucidating general viral mechanisms. In particular, the replicative mechanism and the multishell structure of cystoviruses make them analogous to members of reoviridae. With only three double-stranded RNA genomic segments they all have a very similar genetic organization and express similar proteins. However, the amino acid sequences of comparable proteins from different species vary considerably and thus constitute a ready-made mutant library. They all infect strains of the plant pathogen *Pseudomonas phaseolicola* and are enveloped by a phospholipid membrane. The P3 complex is responsible for binding the host cells with receptors, either on the pili or in the rough lipopolysaccharide, depending on the particular species. In all species, the polymerase complex is composed of four proteins, P1, P2, P4, and P7 arranged in a dodecahedral assembly that packages and then replicates the viral RNA.

Here we present recent structural work on the phi12 species of cystoviruses. We used electron cryo-tomography and image processing to visualize the overall architecture of the intact virus particle. This particle was seen to have two discreet shells resulting from the nucleocapsid and the membranous envelope, respectively. The tomograms suggested connections between the nucleocapsid and the inner surface of the envelope. The tomograms indicated that the radius for the membrane envelope was ~35 nm with thickness of ~5 nm, and the radius for the nucleocapsid was ~25 nm with thickness of ~4 nm; the gap between nucleocapsid and the membrane envelope was ~5 nm. For the first time, the P3 attachment proteins, so called spikes, were revealed to form a ring like structure or 'donut' that protrudes from the outer surface of the membrane envelope. The donuts adopted a regular distance and orientation relative to the outer surface of the membrane envelope, and appeared to be connected to the envelope by a flexible low-density stem structure. Based on its volume, the molecular weight of the donut was estimated to be 650 kDa, thus implying a large oligomeric assembly.

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## ELECTRON CRYOTOMOGRAPHY OF SYNECHOCOCCUS STRAIN WH8102 CARBOXYSOMES

Presenting Author: CRISTINA IANCU, Postdoc

Cristina V. Iancu, California Institute of Technology H. Jane Ding, California Institute of Technology Dylan M. Morris, California Institute of Technology Arlene Gonzalez, Sandia National Laboratories Anthony Martino, Sandia National Laboratories Grant J. Jensen, California Institute of Technology

Carboxysomes are organelle-like polyhedral bodies enclosed by a proteinaceous shell and filled with ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) molecules, a key enzyme in CO<sub>2</sub> fixation. They are part of the CO<sub>2</sub> concentrating machinery (CCM) in cyanobacteria and many chemoautotrophic bacteria, and are produced in a greater number when the organisms are grown under low-CO<sub>2</sub> conditions. Exactly how carboxysomes help the organisms compete when CO<sub>2</sub> is limited is still unclear. Electron cryotomographic studies of purified carboxysomes from *Synechococcus* strain WH8102 reveal the 3D structure of these organelles. While the size of individual carboxysomes in this strain varies from 114 to 137 nm, surprisingly, all are approximately icosahedral. Average density profiles showed that RuBisCO molecules are organized in 3-4 shells spaced about 12 nm apart. Preliminary analysis of the relative distances between neighboring RuBisCO molecules suggests that, for the most part, they are equally spaced. Simulations of carboxysomes in which RuBisCO molecules are randomly placed revealed that, as the number of enzymes increased, shells spontaneously arise similarly to the ones in the data. Recent studies on the shell proteins showed that two of these proteins form charged pores that probably provide charged metabolites to the RuBisCOs, and another shell protein is a novel carbonic anhydrase supposed to concentrate CO<sub>2</sub> proximally to RuBisCOs. Thus it appears that the carboxysome outer shell acts both as a permeable membrane for substrates and as a template, organizing RuBisCO in specific patterns that optimize CO<sub>2</sub> fixation.

# STRUCTURAL ANALYSIS BY CRYO-ELECTRON MICROSCOPY OF HERPES SIMPLEX VIRUS 1 TRANSPORT INSIDE HOST CELLS

Presenting Author: IOSUNE IBIRICU

Iosune Ibiricu Kay Gruenewald Department of Molecular Structural Biology, Max-Planck-Institute of Biochemistry, Martinsried, Germany

After entry into host cells by fusion of the viral envelope with the plasma membrane, capsids of Herpes simplex virus 1 (HSV1) are transported along microtubules from the cell periphery to the nucleus by the minus end directed cellular motor protein dynein. The nature and identity of the protein(s) making the contact between the dynein complex and its viral cargo are still not fully known. To understand the processes involved we apply electron cryo-tomography allowing for direct three-dimensional visualization of capsid transport. Correlation between fluorescence microscopy and cryo-electron tomography helps us to determine time points of infection. Therefore the cells are infected with HSV-1 -GFP-VP26 viruses and then vitrified at different post-infection states characterized by highly active capsid transport. We will present preliminary data allowing first structural insights into this transport at the level of macromolecular complexes.

## DO ALL THREE-DIMENSIONAL ACTIN BUNDLES LOOK ALIKE?

Presenting Author: MANDY JANSSEN, Student

Mandy Janssen\*, Eldar Kim\*, Paul Matsudaira+, Steve Almo#, Niels Volkmann\* and Dorit Hanein\* \*The Burnham Institute for Medical Research +Massachusetts Institute of Technology #Albert Einstein College of Medicine

Actin bundles have profound effects on cellular shape, division, adhesion, motility, and signaling. Fimbrin belongs to a large family of actin-bundling proteins and is involved in the formation of tightly ordered cross-linked bundles in the brush border microvilli and in the stereocilia of inner ear hair cells. Fimbrin bundling activity is determined by its directly linked tandem repeat of actin-binding domains. Fascin, similar to fimbrin, forms rigid actin bundles in structures such as microspikes, filopodia and stress fibers. Fascin's actin-binding domains are also expressed in a directly linked tandem. We used electron tomography, image reconstruction, and segmentation protocols to isolate individual fimbrin and fascin crosslinkers and to determine their structures at approximately 3-nm resolution. Using the available X-ray structures of the crosslink components for statistics-based density docking, we characterized the respective actin-binding interfaces and the corresponding counterparts in the actin filaments at the residue level.

## A NEW DIRECT DETECTION DETECTOR FOR ELECTRON MICROSCOPY

Presenting Author: LIANG JIN, Student

Liang Jin, Mark Ellisman, Philippe Leblanc, Fred Duttweiler, James C. Bouwer, Steven T. Peltier, Anna-Clare Milazzo, Nguyen-Huu Xuong University of California, San Diego Stuart Kleinfelder, Shengdong Li University of California, Irvine

In a longstanding effort to overcome limits of the current charge coupled devices (CCDs) systems in electron microscopy, we have developed a radiation-tolerant system that can withstand direct electron bombardment. A prototype system based on an Active Pixel Sensor (APS) has already delivered unprecedented performance-in many areas exceeding the fundamental capabilities of CCD based systems. This implementation features a 512x550 pixel format of 5 $\mu$ m pitch with an excellent signal-to-noise ratio (approximately 13/1 for a single incident electron in the range of 200-400 keV) and a very high spatial resolution (2-4  $\mu$ m). Radiation tolerance with high-energy exposure is also impressive, especially with cooling to -15 $^{\circ}$  C. Stable performance has been demonstrated over a period of several months of continuous use before a thermal annealing process is required to restore the device. The characteristics of this new detector have exciting implications for transmission electron microscopy, especially for cryo-EM as applied to biomolecular structures.

# CRYO ELECTRON TOMOGRAPHIC VISUALIZATION OF MICROTUBULE PLUS ENDS IN VITRO AND IN SITU.

Presenting Author: ROMAN I. KONING, Postdoc

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Microtubules (MTs) are cytoskeletal biopolymers consisting of  $\alpha\beta$ -tubulin heterodimers that are structured like hollow tubes. MTs are dynamic instable structures that constantly grow and shrink. This dynamic instability is important for MT functions in eukaryotic cells. While MTs function as a scaffold, MT associated proteins (MAPs) mediate their functions inside the cell. One special type of MAP are +TIPs (plus end TrackIng Proteins), proteins that specifically bind growing plus ends of MTs and therefore can functionalize MT plus end growth. These +TIPs can bind to other proteins (e.g. CLASPs), forming functional complexes that are involved in various cellular process like e.g. cargo transport, cell motility and cell division. Furthermore they influence MT dynamics, possibly by influencing the structure of the MT plus end.

Our goal is to visualize CLIP-170 and CLIP-115, two +TIPs that have shown to be preferentially located at the plus end of growing MTs<sup>2,3</sup>. Furthermore we want to investigate to study their influence on the structure of the MT plus ends. MT plus end conformations in situ were already visualized using electron microscopy and tomography on cryo-fixed, freeze-substituted, plastic embedded 3T3 mouse fibroblasts (See poster S. Zovko et al.). We now also have imaged MT plus ends in vivo and in situ using cryo electron tomography. For in situ localization and structure determination we use mouse embryonic fibroblasts (MEF) cells, wild-type and double knock out for CLIP-115 and CLIP-170, which are grown on electron microscopy grids and vitrified in liquid ethane. In addition we also perform cryo electron tomography on in vitro grown MTs with and without bound CLIPs.

Preliminary data using wild type MEFs showed that we were able to visualize MT plus ends in situ. In these cells we also could distinguish several types of plus end conformations. Furthermore, near the MT plus ends we observed densities that probably are proteins or protein complexes. Future work will focus on improving the number and quality of datasets and on visualization of MTs in MEF cells that are double knock out for CLIP-115 and CLIP-170.

<sup>1</sup> We would like to acknowledge Daniel de Geus en Jan Pieter Abrahams (Biophysical Structural Chemistry, Leiden University), who are participating in the - Molecule to Cell-program

## VISUALIZING THE SPATIAL AND TEMPORAL RELATIONSHIP OF VIRUS ENTRY AND ASSEMBLY IN CELLS

Presenting Author: JASON LANMAN, Postdoc

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Virus entry and assembly occur in complex cellular environments and are dependent upon both viral components and cellular components being in the correct position at the proper time. To develop a better understanding of the spatial and temporal organization of non-enveloped virus entry and assembly, fluorescent microscopy correlated with electron microscopy (EM) and EM tomography were used to visualize FHV infections in *Drosophila* cells.

# VISUALIZING THE CELL: ANNOTATION OF STAINED AND RESIN-EMBEDDED TOMOGRAPHIC RECONSTRUCTIONS BY TEMPLATE MATCHING

Presenting Author: MISJAEL N. LEBBINK, Student

Misjael N. Lebbink [1] Theo P. van der Krift [1] Maurice Bouwhuis [2] Bart Heupert [2] Willie J.C. Geerts [1] Bob L.O. Herzberger [3] Arie J. Verkleij [1] Abraham J. Koster [4] [1] Cellular Architecture & Dynamics, Utrecht University. [2] SARA Computing and Networking Services, Amsterdam. [3] Department of Computer Science, University of Amsterdam. [4] Molecular Cell Biology - Electron Microscopy, Leiden University Medical Center

In recent years electron tomography (ET) improved three-dimensional (3D) insight in the architecture of cells and organelles. However, the amount of information in a tomogram can be overwhelming. Therefore modeling steps are performed. Often, this is done manually by computer aided tracing of membranous structures. The contours are combined into a 3D model of the structure of interest. While shown to be very useful to the field, there are limitations caused by the need to make decisions based upon interpretation of the data. Recent developments in computer guided analysis combined with an increase in computational power allow for the possibility of using template matching (developed by Frangakis et al, 2002) as an additional tool for analyzing TEM-tomographic data-sets. At the moment we are evaluating the template matching approach to locate structures in thick sections. First results are obtained and presented.

An endosomal structure with clearly visible clathrin coats present on the membrane (Murk et al, 2003) was used as a model system. We made multiple cuboid-shaped templates of increased thicknesses based on the membrane thickness observed in the tomogram contained in a cube of 323 pixels (figure A). These were matched over  $180^\circ$  in  $\varphi$ ; ( $\phi$ ) and  $\theta$  ( $\theta$ ) with an increment of  $5^\circ$  (no rotation was performed over  $\psi$  ( $\psi$ ) because of the shape of the cuboid template). The cross-correlation values of the different matchings were combined and visualized in Amira. First results show that it is possible to localize coated patches on the endosomal membrane by using two distinct templates. Furthermore, by increasing the number of different templates, both the clathrin coats, and regions within uncoated membrane could be observed (figure B).

We conclude that template matching is capable of annotating structures within a stained biological background with high enough accuracy to be used as a valuable addition to classical visualization techniques.

References: Frangakis A.S. et al. (2002) PNAS 99:14153 Murk J.L.A.N. et al. (2003) PNAS 100:13332

## IN VIVO FTSZ CYTOSKELETON IN CAULOBACTER CRESCENTUS AND ITS DYNAMICS DURING CELL DIVISION

Presenting Author: ZHUO LI, Postdoc

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In bacteria, FtsZ forms a ring-like structure at midcell in the initial phase of cell division. The FtsZ ring (Z-ring) acts as a scaffold for other cell division proteins that function sequentially when the cell divides. It is thought that the Z-ring itself provides the driving force for cell membrane constriction. X-ray and electron crystallography have revealed that FtsZ and tubulin are structural homologs, and electron microscopy has shown that FtsZ polymerizes in vitro into protofilaments, mini-rings, bundles and even 2-D sheets. The Z-ring has not yet been visualized in vivo, however, beyond the light microscopic resolution level. Here, we imaged the Z-ring of *Caulobacter crescentus* in an unperturbed, close-to-native state by electron cryotomography. During cell division, the number and localization pattern of Z-rings are highly dynamic. We also imaged a mutant strain where FtsZ hydrolysis was blocked, producing elongated constrictions between dividing cells. Many tens of filaments were visible in this case, allowing us to characterize more completely the interactions between filaments in a cellular context.

# CYBERINFRASTRUCTURE FOR PARALLEL TOMOGRAPHIC RECONSTRUCTION AND ANALYSIS

Presenting Author: ABEL W. LIN, Programmer Analyst

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Electron tomography is a powerful tool for deriving three-dimensional (3D) structural information about biological systems within the spatial scale spanning 1 nm<sup>3</sup> and 10 mm<sup>3</sup>. Exponentially growing data sizes have necessitated the need to integrate high-performance computing (HPC) and Grid technologies with traditional electron tomography processes and algorithms. Also growing is the number of novel mathematical algorithms that aim to improve data fidelity. As a result there is an immense need to accelerate the rate at which new algorithms can be integrated with HPC and Grid technologies. Traditionally, however, the task of integrating these algorithms with HPC/Grid technologies is considered a non-trivial endeavor, often taking several months. Here we describe the architecture of the Telescience Project (<http://telescience.ucsd.edu>), specifically the use of complementary ATOMIC (Telescience Applications to Middleware Interaction Components) and workflow technologies to vastly reducing the time-to-solution for introducing new algorithms to HPC/Grid technologies from months to days and weeks.

# HUMAN PLATELETS: A MODEL FOR EUKARYOTIC ULTRASTRUCTURE ANALYSIS

Presenting Author: MIKE MARSH (1), Student

Junmei Chen (2), Juan Chang (1), Jose Lopez (2), Wah Chiu (1) (1) Program in Structural and Computational Biology and Molecular Biophysics. Verna and Marrs McLean Department of Biochemistry and Molecular Biology. Baylor College of Medicine, Houston TX. (2) Puget Sound Blood Center, Seattle WA.

Platelets are circulating mammalian cells that play a major role in primary hemostasis. Platelet deficiencies can lead to an array of bleeding disorders, while platelet hyperactivity can significantly increase risk for embolic events such as heart attack and stroke. Platelets naturally lack nuclei, rendering them much flatter than typical eukaryotic cells and therefore electron-transparent to even low-voltage electrons. Yet because they carry mitochondria, lysosomes, microtubules, an actin cytoskeleton and other eukaryotic features, they are an excellent target for structural analysis by electron cryotomography. We have begun imaging and reconstructing tomograms of frozen-hydrated human platelets under a variety of conditions which illustrate their activation pathway. These tomograms show significant cellular morphological rearrangements associated with platelet activation and point to the cytoskeletal components responsible for those rearrangements. Furthermore, the tomograms show major eukaryotic organelles in their native 3D cytoplasmic milieu for the first time. Platelets in citrated plasma isolated from healthy human subjects were allowed to settle onto a holey carbon grid and frozen immediately in liquid ethane or were allowed to settle for different time periods on a grid coated with fibrillar type 1 collagen before being frozen. Using Mr T cryotomography package, tilt-series were recorded at 200 keV under low dose conditions on a Gatan 4K CCD camera in a JEM2010F electron microscope. Additional tilt-series were collected at 300 keV also on a Gatan 4K CCD camera in a JEM3200FSC electron microscope at liquid N<sub>2</sub> temperature with an in-column energy filter. Images were aligned and reconstructed with IMOD, yielding a 3D density map with an 4.0 gigavoxel (32 GB) reconstruction. Subvolumes were interactively selected and cropped with VolRover. Final rendering and visualization was performed in Amira and Chimera.

The reconstructions were annotated in terms of the sizes, shapes and relative locations of the subcellular components, including mitochondria,  $\alpha$  and dense granules, dense clusters, the dense tubular system, microtubules, and the surface-connected canalicular system. We observed great heterogeneity in  $\alpha$  granules and in the filopodia adhering to the grid surface. The tomograms resolve the fibrous actin bundles driving filopod extension and, in some cases, the individual actin cross-links within the bundles. In some platelets, we observed membrane tethers that attached to the surface but were devoid of actin. This represents the first 3D visualization of hydrated human platelets under native conditions. These observations suggest that electron cryotomography can become a useful tool for dissecting the molecular sequence of cytoskeletal rearrangements occurring during platelet activation and granule secretion and for understanding the structural basis of platelet diseases.

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## ELECTRON TOMOGRAPHY OF THE MAMMALIAN KINETOCHORE

Presenting Author: BRUCE F. MCEWEN, P.I.

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A critical and long-standing question is how the dynamics of individual kMTs within the K-fiber are coordinated. We have addressed this question by using electron tomography to determine the polymerization/depolymerization status of individual kMTs in the K-fibers of PtK1 and *Drosophila* S2 cells. Surprisingly, we find that the plus-ends of two-thirds of kMTs are in a depolymerizing state, even when the K-fiber exhibits net tubulin incorporation at the plus end. Furthermore, almost all individual K-fibers examined had a mixture of kMTs in the polymerizing and depolymerizing states. Therefore, although K-fibers elongate and shrink as a unit, the dynamics of individual kMTs within a K-fiber are not coordinated at any given moment. Our results suggest a novel control mechanism through which attachment to the kinetochore outer plate prevents shrinkage of kMTs. We discuss the ramifications of this new model on the regulation of chromosome movement and the stability of K-fibers.

## RECENT PROGRESS IN UNCONVENTIONAL ELECTRON TOMOGRAPHY

Presenting Author: PAUL MIDGLEY, P.I.

Paul Midgley, Ana Hungria, Jon Barnard, Jo Sharp, Ilke Arslan\*, Jenna Tong, Mhairi Gass and Alison Twitchett Department of Materials Science and Metallurgy, University of Cambridge, Pembroke Street, Cambridge, CB2 3QZ, UK. \* now at: Sandia National Laboratories, 7011 East Avenue, Livermore, CA 94550, USA.

Electron tomography has become established as an important and powerful tool in materials science and in particular for the study of 3D nanomaterials and devices. In many cases, particularly for crystalline specimens, bright field (BF) imaging, so often the norm in the life sciences, does not satisfy the projection requirement and cannot be used for tomography. In materials science, the use of scanning transmission electron microscopy (STEM) high-angle annular dark field (HAADF) images for electron tomographic reconstructions is becoming the conventional mode. Recently, however, we have also developed complementary, somewhat more unconventional, approaches to electron tomography. Here we report on some of these: (i) STEM tomography of intra-cellular inorganic particles. We will show two examples of this, firstly the reconstruction of magnetite crystals within magneto-tactic bacteria in which the faceting of the cubic magnetite is revealed, and secondly the distribution of ferritin within a liver cell of a haemochromatosis patient and the remarkable 3D array of the iron-rich cores. (ii) 3D chemical mapping using low-loss electrons and volume-spectroscopy. The low loss region of the EELS spectrum (e.g. volume plasmons) can be used to retrieve chemical information. We will show one example, of a composite structure of multi-walled carbon nanotubes (MWNTs) in a nylon matrix, where conventional 3D energy-filtered TEM (EFTEM) is not appropriate, because both components are carbon-based, and a more unconventional approach has had to be used. By recording an energy series of EFTEM images at every tilt, it is possible to reconstruct a 3D volume at every energy increment and therefore interrogate the energy loss spectrum at every voxel, thus enabling a form of 'volume-spectroscopy'. (iii) Tomographic holography. In the absence of magnetic fields and strong diffraction, the phase change recorded by an electron hologram of a semiconducting p-n junction is proportional to the built-in potential of the junction and the projected thickness of the specimen. As such, the holographic phase signal can be used for tomographic reconstructions. We will show an example of the 3D reconstruction of a biased and unbiased silicon p-n junction. The strong influence of the electronic properties of the surface is clearly revealed and in particular the surface depletion that forms when the sample has been prepared using a focused ion beam (FIB) instrument. (iv) Weak-beam dark-field (WBDF) tomography of dislocations. We will show, using weak-beam dark field (WBDF) imaging and keeping the diffraction conditions constant throughout a tilt series, that it is possible to reconstruct the 3D network of dislocations with high spatial resolution. We reveal how threading dislocations in a GaN epilayer turn over and become in-plane dislocations, how threading dislocations populate sub-grain domains and reveal the presence of dislocation bundles emanating from a crack.

# AUTOMATIC PROJECTIVE MODEL ESTIMATION AND RECONSTRUCTION IN CRYOGENIC ELECTRON TOMOGRAPHY

Presenting Author: FARSHID MOUSSAVI, Student

Farshid Moussavi - Stanford University Fernando Amat - Stanford University Dr. Mark Horowitz - Stanford University Dr. Luis R. Comolli- Lawrence Berkeley Laboratory

In recent years there has been increasing interest in using cryo TEM tomography to study cells in close to their 'native' environment. One limitation of this technique is the relatively low signal to noise ratio in each of the TEM images, since the total electron dose through the sample must be constrained to limit structure damage to the cell. Even with gold markers added to the sample, robust automatic alignment of the TEM slice data for reconstruction remains difficult. We have tried to address this problem by leveraging recent work in probabilistic analysis, and have constructed a prototype alignment system using Markov random fields (MRF's) for alignment, and robust optimization methods for projective model estimation. With markers, there are three basic steps required to align the TEM dataset: marker feature identification, correspondence and tracking of these features throughout the image set, and projective model estimation from these feature tracks. In our framework, features are extracted initially using standard template matching techniques like cross correlation. Feature correspondence and tracking is accomplished by constructing a Markov random field (MRF) probabilistic model where contour labels are random variables which take on values of candidate marker feature locations. We use mutual information and the relative geometric positions to estimate a priori marker correspondence probabilities between two images. An approximate probabilistic inference technique called loopy belief propagation (LBP) is then used to calculate the maximum a posteriori assignment of features to contours in the image set. In this technique, rather than a joint distribution (whose complexity is exponential in the number of random variables), a collection of singleton and pairwise distributions is maintained in a special data structure. This data structure contains cycles, and is called a cluster graph. The a priori estimates for these distributions (initial beliefs) are refined by belief propagation, until they converge to roughly the true pairwise distributions (final beliefs). The correspondences of candidate markers to contours are taken directly from these beliefs. Errors in the correspondence are possible due to feature location mistakes as well as inaccurate inference results. Therefore, the projective model estimation uses a robust fitting method as opposed to least squares (the traditionally applied fitting) and is tolerant to outliers. Once we have an estimate of the projective model, the model is iterated using expectation maximization (EM) to re-estimate perceived outliers with improved reprojection data from the current model. This iteration is performed as many times as necessary before a stopping criterion is satisfied, but in our example a small number of iterations is needed (often only one). This robust framework has allowed us to fully automatically recover dozens of contours (both complete and piecewise) with subpixel accuracy from several challenging cryo datasets of bacteria *Caulobacter crescentus*. The results were used to create 3D reconstructions comparable to results previously obtainable only by extensive manual intervention.

## IN SITU STRUCTURE OF THE COMPLETE TREPONEMA PRIMITIA FLAGELLAR MOTOR

Presenting Author: GAVIN E. MURPHY, Student

Jared R. Leadbetter, Asst. Prof. of Environmental Microbiology, Caltech; Grant J. Jensen, Asst. Prof. of Biology, Caltech

The bacterial flagellar motor is an amazing nanomachine: built from more than twenty different proteins, it uses an electrochemical ion gradient to drive rotation at speeds of up to 300 Hz in some organisms. The flagellar motor consists of a fixed, membrane-embedded, torque-generating stator; a spinning rotor and a cytoplasmic C-ring, which controls the direction of rotation in response to chemotactic signals. While previous work has characterized the structure of those components that purify with the rotor, little is known about the stator and its interactions. Using electron cryotomography of whole cells, we show the 3-D, in situ structure of the complete flagellar motor from the spirochete *Treponema primitia* at 7 nm resolution. The structure of the elusive stators is present, and their natural interactions with other components and the membrane are preserved. Twenty individual motor particles were computationally extracted from the 3-D reconstructions, aligned, and then averaged. The stator assembly was observed to have 16-fold symmetry and was connected directly to the rotor in two places through finger-like densities. Connections between the stators and C-ring were also visible, but not directly between the C-ring and rotor. A novel feature called the P-collar rests upon the stators and functions in place of the typical peptidoglycan P-ring. The unusually large size of the motor suggests mechanisms for increasing torque and supports models wherein critical interactions occur atop the C-ring. These results highlight how electron cryotomography can make possible both the visualization of individual, complete, large protein complexes in their native cellular context and their averaging in 3-D for improved clarity.

## THREE-DIMENSIONAL STRUCTURES OF ADHESIVE JUNCTIONS REVEALED BY ELECTRON TOMOGRAPHY.

Presenting Author: ANDREA S. NANS, Student

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Adhesive junctions participate in a broad range of functions that include cell-cell recognition, tissue formation and integrity, homeostasis, and embryogenesis. Cell-cell adherence is maintained by linking transmembrane proteins on adjacent cells, resulting in the fusion of adjoining membranes. While there are numerous studies on the biochemical and structural aspects of isolated components, little is known about the molecular organization of adhesive junctions in a cellular context. To shed light on this organization, we are investigating the structural arrangement of adhesive junctions in mouse cardiac muscle and mouse nerve tissue using electron tomography. Intercalated discs within cardiomyocytes mechanically stabilize the sarcolemmas of adjacent cells, allowing the cells to remain anchored together despite the contractions of the heart. The intercalated discs are composed of fascia adherens junctions, which contain the transmembrane adhesion molecule N-cadherin. The intracellular tails of N-cadherin are anchored to actin filaments through linker proteins such as plakoglobin,  $\beta$ -catenin, and  $\alpha$ -actinin. Interestingly, defects in any single component cause discontinuities in the network of cell-cell adhesion, resulting in cardiomyopathies. We have obtained a preliminary tomographic map and surface-rendered depiction of a fascia adherens junction. While these results provide a first look into the molecular arrangement of the intercellular material, we expect better tissue preservation will yield more detailed tomographic maps to resolve individual cadherin molecules. The second tissue of interest is specifically the corpus callosum from mouse brain. The corpus callosum is composed of bundles of myelinated nerve fibers, with myelin serving as an insulator to axons. The tight association of myelin and axons is maintained at the paranode by a critical series of cell-adhesion junctions termed "septate-like junctions." It is currently theorized that septate-like junctions are comprised of three cell-adhesion molecules that form a tripartite complex. The presence of septate-like junctions is crucial, as mice lacking these junctions have a slowed nerve impulse conduction rate and suffer from severe neurological deficits. Preliminary tomographic maps and surface-rendered depictions are providing insight into the spatial organization of the septate-like junctions. Again, we anticipate obtaining more detailed maps after improving the tissue preservation. Currently, these studies utilize tissues prepared using conventional methods in electron microscopy such as room temperature chemical fixation and dehydration; we will obtain better tissue preservation by using high-pressure freezing and freeze substitution methods. Together, these preliminary tomographic studies provide a first look into the spatial and molecular organization of the fascia and septate-like adhesive junctions.

# ELECTRON TOMOGRAPHY REVEALS NOVEL FEATURES OF THE MITOCHONDRIAL ASSOCIATED ADHERENS COMPLEX

Presenting Author: GUY PERKINS, Faculty

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We know little about the functional significance of substructural components of mitochondria such as crista junctions. The 3-D structure of these small components can be described using electron microscopic tomography (EMT). In this work we determined that there are systematic variations of mitochondrial substructure within mitochondria found tethered to presynaptic membrane near active zones in the calyx of Held. This nerve terminal follows the afferent spike train, typically at rates of hundreds of Hz, and transmits this activity to its postsynaptic target with excellent fidelity to preserve temporal structure of the incident sound wave. It achieves this result in part because a single nerve terminal (in cats, our experimental animal) can contain over 2000 active zones. Within the calyx of Held resides a unique organelle complex named the mitochondria-associated adherens complex (MAC). The MAC contains a mitochondrion with cristae oriented perpendicular to the cell membrane. There is a flat plaque close to the mitochondrion that is tethered by filaments to a punctum adherens. The MAC mitochondrion is consistently anchored 210 nm from the punctum adherens. Interspersed within the filaments are membranous elements called the vesicular chain that appear vesicular or tubular using conventional, low-voltage transmission electron microscopy. We have observed the close proximity of MACs and synapses and have therefore proposed roles for MACs in synaptic vesicle recycling (Rowland et al., 2000). With the added power of electron tomography, we have made a number of novel observations concerning MAC structures. First, in the mitochondrion itself: The crista junctions exhibit a polarity that has never been noted in mitochondria from any cells or tissues. The MAC mitochondrion has a flat edge that faces the presynaptic membrane. There are significantly more junctions along this flat edge than anywhere else along the periphery of the mitochondrion. Moreover, there are more crista junctions in MAC mitochondria than in the surrounding non-MAC mitochondria. Even so, the size of the crista junction opening was no different from non-MAC mitochondria. Could the propensity of junctions facing the synapse facilitate the transfer of glutamate generated inside mitochondria for use by the glutamatergic calyx? The cristae in MAC mitochondria occupy a large volume and have about twice the membrane surface area compared to neighboring mitochondria. They are lamellar rather than tubular. This points to a high rate of ATP synthesis. Second, in the mitochondrial plaque: The surface area of this plaque is smaller than the punctum adherens. There are fibrous linkages from the plaque to the mitochondrion. Third, in the vesicular chain: This chain appears to be continuous and tubular in nature. We have not found evidence for a role in vesicle recycling. Fourth, the filamentous meshwork: The meshwork consists of threads of varying thickness and length. Yet, this meshwork appears to hold the distance from the punctum adherens to mitochondrial plaque constant. Fifth, the synaptic vesicles: They are linked to one another via rod-like structures. They are found linked to the side of MAC mitochondria via the same rod-like linkages, but not to the nearby non-MAC mitochondria. It is tempting to speculate that local variation in mitochondrial substructure may relate to special requirements of the subcellular domain. The physiology of this region may require greater output of ATP, more glutamate and augmented capabilities for calcium sequestration and release as the associated synapse is at the extreme end of physiological variation, being very frequently activated for comparatively long time periods. A notion central to this work is that one may be able to derive new information about the functional role of mesoscale substructural features of organelles like mitochondrial crista junctions from assessment of the location-specific variations in their structure. Windows into the complex relationships of this sort are being enabled by intermediate voltage EMT.

# DETERMINATION OF RESOLUTION IN TOMOGRAPHY BY SPECTRAL COHERENCE OF THE PROJECTIONS.

Presenting Author: CHRISTIAN RENKEN, Postdoc

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Determining resolution in electron tomography has been problematic. The original estimation formula of  $r = \frac{\pi D}{N}$  by Crowther et al. 1975, reflected a purely cylindrical geometry and was modified to  $r = \frac{\pi}{N \cos(\theta_\alpha)}$  by Radermacher, 1992 to reflect the infinite slab geometry of electron microscopy data. Both these measures of resolution effectively describe the portion of Fourier space that in which each pixel is sampled at least once. They do not take into account the accuracy of the sampled points and thus are only ever theoretical, not actual estimations of resolution. Spectral signal to noise ratios as first introduced by Unser et al. 1989, and extended to 3D by Unser et al. 1996, Penczeck 2002, and Unser et al. 2005, calculate resolution based on the quality and nature of the data and work very well for single particle reconstruction data. However these methods only work in cases where a pixel in Fourier space has been sampled more than once, which is true for less than 2% of Fourier pixels in a typical electron tomogram. Cardone et al. 2005 proposed a noise-compensated leave-one-out Fourier ring correlation between input projections and the resultant re-projections that not only accounts for the input data but also for the fidelity of the reconstruction and is applicable to electron tomography data. For tomography data the NLOO method can give resolutions beyond that of completely sampled Fourier space if sampled pixels vary minimally. This extended resolution agrees better with observation in many tomograms where Crowther et al. and Radermacher's estimates seem overly conservative.

The drawback to the method of Cardone et al. 2005 is that it is compute intensive. We propose an alternative estimation of resolution based solely on Fourier ring correlation of adjacent projections, which results in very similar estimations of resolution without the need to compute separate reconstructions for each projection. Our method computes the Fourier ring correlation between each projection and the two adjacent projections to determine the spectral coherence of the data. This spectral coherence differs from the NLOO-2D at low frequencies where the number of images that contributed to the left-out projection is high but approaches the NLOO-2D FRC at higher frequencies where the contribution to the left-out projection is almost entirely from the adjacent projections.

While the spectral coherence method is less compute intensive, it does not consider the reconstruction algorithm used and thus does not account for any noise reduction within the reconstruction. For this reason it's estimates of resolution are more conservative than the NLOO-2D method. However as the quality of the data is the main criterion to resolution the difference between the two is generally minute.

## INVESTIGATION OF MOTOR SKILL LEARNING CIRCUIT WITH SYNAPSE TOMOGRAPHY

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Motor skill learning increases the number of parallel fiber to Purkinje cell synapses in the adult rat cerebellum. Interestingly, the number of multiple synaptic varicosities (MSVs), formed between a single parallel fiber varicosity and two Purkinje cell spines, was markedly increased following motor skill learning. However, whether two spines on MSVs induced by motor learning originate from the same dendrite or different ones has not been determined. Here we investigated this issue using serial section electron microscopy. After 26 days of motor skill training, rats were perfused and the cerebella were embedded in Epon-Araldite mixture. The serial 70-nm sections or 250-nm sections were mounted on formvar-coated slot grids. MSVs were scanned under the transmission electron microscopy (Hitachi H-7500) or high voltage electron microscopy (JEM-ARM 1300S) and serial images were acquired on the sections adjacent to the MSVs. The serial images were aligned, traced, and reconstructed with Reconstruct software, which is written by John C. Fiala and available at <http://synapses.bu.edu/tools>. The reconstruction of MSVs clearly showed the three-dimensional configuration of the learning-induced MSVs. The classification of the MSVs into same or different dendrite MSVs demonstrated that majority (approximately 97 %) of motor learning-induced MSVs make contacts with two spines originating from the same dendrite, indicating strengthening of local synaptic efficacy. We conclude that motor learning, at least in part, enhances existing synaptic circuits rather than forming new ones, and propose that an increase in same dendrite MSVs following motor skill learning plays a crucial role in the formation of motor memory via the change of synaptic efficacy.

# INTERPHASE MICROTUBULES ARRAYS IN A *S. POMBE* STRAIN LACKING ASE1P BUNDLING FACTOR STUDIED BY ELECTRON TOMOGRAPHY

Presenting Author: HELIO D. ROQUE, Student

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Ase1p (Anaphase spindle elongation) function in *S. pombe* is to organize microtubules (MTs) into overlapping antiparallel bundles in interphase (1). Using Electron Tomography (ET) and IMOD modeling software package (2, 3) we investigated the organization of the interphase MTs arrays in Ase1p deletion strain. Our analysis showed that MT bundles are mainly disorganized losing the parallel orientation to the cell main axis. The SPB (Spindle Pole Body) bundles present less MTs than in wt. Most iMTOCs (interphasic Microtubule Organizing Center) bundles present only one MT that in some cases displays an orientation perpendicular to the cell growth axis, which is not observed in wt cells. Nonetheless some overlap alignment of MTs is observed in both antiparallel and parallel orientations. In these regions, we also performed NDA (Neighboring Density Analysis) to assess the spacing of the MTs. We found that the preferred minimal distance between MTs is smaller than in wt. Our results also show that a few anchoring points between MTs are possibly remaining, which suggests that other bundling proteins may also have a role in the organization of the interphase MT arrays.

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THE CELL CENTERED DATABASE - DEVELOPING SMART TOOLS TO IMPROVE  
ACCESS TO AND INTEGRATION AND ORGANIZATION OF A LIGHT AND ELECTRON  
MICROSCOPY DATA MANAGEMENT SYSTEM

Presenting Author: JOY SARGIS, Director of Knowledge Engineering

Joy Sargis, NCMIR, UCSD Maryann Martone, NCMIR, UCSD

The Cell Centered Database (CCDB) is a microscopy imaging database management system using a Portal environment to track projects involving multi-scale 3D structural and protein localization imaging data with multiple methods. The system includes a web-accessible database for 2D, 3D and 4D imaging data from light and electron microscopy. The system is designed to address various issues involved in the creation of on-line community databases for electron microscopy data and includes multiple applications for depositing and withdrawing images and associated complex metadata to facilitate data sharing among groups located in disparate locations. Microscopic images at the light and electron microscopic levels are accessible via a user friendly interface on a web site. The database allows the user to capture descriptive imaging data pertaining to the important details of the experiment, the type of specimen processing performed, and instrument parameters selected in the process of creating three dimensional reconstructions. The system is flexible enough to be modified to track specific experiments, subjects and effects observed by participating teams. Analysis tools created for this project can search, access, download and analyze images and data via one of the existing interfaces to the CCDB.

# CORRELATIVE MICROSCOPY AT CRYO-TEMPERATURES: STRUCTURAL AND MORPHOLOGICAL STUDIES OF LIVING CELLS

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Cryo-electron tomography (cryo-ET) of frozen, hydrated, biological samples on carbon-coated copper grids is a powerful technique for quasi in vivo studies of cellular structures with nanometer-scale resolution [1]. A major inconvenience of this method exists in the difficulty of locating and unequivocally identifying the structures of interest within an ice embedded sample. By establishing a direct correlation between cryo-fluorescence microscopy and cryo-electron microscopy (cryo-EM), we propose an effective way to overcome these limitations, opening the way for a wide spectrum of novel applications. Our concept of correlation microscopy is based on the possibility of identifying and determining the position of fluorescently-labelled structures in vitrified samples directly on the TEM grid by means of cryo-fluorescence microscopy, and the recovery of these positions during subsequent investigations with cryo-EM [2]. The entire vitrified grid can be imaged and mapped with epifluorescence under cryo-conditions thanks to a new designed and homemade cryo-holder for TEM grids, adapted to a fully automated, inverted light microscope (Zeiss Axiovert 200) with long working-distance objectives (20x and 40x magnification). The cryo-holder is devised to keep the sample at liquid nitrogen temperature (LN<sub>2</sub>) and it is efficiently isolated from the external environment to ensure a thermal equilibrium and to prevent undesirable contamination during investigations. The absolute coordinates of the area of interest on the grid can be determined and recorded by cryo-light microscopy and directly recovered in the electron microscope with a MatLab-based program, integrated within the TOM toolbox [3]. Fluorescently labelled neurons and neuroblastoma/glioma cells (NG108-15) grown on carbon-coated EM indexed grids have been embedded in vitreous ice and successfully used for first studies. In the case of thin specimens suitable for cryo-electron tomography (where the thickness of the amorphous ice does not exceed 200-400 nm), the resolution is comparable to investigations in buffer solutions. The 'blurring' of the fluorescence signal and the decrease in resolution is directly related to the inhomogeneous composition and thickness of the ice, which can be estimated from transmitted light measurements. Our current applications involve fluorescent labelling and immunolabelling of extra-cellular molecules, structures and receptors in living cells with anti-bodies conjugated to fluorescent colloidal gold or Quantum Dots [4], which are ideal candidates for correlation microscopy due to their fluorescence and electron-dense characteristics, which allow visualisation and targeting of the label both with cryo-light microscopy and cryo-EM, especially cryo-ET. We focus on one side on the study of the actin organisation in retracting fibers and at focal adhesion points for keratinocytes grown on EM grids coated with Fibronectin. On the other side, we concentrate on the localisation of excitatory synapses in living hippocampal primary neuronal cultures by immunolabelling of glutamate receptors and on the study of cytoskeleton organisation in neuronal growth cones. References [1] W. Baumeister, *Biol. Chem.*, 385 (2004) 865-872. [2] A. Sartori et al., to be submitted to *J. Struct. Biol.* [3] S. Nickell et al., *J. Struct. Biol.*, 149 (2005) 227-234. [4] D. R. Larson et al., *Science*, 300 (2003) 1434-1436. [5] This project is supported by the Deutsche Forschungsgesellschaft (SFB 563)

## ALIGNING AND AVERAGING SINGLE PARTICLES FROM A TOMOGRAM

Presenting Author: MICHAEL F. SCHMID, P.I.

Michael F. Schmid Mike Marsh Angela Cruciano Juan Chang Htet Khant Wah Chiu All Authors: National Center for Macromolecular Imaging, Dept of Biochemistry, Program in Structural and Computational Biology and Molecular Biophysics, BCM

Electron cryotomography gives insights into the structure of cells and organelles, and thus it is usually thought of as being applicable to pleiomorphic structures which are unique. This contrasts with the concept of 'single particle' reconstruction, where a molecule or macromolecular complex is imaged in a field containing numerous identical versions of the specimen in various orientations.

However, there are certain situations in the analysis of 'single particles' where tomography may also be useful. For instance, a valid starting model for the object may be difficult to derive. This may be because the symmetry is ambiguous, or the size distribution is heterogeneous, necessitating multiple starting models. The possibility of initial model bias in these cases is non-trivial. Tomography can give a low resolution 3D starting model or models without any assumptions about the structure or its orientation.

There are significant challenges to overcome in the use of tomography for the reconstruction of multiple copies of macromolecular complexes. The inability to tilt the specimen as far as 90° leads to a missing wedge of data in Fourier space that in turn leads to distortion of the reconstruction. When 3D cross-correlation is used to determine the relative orientation of two particles extracted from a tomographic reconstruction, a trivial, but wrong, solution is one that aligns their missing wedges with each other. Yet this is often the orientation that yields the highest cross-correlation coefficient. We are investigating methods of scaling the correlation maps to determine the proper relative orientations of particles when both maps contain a missing wedge component, and for weighting such aligned particles when computing an average.

The Fourier components in the 'missing wedge' are nominally zero, or at least small, depending on the details of the reconstruction method. In the complex multiplication performed during cross-correlation, if either data set has such zeros, the complex product will also. The proportion of zeros in Fourier space will vary due to the change of overlap of the missing wedges with different orientations. To a first approximation, the height of the cross-correlation peak will depend on the total power of the complex product in Fourier space. Therefore we scaled the peaks in the cross-correlation map by the reciprocal of the fraction of non-zero Fourier components. This greatly reduced the tendency to mutually align the missing wedges of the data. As opposed to methods which explicitly treat the geometry of a missing wedge, we can use this criterion even when one or both of the maps is in turn composed of an average of several individual mutually aligned particles.

For averaging aligned particles in 3D, we average their Fourier components, amplitude-weighting each pixel in Fourier space. This reduces the effect of particles whose missing wedge contributes at a certain pixel, versus other particles which are contributing real data.

We are applying these techniques to 3D particle volumes extracted from tomograms of carboxysomes from marine chemoautotrophs, pentonless capsids of herpes simplex virus which contain the portal protein, and non-icosahedral cyanophages.

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# ACCURATE AND FAST DUAL AXIS SIRT RECONSTRUCTION FOR ELECTRON TOMOGRAPHY

Presenting Author: AUKE VAN BALEN, FEI R&D

R.H.M. Schoenmakers FEI Company

There is a clear trend in both research and industrial use of electron tomography (ET) to move from a descriptive technique into a standard analytical technique. Iterative reconstruction techniques that preserve the frequency information of the input data (like e.g. SIRT) are now accepted as mainstream reconstruction techniques. The use of dual axis tomography as a means to get more uniform resolution in the X-Y planes of the reconstructions is seen as a must. The combination of both techniques makes the resulting 3D volume easier to visualize, segment and therefore interpret.

We have developed an easy-to-use software package for producing 3D tomograms by dual axis SIRT reconstruction techniques. In this paper, we describe a novel registration approach to calculate the transformation parameters between the two data sets of a dual axis tomography acquisition experiment. We also describe how we combined GPU accelerated SIRT with this dual axis tomography. By doing so, large (Gigabyte sized) uniform resolution tomograms can be obtained in less than an hour. Without such accelerations, this will take up to several days. Finally, we describe a novel algorithm to remove gold bead artifacts in a tomography data set, thus improving the interpretation and analysis of parts of the tomogram that otherwise would have suffered from these artifacts ('streaks').

## 2D AND 3D CRYO-TEM OF DNA-BASED BIOMATERIALS

Presenting Author: NICO A.J.M. SOMMERDIJK

Due to its intriguing properties DNA has been explored in various different applications ranging from sensors and assays to building blocks in supramolecular architectures. Its use not as a carrier of information but as a biomaterial, however, is essentially unexplored. Nevertheless, the favorable non-immunogenic properties of this degradable biopolymer have prompted us to develop biomaterials coating based on DNA. For this we apply the Layer-by-Layer assembly technique that allows to build a coating by the repeated sequential deposition of negatively charged and positively charged components on to substrates of any shape or form.<sup>1</sup> It was proposed that the layered build up of these coatings that could be used for the localized entrapment of drug or growth factors released during the degradation process. To ensure a truly layered coating structure we designed a self-assembling surfactant based on the bis-urea motif.<sup>2</sup> The DNA/surfactant assemblies as well as the individual components were extensively analyzed by cryoTEM and cryotomography using the worlds first FEI “life science” TITAN system. We demonstrate that this compound formed ultra thin ribbon-like aggregates. The complexation of DNA was first visualized using Langmuir monolayers of this surfactant both by 2D and 3D cryoTEM. In addition cryotomography demonstrates the formation of a layered coating in which bioactive molecules can be entrapped at predefined positions.

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# THREE DIMENSIONAL ARCHITECTURE OF NUCLEAR LAMINARIS NODES OF RANVIER FROM THE AUDITORY SYSTEM OF THE BARN OWL

Presenting Author: GINA SOSINSKY, P.I.

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The node of Ranvier is one of the most specialized and regulated domains in cellular neurobiology and relies on a complex synergy between glial and neuronal cells. An extreme example of specialized myelinated fibers exists in the auditory system of birds. The axons in the nucleus laminaris (NL) in the central nervous system (CNS) of the barn owl maintain very long periods of high frequency activity without latency shifting. The nodes of the NL are quite unusual with extreme differentiation of the astroglial and oligodendroglial processes as well as high local concentrations of mitochondria. The NL neurons in the barn owl represent an excellent system to test the hypothesis that nodal structure is directly moderated by physiological characteristics of the conduction properties such as its penchant for firing accurately and often. We used serial section electron tomography to produce accurate reconstructions of these nodes. We synthesized serial section electron microscopic tomograms of NL nodes of Ranvier from 0.5 mm sections of conventionally prepared brain tissue. Each NL node reconstruction is made up of four serial volumes encompassing 90-95% of the node of Ranvier. We have traced the axon, paranodal loops, compact myelin and several surrounding astrocytic processes in two owl node of Ranvier reconstructions. In these reconstructions and models, the paranodal loops are highly elaborated and invested on the axolemma, extending well into area underneath the compact myelin. In addition, numerous astrocytic processes are in close apposition with the nodal domain. These new reconstructions are compared to tomograms from an owl CNS non-NL node and to published mammalian peripheral nerve (PNS) node reconstructions (Sosinsky et al., 2005, *Neuroinformatics* 3:133-162) that show a much less elaborate investiture of the glial processes.

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### 3D IMAGING OF THE CONTACT BETWEEN T-LYMPHOCYTES AND THE AIDS VIRUS

Presenting Author: RACHID SOUGRAT, Postdoc

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We have used electron tomography at room and liquid nitrogen temperatures to investigate the structures of intact simian immunodeficiency viruses both after isolation and in infected lymphocytes. Analysis of tomograms from acute and chronically infected T-cells demonstrate the presence in the extracellular medium of free virions as well as virions contacting the T-cell plasma membrane that are captured in a state that may immediately precede viral fusion. A three-dimensional density map of the neck-shaped contact region shows that it is approximately 30 nm in width, and contains rod-shaped densities that span the two lipid bilayer membranes, presumably reflecting the molecular complex formed between gp120 and surface receptors on the target T-cell. Substantially reduced numbers of gp120 trimers are observed in rest of the virion surface only in those viruses that form this unique contact region. These studies provide a glimpse into the architecture of what we propose is the first stage of interaction between SIV and target T-cells, representing the "pre-fusion" complex.

## STRUCTURAL STUDY OF MICROTUBULE DOUBLETS BY ELECTRON TOMOGRAPHY

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Microtubule doublets are the major structural components of eukaryotic flagella and cilia axonemes, which are among the largest macromolecular machineries and are conserved among eukaryotes from protists to mammals. In the axoneme, nine microtubule doublets are evenly distributed around a group of two singlet microtubules (central pair), with power stroke complexes bracing in between to give a wheel-like cross-section. Coordinated sliding between the microtubule doublets driven by dyneins facilitates the movement of flagella and cilia. Structure information on microtubule doublets is essential to understanding the molecular basis of their function in axonemes and their roles in flagellar movement.

We have studied the structure of intact microtubule doublets by cryo-electron tomography followed by 3D image averaging. The grids of microtubule doublets from axonemes of sea urchin sperm were plunge-frozen in liquid ethane. Tomographic data from the frozen hydrated specimens were collected using a JEOL 3100 microscope equipped with an omega energy filter. The power spectrum of reconstructed tomograms showed distinctive layer lines at 4nm. Taking advantage of the periodicity in the longitudinal direction of the doublets, we aligned and averaged 3D volumes extracted along several doublets in the tomographic reconstructions and achieved a 3D map with a resolution better than 3nm. The density map allowed a precise docking of atomic models for all tubulin protofilaments. Further analysis of the density map provided insight into locations of particular proteins within the doublets. We identify likely candidates for several of these non-tubulin components of the doublets.

This work offers novel insight on how tubulin protofilaments and accessory proteins attach together to form the doublets and provides a structural basis for understanding doublet function in axonemes.

## CORRELATED LIGHT AND ELECTRON MICROSCOPY/TOMOGRAPHY REVEALS CHANGES IN MITOCHONDRIA DURING RELEASE OF CYT. C

Presenting Author: MEI SUN, Student

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Mitochondria initiate and/or regulate the apoptosis (programmed cell death) through the release of cytochrome c and other proteins from the intermembrane and intracristal compartments. Two principal mechanisms have been proposed by which cytochrome c can cross the outer membrane: (1) Opening of the mitochondrial permeability transition pore (MPTP) that stimulates large amplitude swelling of the mitochondrial matrix rupturing the outer membrane, or (2) Formation of a large pore in the outer membrane without mitochondrial swelling. More recently a variation of the second mechanism has been suggested in which 'remodeling' of the inner membrane induced by opening of the MPTP increases the diameters of crista junctions facilitating release of cytochrome c from the intracristal compartment. We have studied this process by correlated light and electron microscopy in HeLa cells in which apoptosis was initiated by treatment with etoposide causing DNA fragmentation. We monitor release of cytochrome c using cells transfected with fluorescent cytochrome c fusion proteins and membrane potential by staining with TMRE, a fluorescent cation. By growing HeLa cells in special Petri dishes containing an etched grid, we can monitor cytochrome c release and membrane potential simultaneously by confocal microscopy and subsequently fix and embed the identical cells for examination by electron microscopy and by electron tomography. We find that in HeLa cells treated with etoposide loss of cytochrome c precedes loss of membrane potential. Examination of these cells by electron microscopy showed that immediately after cytochrome c release few mitochondria were swollen with most mitochondria appearing either normal or displaying a dramatic change in crista morphology. Electron tomograms reveal that this change is characterized by the formation of multiple vesicular cristae, a membrane conformation distinct from the 'remodeling' previously reported by others. The number of swollen mitochondria increases in the later stages of apoptosis after mitochondria have both released cytochrome c and lost their membrane potential. The timing of these changes indicates that normal mitochondria first change to the vesicular form followed by swelling. The results of these studies indicates that in HeLa cells treated with etoposide to initiate apoptosis, cytochrome c is released by the formation of a large pore in the outer membrane, probably by pro-apoptotic proteins of the BCL-2 family. Both the MPTP inhibitor, cyclosporine A, and the general caspase inhibitor, z-VAD, inhibit the formation of vesicular mitochondria, but do not inhibit cytochrome c release. Thus, inner membrane remodeling is not required for release of cytochrome c in agreement with theoretical models. The transformation of mitochondria from the normal conformation to the vesicular conformation fragments the matrix compartment and may be associated with mitochondrial fragmentation that accompanies apoptosis.

## FEATURE OF ULTRA-HVEM TOMOGRAPHY AND APPLICATIONS

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Akio Takaoka, Toshiaki Hasegawa, Kiyokazu Yoshida and Hirotarō Mori Research Center for ultra-HVEM, Osaka University

The ultra-HVEM with 3MV in Osaka University is excellent in both the penetration and the resolution for thick specimens. We can observe 5 microns slice tilted 60 degree for biological samples and 2 microns section for LSI device samples. The CT with ultra-HVEM is suitable to analyze the 3D structure in the magnification of several thousands or more, where the resolution is about ten times higher than optical microscopy. The resolution holds in a value about 1/1000 of the thickness for well-stained samples. [Key factors in ultra-HVEM CT] (1) In the preparation of biological sample, electron staining is quite important to get a fine 3D image because the contrast of images becomes weak at such high electron energy and it needs some technique to stain thick samples uniformly. We tested various kinds of solution and condition, and it was found that 3% uranyl acetate 70% methanol solution is best under the heating by a microwave oven in 30 seconds. (2) To reconstruct tomogram with high quality from a tilt-series, it is important to minimize the missing zone, so we developed a 360-degree rotating holder and also we use a high quality 4k x 4k CCD camera. In the case of device samples, the sample is shaped like a thin stick with a focused ion beam machine. Then, we can collect the projections from every direction. (3) Though the size of sliced biological samples shrinks several % during the micrograph of a tilt-series, the detail configuration in image hardly changes by beam irradiation in the case of stained samples. We observed the stained sample in room temperature. (4) The alignment between tilt-series images is very important to obtain a high resolution on the reconstruction; we need the geometrical modification to the shrinkage and the image rotation due to the deviation from eucentric condition. We use some gold particles as the fiducial marker and reconstruct with the software "IMOD" developed in Colorado University. [Application of ultra-HVEM tomography] In our research center, we analyzed the 3D structure of the following samples in the collaboration with researchers in various fields. (a) Unicellular organism such as bacteria and plankton. (b) Cell organelle such as nucleus, Golgi system, mitochondria, chromosome etc. (c) Sections of plant or animal tissue such as human hair, papilla cell, osteoblast, etc. (d) Pathological injury texture such as nervous system, capillary, etc. (e) Compound or hybrid materials such as tire rubber etc. (f) Polymer such as block copolymer. (g) Electronic integrated devices such as LSI. LSI devices consist of some materials including polycrystalline substances and Bragg reflection changes the contrast of the grains by the tilt angle. The influence of each reflection on the tomogram is reduced in inverse proportion to the number of images in the tilt series.

## ELECTRON TOMOGRAPHIC ANALYSIS OF OUTER HAIR CELL STRUCTURE

Presenting Author: WILLIAM J TRIFFO, Student

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In the mammalian cochlea, the outer hair cell (OHC) is capable of generating axial deformations in response to variations in transmembrane potential. Our understanding of electromechanical transduction in the OHC depends on precise knowledge of intracellular structure, and efforts to model OHC physiology are likewise limited to the fidelity of known intracellular geometry. The cortex of the OHC, referred to as the lateral wall, can be viewed as a trilaminar composite made up of (1) the plasma membrane, (2) a network of actin and spectrin termed the cortical lattice, and (3) lamellar stacks known as the subsurface cisternae (SSC). Previous studies of the cortical lattice using conventional TEM and AFM techniques relied on protocols that removed the lattice from its native environment. Moreover, depending on the exact fixation protocols, conflicting depictions of SSC ultrastructure have been reported. We have therefore tested a variety of sample preparation methods, including iso-osmotic fixation protocols as well as high-pressure freezing (HPF) and freeze-substitution (FS). Using cochlear samples from mouse and guinea pig, we have employed Electron Tomography (ET) to study the cortical lattice and its structural relationship to the plasma membrane, resolving the pillar proteins known to span the extracisternal space between the plasma membrane and cortical lattice. We are also positioned to address whether the SSC is continuous or fenestrated in vivo, and whether the fenestration commonly observed represents a fixation artifact. We discuss the usage of cellulose tubing to preserve hair cell architecture during HPF, and also comment on our progress in the visualization and analysis of rootlet architecture at the junction of the stereocilia bundle and apical region of the cell.

# HIGH-THROUGHPUT ELECTRON MICROSCOPE TOMOGRAPHY OF THE INSULIN-SECRETING PANCREATIC BETA CELL

Presenting Author: PETER VAN DER HEIDE, Student

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We have employed electron microscope tomography (ET) to qualitatively and quantitatively characterize the subcellular organization of insulin-secreting pancreatic beta cells high pressure frozen in situ in murine islets within 24 hours of isolation from adult mice. Following freeze-substitution, resin embedding and post-staining, thick (300-400nm) sections are then imaged for dual-axis tomography at 300keV using a Tecnai F30 TEM (FEI). Such tomograms have already provided insight into important structure-function relationships in the beta cell, most notably with regard to compartments along the insulin biosynthetic pathway. However, conventional CCD arrays and ET methodology typically limit the volume that is imaged and reconstructed in 3D at high resolution to ~1% of an islet beta cell. To properly understand structure-function relationships with respect to cells as complex systems, the development of new methods for reconstructing and interpreting high resolution tomograms of cells in toto is required. Although stitching reconstructed volumes serially in Z can increase the imaged volume incrementally, new methods to accurately stitch tomograms along all 3 axes must be developed as the beta cell's cross section is invariably larger than the microscope field at appropriate magnification. Acquisition software must also be extended to provide easy and reliable imaging of grids of neighboring montaged panels. As proof-of-concept for the construction of a whole cell tomogram at high resolution (~5nm), we have begun imaging and reconstructing a tiled series of dual-axis montaged tomograms (i.e., a 'super-montage' comprising 3x3 montages in X and Y) through at least 5 serial sections in Z. The final reconstructed volume will encompass ~10% of the volume of an islet beta cell, along with portions of adjacent neighboring cells that include both beta and non-beta cells of the endocrine pancreas. Imaging these neighboring cells offers an opportunity to study potential autocrine and paracrine activities at the intercellular interface. Manual segmentation of membranes and other features to delineate the geometries of subcellular compartments and the cytoskeleton, along with their internal structure remains the primary obstacle to being able to rapidly and reliably visualize, quantify and interpret analyzing cellular tomographic data. Our previous studies have demonstrated that to manually segment ~1% of the volume of a beta cell at high fidelity takes approximately 9-12 months. Based on this, manually contouring a whole cell at similar detail would take more than 50 years; thus, it is vital that automated and semi-automated segmentation techniques are developed in tandem with such large scale/high-throughput tomography projects. Although a number of segmentation techniques will be required to handle all entities of interest, we have found that a 3D variant of the Watershed transform applied following pre-processing with different combinations of filters including median filtering delivers accurate results for a broad range of organelles, including those with highly irregular geometries such as the Golgi. This semi-automated approach also deals well with the high levels of image noise inherent to ET, and requires no a priori knowledge of the volume, such as object location or shape.

## COMBINED 3D STRUCTURAL ANALYSIS OF A BACTERIUM WITH AN ORGANELLE

Presenting Author: LAURA VAN NIFTRIK, Student

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Anaerobic ammonium oxidation (anammox) is a recently discovered pathway of the biological nitrogen cycle [1] and is currently estimated to be the major source of gaseous nitrogen on Earth [2]. Anammox is performed by a clade of planctomycete bacteria, which divide only once per two weeks at maximum speed. These bacteria possess an organelle, the 'anammoxosome', an intracytoplasmic compartment surrounded by a bilayer membrane. This organelle is dedicated to anammox catabolism and is the locus of respiration by these bacteria, analogous to the mitochondria in Eukaryotes. The anammoxosome membrane consists mainly of so-called ladderane lipids [3] that make this membrane almost impermeable to protons and the toxic intermediates of anammox catabolism.

With their small size ( $< 1 \mu\text{m}$ ) and spectacular cell biology, these prokaryotes are an ideal target for tomographical investigation. Here we used electron tomography to compile a complete image of the anammox cell and its organelle. Different stages of the one-month cell cycle were investigated and compared to resolve how the anammoxosome divides and is passed on to the daughter cells. Many structures were observed in the cytoplasm and in the anammoxosome, such as glycogen storage particles, iron containing particles, membrane foldings and fibrous protein structures. Electron tomography was combined with energy dispersive x-ray analysis, immunogold localization of anammox enzymes, and histochemical staining methods to provide the link to the functional significance of the structures visualized. The recently completed genome of the anammox bacterium *Kuenenia stuttgartiensis* [4] provided us with many leads for the functional unraveling of anammox cell biology. [1] Strous et al (1999) *Nature* 400: 446-449 [2] Kuypers et al (2003) *Nature* 422: 608-611 [3] Sinninghe Damst, et al (2002) *Nature* 419: 708-71 [4] Strous et al (2006) *Nature* 440: 790-794

# ASSESSMENT OF THE PERFORMANCE OF SIRT, ART AND WEIGHTED BACKPROJECTION ALGORITHMS OF TOMOJ

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The use of iterative methods for tomographic reconstruction such as ART (algebraic reconstruction technique) and SIRT (simultaneous iterative reconstruction technique) are providing an alternative to the established algorithm of weighted backprojection. These algorithms iteratively refine the result and consist of a 3-step workflow. First, a projection  $P$  is backprojected at its corresponding angle. Then, the difference  $D$  between the existing volume  $V$  and this backprojection is calculated. Finally, this difference  $D$  replaces the content of the volume  $V$  (Kak and Slaney 2001). One iteration (cycle) consists of a backprojection of each projection in the tilt series. Each iteration verifies the result with the source and thereby amplifies signal and reduces noise. The SIRT algorithm allows to calculate several backprojections (step 1) simultaneously, depending on the number of available processors. We considered the TomoJ 3D reconstruction software (Messaoudi et al. 2006), implied as a plugin in the public-domain Java image processing program ImageJ inspired by NIH Image. We assessed several parameters of its performance: computing speed, convergence speed towards a stable volume (thereby comparing the SIRT with the ART algorithm) and the influence of the relaxation coefficient for the SIRT algorithm. The objective parameters were complemented with our judgement of the reconstructed volume in terms of ultrastructural information.

The calculations were performed on a dual core AMD Opteron 252 biprocessor, 2.6 Ghz with AMD64 architecture and 4 GB RAM. We tested 200 nm thick Epon sections with Walker carcinosarcoma cells embedded on a LEO912 (120 kV) transmission electron microscope with an in-column energy filter over a  $120^\circ$  tilting angle ( $-60^\circ$  to  $+60^\circ$ ). The results show that there is a significant difference between the calculation time for one iteration by ART and by SIRT: the first step of the SIRT algorithm is divided over the two processors, considerably reducing the time needed to perform 1 iteration. The error values produced by the algorithms, however, show a smoother convergence for ART than for SIRT: the less iterations implied, the more robust the stack is converging to a stable volume. The weighted back projection requires the least time to produce the reconstruction, but the result is of lower ultrastructural quality compared to ART and SIRT reconstructions with at least 10 iterations. The relaxation coefficient is a pixel greyscale multiplier ranging from zero to one. The relaxation coefficient changes directly the speed of the convergence. A relaxation coefficient of one for a one-iteration SIRT produces a back projection. Near zero values will slow down the convergence considerably, but will produce a qualitatively superior reconstruction.

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# MORPHOLOGICAL AND QUANTITATIVE ANALYSIS OF ENDOPLASMIC RETICULUM DURING CELL DIVISION

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The fate of endoplasmic reticulum (ER) and its subcompartments during cell division is poorly understood. Nuclear envelope (NE) disassembles during mitosis and light microscopy studies suggest that the inner NE membrane proteins are dispersed into the ER. Here we have studied the morphology of the ER in more detail using live cell confocal microscopy, electron microscopy and electron tomography throughout the whole cell cycle. Live cell confocal microscopy revealed ER network with tubular profiles in mitotic as well as in interphase cells. To measure the degree of ER networking during different phases of cell cycle three-way-junctions and tubule lengths were determined. Quantification analysis of these tubular profiles showed increase in tightness of the ER network during cell division. Electron tomography clarified the structural appearance of ER showing the existence of ER sheets in addition to tubular structures in interphase cells. When entering mitosis the ER sheets disappear while leaving the ER tubular network to remain continuous throughout cell division. NE marker proteins were dispersed into the ER at the beginning of mitosis as revealed by confocal microscopy and electron tomography. At the late anaphase NE marker proteins were accumulated in the ER sheets surrounding the chromosomes, and stained and unstained ER profiles were directly continuous with each other. These results support the earlier observations suggesting that NE loses its identity while becoming a part of ER network and thereby the partitioning of NE is subordinated to the ER network during cell division. The reorganization of ER network may be required to ensure even distribution of ER and all its subcompartments to both daughter cells.

## A STUDY OF THE STRIATED ORGANELLE IN VESTIBULAR ENDORGANS

Presenting Author: FLORIN VRANCEANU, PhD Student

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Striated organelles (STOs, also known as laminated or striated bodies, or Friedmann's organelle) are structures that have been described in the auditory and vestibular hair cells of human and several animal species.

The detailed structure of STOs has not yet been described, especially in light of new cell biological knowledge about the cytoskeleton. The purpose of our study is to fill the existing gaps and to complete the description of the STO structure and if possible, to identify its composition.

We already know that the STO is connected to microtubules, smooth endoplasmic reticulum, mitochondria, and the cell membrane. We will attempt to derive its function, which we hypothesize to be related to the action of the hair bundle, from a detailed three-dimensional reconstruction of it and its connections to the hair cell cytoskeleton and, possibly, to the hair cell stereociliary bundle itself. Furthermore, given the striated aspect of the organelle, one possible function may be to modulate the "stiffness" of the hair bundle which would influence the sensitivity of hair cells to external stimuli.

We expect to be able to produce better hypotheses about the function of STOs once we have more information about its detailed structure.

## ANALYSIS OF VOLUME DATA OBTAINED BY ELECTRON TOMOGRAPHY

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The quality of tomograms is affected by various factors. Not considering the preservation of the specimen itself or degradations during the imaging, proper sampling of the three-dimensional structural information is crucial. A specific problem is the limited angular range of tilting, which results in a missing wedge of data in Fourier space for single axis tilt series, or a missing pyramid for dual axis series. For a further data analysis with methods derived from single particle analysis, the missing wedge has the potential to affect the three-dimensional alignment, the classification, and the formation of averages. In applications to the crossbridge motifs in insect flight muscle the effects could largely be ignored, because the motifs are regularly arranged in the same orientation and the tomograms had missing wedges that were very similar, because the tilt axis was chosen to be in the direction of the filament axes. With other classes of specimens, the orientation of the missing wedge relative to the specimen structure must be taken into account. It will have one rotational degree of freedom for proteins adsorbed on lipid monolayers, three degrees of freedom in the general case. Results in the monolayer case indicate that classification does not seem to bias the separation in favor of orientation instead of reporting structural variability. For the investigation of the general case, specimens consisting of integrin reconstituted into phospholipid vesicles are used. The distribution of the integrin on the vesicle surface allows motifs to be extracted in all orientations in space. Classification tends to separate motifs from the top and bottom from those extracted from the sides. Averaging with proper weighting according to the missing wedge orientation produces images with more isotropic resolution.

## ELECTRON CRYOTOMOGRAPHY OF IMMATURE HIV-1 REVEALS THE STRUCTURE OF THE CA AND SP1 DOMAINS OF GAG

Presenting Author: ELIZABETH R. WRIGHT, Postdoc

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The major structural elements of retroviruses are contained in a single polyprotein, Gag, which in HIV-1 comprises the matrix (MA), capsid (CA), SP1, nucleocapsid (NC), SP2, and p6 proteins. In the immature HIV-1 virion, the domains of Gag are arranged radially with the amino-terminus of MA at the membrane and NC-p6 facing the particle center. While assembly of the mature virion from the immature form has been studied extensively, and the structural elements of both have been identified and determined, a complete understanding of the immature virion has remained elusive. Here, we report the first three-dimensional structure of individual immature HIV-1 virions obtained by electron cryotomography. We measured the virions and found the diameters to range from 115 to 140 nm with a mean of 127 nm. The radial spoke arrangement of the Gag polyprotein was clearly visible in the tomograms. The thicknesses of the domains of the Gag polyprotein were measured as  $78 \pm 10$  Å for membrane-MA,  $77 \pm 10$  Å for CA, and  $43 \pm 10$  Å for NC. Upon producing surface projections of each layer of Gag, it was determined that only the CA domains contained regions of order within the virions. The CA domains were arranged in hexagonal lattices interspersed between patches of disorder. We developed a novel method for detection and averaging of unit cells from highly ordered regions within the Gag shell. A three-dimensional map produced by averaging 120 unit cells revealed a two-layer hexagonal lattice in the CA and SP1 domains. The ring-to-ring spacing of the CA NTD lattice was 8 nm and the ring-to-ring spacing of the SP1 lattice was 7.5 nm. From our data, we describe a pseudoatomic model of the CA and SP1 domains of immature HIV-1 in which SP1 hexamers appear to stabilize individual CA hexamers (Figure 1).

## TOWARDS SHARING DEVICE MEASUREMENT INFORMATION: ARE WE READY AND WILLING?

Presenting Author: H. HAKAN YAKALI, Researcher

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Different device measurement techniques, like electron and light tomography and mass spectroscopy, provide information about the different aspects of a biological sample. If we seek to gain more insight about a specific sample, we first need a platform where we can find other relevant works and activities. We propose an abstract high-level schema for representing device measurements experimentations, like electron tomography and mass-spectroscopy, and facilitate that for searching relevant other experiments.

Our goal in this work is in two folds:

- a. to check if there is sufficient consensus on this view - by conducting a small survey among the visitors during the conference -, and if not what is missing
- b. provided that such a view exist, to prepare the ground for future collaborations by creating a data exchange point around this abstract view.

Currently, many institutes and academies are conducting various device measurement experiments to capture certain aspects and properties of variety of biological samples. In these experiments, scientist use different measurement devices such as light microscopy, electron microscopy, mass spectroscopy and others. In order to be able to search, repeat and understand the results of these experiments, all the relevant data regarding these experiments are stored in a metadata database whose structure is defined using some kind of database schema. Many institutes have developed their own database schemas based on their own experience, rather than choosing an existing schema and adapting it to their needs. This resulted in fragmentations along across and even in similar domains.

When looked closely to these experiments, one can see that the procedures followed in them are very identical to each other, if not the same. They all start with a sample; it is treated, prepared and measured. Each experiment performed using a different experiment device provides more, but most importantly, complementary information about a specific aspect of that sample. Some scientists have already started considering these different measurement data sets, for instance, from electron-tomography and mass-spectroscopy, to gain more insight about a sample [1, 2]. It is only matter of time before other scientist start thinking in this ever growing multi dimensional space, to push the boundaries. Clearly, starting with large scale collaboration initiatives are extremely challenging and a complex undertaking due to organizational policies. In an attempt to break these walls, we first want to stimulate sharing, centered on a high-level device experimentation schema. We want to use this opportunity to introduce our high level device experimentation schema to electron tomography community, to get a feedback. If we achieve a sufficient consensus and momentum, we would like to start with building this portal where scientist can find relevant information, if not the data itself, at the least a pointer to where they can get it. We will report our findings in the following conference.

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## OLINGO: DATA STRUCTURE GENERATION FROM ONTOLOGY

Presenting Author: H. HAKAN YAKALI, Researcher

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Cell-Centered Database (CCDB) has a large schema that needs continuous update to accommodate users demand. Because of the size of the schema, this management task is not trivial and is performed ad-hoc basis. In order to bring a structure in this task, we propose an ontology based approach. We report about our ontology based OLINGO tool. Using an ontology, this tool can generate data structures. Database schemata, java classes, object relational mappings are among the few to be named. Among others, OLINGO provides an alternative for managing database schemas centrally and structurally.

Since the mid 2005, we are collaborating with Cell-Centered Database (CCDB) group from UC San Diego within CellTom sub-project, one of many in Virtual Laboratory e-Science (VL-e) project. It has complex schema with many tables (around 100) and attributes. It is constantly being updated in direction of the user requirements. Because of the size and continues demands of the user, its management becomes a growing problem. Due to the multidisciplinary nature of the VL e project, we are also interacting with other bio-informatics groups and bio-diversity domain. Suffice to say that this, management of large and complex database schemas, is a problem at large. We propose our OLINGO tool; an ontology based data-structure generation tool to address this in a structured way. An ontology describes concepts and their relationships, in term of both human and machine understandable format, with the basic principle of enabling sharing of knowledge and information. A domain ontology essentially describes entities, their properties, and their relations (and subsets) among them, within a domain of disclosure. It supports the process of conceptual modeling and provides a common collection of semantic information for a particular domain, involving a vocabulary with the set of terms used for modeling, a taxonomy, including some structure of the entities, and the semantic interpretation of the terms and concepts. In a way, a domain ontology is explicit conceptualization of a specific domain. A database schema is a structured collection of data within a problem domain. Despite the differences in modeling techniques, it contains tables or objects with the relationships among them. In our view, a database schema is a implicit (data-centric) conceptualization of a problem space. The conversion, between the concepts (or objects) described in the ontology and the database structures, is not always flawless or perfect. The underlying paradigms are indeed different and two sets of structures cannot be converted seamlessly, without leading to a conflict or discrepancy between the models known as impedance mismatch. Nevertheless, it is possible to make intelligent tradeoffs to tackle these differences, allowing a smooth translation between the objects and their relational counterparts. Even though our prototype based on Prot,g, is capable of creating various data structures, here we focused on its use from database point of view. In this respect, we utilize this tool to automatically generate a set of SQL statements from a given ontology for the purpose of creating database schemas. This functionality provides the basis for a tool for managing the database schema centrally.

# STEM TOMOGRAPHY: A NEW TECHNIQUE IN LIFE SCIENCE APPLICATIONS

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In material science, scanning transmission electron microscopy (STEM) is a well characterized technique for two-dimensional (2D) imaging with high resolution (within nm scale). In combination with a high-angle annular dark field (HAADF) detector, heavily scattered electrons can be detected and the recorded signal is proportional to the square of the atomic number ( $Z^2$ ) of an element. This allows the detection of heavy atoms, like for instance ultra small gold particles. By using electron tomography, (ET) 3D information can be restored from a set of 2D projection images recorded at different tilt angles. Although transmission electron tomography (TEM-ET) has been used in biological sciences for quite some time and proven to be a valuable tool, the STEM-ET mode is not often applied in this field of research [1]. In this contribution, we report on the potential of the HAADF-STEM tomography mode on biological material. Bright Field TEM (BF-TEM) and HAADF STEM tomograms from the same areas are compared and contrast and signal-to-noise ratios are calculated. We conclude that HAADF-STEM images have five times better contrast and signal-to-noise ratio than BF-TEM images. Our biological interest focuses on the organization of the endothelial cell junctions under different conditions. Endothelial cells form the main cellular barrier in blood vessels. Their major function is to regulate the infiltration of blood proteins and cells into the vessel wall. This unique characteristic is achieved through specialized transcellular systems of transport vesicles and by the coordinated opening and closure of cell-cell junctions [2]. For this purpose, endothelial cells have several types of specialized junctional regions that are comparable to adherens junctions (AJs) and tight junctions (TJs). The first 3D structure of TJs of chemically fixed capillary tissue has been published in 1984 by Bundgaard [3]. We employ an alternative method of high-pressure freezing followed by dehydration during freeze substitution and embedding in Epon and use HAADF-STEM and BF-TEM tomography, to study the 3D architecture of the tight junction between endothelial cells in Human Umbilical Vein Endothelial Cells (HUVEC) cells.

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# DESIGN OF A HIGH PRECISION TILTING APPARATUS FOR ELECTRON TOMOGRAPHY WITH THE 3MV HVEM

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In electron tomography, it is necessary to take a series of micrographs of a sample while it is systematically tilted from +90 degree to -90 degree. This process consumes a considerable amount of time, because every time the sample is tilted, a shift of the sample image and a deviation from the in focus condition take place and therefore both the shift and the deviation need to be appropriately adjusted before taking a micrograph. In the case of conventional intermediate voltage electron microscopes, systems in which these adjustments can be automatically done by employing the image recognition technique have been developed and adopted in commercial microscopes. Although the adjustment is effective and important in an automatic photographing control, basically, emphasis should be placed not upon the adjustment but upon the reduction (on the elimination) of the fluctuations (such as a shift of the image and a deviation in the focus). Based upon this premise, in the present work, a high precision tilting apparatus for electron tomography with the 3MV electron microscope, has been designed in an attempt to reduce the fluctuations themselves. In the design, all the mechanical systems of our conventional tilting apparatus have been checked and improvements have been made in the following items with priority. (a) Supplementation of a position adjusting mechanism An independent position adjusting mechanism has been installed in addition to the conventional Z control for the eucentric mechanism. The mechanism possesses a function to shift the whole tilting drum as a unit in the Y and Z directions, independent of the initial value of Z in the conventional height control. A significant improvement in the accuracy of the eucentric mechanism has been achieved by this additional mechanism. Since the operation room is separated from the room that houses the 3MV electron microscope itself, a remote control system for the newly designed eucentric mechanism was developed and attached to the main console in the operation room. This system enables us to finely adjust the mechanism to an optimal point smoothly while observing the monitor screen. (b) Improvements of the spherical socket In our conventional design, a spherical socket is used as the base for both the eucentric mechanism and the sample positioning mechanism. The structure of the spherical socket has been so improved that the axis of the holder rod is fixed at a single point on the base. Furthermore, machining methods for the spherical socket have been improved to get a high mechanical accuracy. By virtue of these improvements, the adjusting accuracy of the eucentric mechanism has remarkably increased. (c) Improvement in the tilting angle accuracy Improvement in the tilting angle accuracy has been made. Digitally controlled stepping motors for the driving system have given us an angle accuracy of 0.05 degree including the backlash. Improvements in the design of gears also contribute to the increase of the tilting angle accuracy.

## AUTOMATING RANDOM CONICAL TILT DATA COLLECTION

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The automation of random conical tilt data collection in an electron microscope presents several challenges, especially in vitreous ice. By integrating modern concepts from the field of computer vision into our current automated single particle collection software Legimon II, we demonstrate how this difficult task might be accomplished.

## ELECTRON CRYOTOMOGRAPHY OF PURIFIED PROTEIN COMPLEXES

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Many protein complexes are not purifiable in a specific conformation as required for single particle analysis. For these, the highest resolution structural technique currently available is electron cryotomography, but it is currently unclear what resolutions might be achievable. We have recently explored two instrumental advances (liquid helium cooling and dual-axis cryotomography) in this context, and applied them to the pyruvate dehydrogenase multienzyme complex and various transcriptional complexes. While liquid helium cooling was actually found to be disadvantageous due to a collapse of the vitreous ice into a denser phase, dual-axis tomography produced more isotropic resolutions as expected. We have systematically scanned different doses and defocuses in reconstructions of test objects to find optimal imaging parameters. In one example application, we imaged the pyruvate dehydrogenase multienzyme complex from *E. coli*. Remarkably, under optimal conditions we were able to resolve individual 80kDa domains without any type of denoising, which can introduce artifacts and obscure interpretability. We have now gone on to image several transcription complexes including the yeast RNA polymerase II and various basal transcription factors. In addition to revealing both quaternary structure and conformational heterogeneity, these reconstructions have proved valuable as initial models for refinement by single particle analysis.

## CONICAL TOMOGRAPHY OF METAL REPLICAS AND PLASTIC-IMBEDDED MATERIAL

Presenting Author: L. M. ZAMPIGHI, Research Associate

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We have used conical tomography with both metal replicas and plastic sections to study the structure of integral proteins and synaptic vesicles in their phospholipid bilayer environments. Complete conical series were collected from metal replicas of the water channel aquaporin-0 (AQP0), a 6.6 nm side tetramer with a molecular weight of 120 kDa that was purified and reconstituted in liposomes. The reconstructions contained information about the size, shape, and molecular volume of the particles representing the cytoplasm and external domains of the AQP0 channels and were studied by sectioning their volume parallel and orthogonal to the z-axis. Sections cut parallel to the z-axis showed the replica as a thin electron-lucent line 2 nm in thickness and the particles as small cup-shaped elevations in the fractured bilayer. Some appeared as deformed squares with sides intersecting at 90 degree angles while other particles exhibited a more rounded overall shape. The difference in particle shape was within the resolution limit and thus likely represents the cytoplasmic and external domains of the AQP0 channel. Through the reconstruction of plastic sections of 50-80 nm in thickness, we studied the structure of chemical synapses in the rat neocortex. We focused on vesicles that were docked to the plasma membrane of the pre-synaptic terminal (the 'active zone') because they represent the pool that release first during stimulation (the 'readily releasable' pool). We found that docked synaptic vesicles contacted the active zone at one side and an adjacent vesicle at the opposite end. At the side contacting the active zone, the docked vesicles merged their outer leaflets with the plasma membrane and formed a hemi-fused region. Fusion pores, shaped as slits of ~5 nm in diameter, opened at the perimeter of the hemi-fused region. At the opposite side, the vesicular membranes were tightly associated but did not hemi-fuse. Based on these observations, we concluded that: a) in accordance with predictions of the lipid pore models, the region of contact between vesicle and active zone undergoes a series of lipid intermediaries that precede neurotransmitter release, b) in contrast with predictions of the protein pore models, the fusion pore crosses only one membrane and is not formed by bridging through gap junction-like channels and, c) the vesicles recruited first during physiological stimulation (the rapidly releasable pool) are those trapped in the hemi-fused state, the lipid intermediary immediately preceding fusion. In conclusion we have determined that conical tomography is a useful method for the imaging of organelles and macromolecular assemblies in their cellular environments

## CRYO-ELECTRON TOMOGRAPHY OF CHEMOTAXIS RECEPTOR CLUSTERS IN E. COLI CELLS

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Bacteria cells respond to chemical changes in their environment beginning with ligand binding to a family of chemotaxis receptors, which ultimately modulate the rotation of the flagellar motors through a signal transduction pathway. There is a remarkable gain achieved in the bacteria chemotaxis system through cooperative signaling within higher order of receptor assemblies. A detailed understanding of the molecular architecture of the receptor assemblies is essential to elucidate the mechanism of signal transduction and amplification. We are using three-dimensional electron microscopy to determine the higher order arrangement of the various signaling components involved in chemotaxis. We have previously reported on the spatial clustering of the chemotaxis receptor Tsr in cells engineered to overproduce Tsr using combination of image information from negatively stained membrane extracts, sections from chemically fixed, cryo-sectioned cells, and frozen-hydrated sections from high pressure frozen cells (Weis et al., 2003, Lefman et al. 2004, Zhang et al. 2005). We now report direct visualization of the location and three-dimensional molecular architecture of chemotaxis receptor assemblies in intact E. coli cells using cryo-electron tomography. Receptors are clustered in a patch near the poles of the cell in wild-type cells in their growth phase, with significant variation in the size and specific location of the cluster even within the same population of cells. Cell growth environment can affect the distribution of the size within the population. The signaling molecules, CheA/W are required for the receptor cluster formation; however excess amount of CheA/W disrupts functional clusters. Expression of receptors at very high levels in the absence of CheA/W also allows formation of clustered receptor assemblies, but these are held together by end-to-end axial interactions of receptors and exclude the access of CheA/W to the receptor. Our experiments demonstrate a crucial role for the signaling molecules CheA/W in receptor clustering, and the stoichiometry of the receptor and signaling molecules is important for optimal functional cluster formation. 1. Weis et al., (2003) *J Bacteriol.* 185:3636-3643 2. Lefman et al., (2004) *J Bacteriol.* 186:5052-5061 3. Zhang et al., (2004) *J Microscopy* 216: 76-83

## ABERRATION CORRECTED MICROSCOPY FOR LIFE SCIENCE APPLICATIONS

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**ABSTRACT** With the advent of aberration correctors, electron monochromators and improved energy filters for the newest generation of transmission electron microscopes (TEMs), disciplines like material science and solid-state physics already demonstrated the radically improved image quality combined with the supreme spatial resolution for structural characterization (Jia et al., 2003), (Batson et al., 2002), (Kabius et al., 2002), (Lentzen et al., 2002), (Haider et al., 1998), (Zach et al. 1995). The two fundamental aberrations of electron lenses, spherical and chromatic aberration (Scherzer, 1936), scale with the dimensions of the lens. Resolution requirements for hard-matter investigations are pushing the designs for lenses with only a few mm to sub-mm space in the pole-piece gap for the specimen. This however, this is in conflict with the requirements for biological cryo-electron microscopy (cryo-EM) investigations, where increasingly more space is needed between the pole pieces for specimen rotation and cooling. One possible solution to the problem emerging when attempting to obtain both, a larger pole-piece gap and higher spatial resolution is the use of aberration correctors. Recently, the spherical aberration barrier has been broken by introducing spherical aberration correctors. The consideration of aberration corrected microscopy to improve biological imaging, especially cryo-EM has not been reported so far. In our study we have explored the possibilities of a Cs-corrected TEM for investigations on biological samples, particularly single macromolecular complexes. For our experiments we utilized a Cs-corrected FEI Titan 80-300 TEM, operating with a field emission gun (FEG) at 300 kV acceleration voltage, and equipped with a Cs-corrector (CEOS GmbH, Heidelberg, Germany) and a Gatan 2kx2k CCD camera. A number of experiments were performed using Cs=1.2, 0 and -0.1mm respectively, to analyze imaging conditions, spatial resolution and image contrast, and the resulting advantages and disadvantages of using a Cs-corrector for biological TEM investigations.

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# CORRELATION OF MICROTUBULE PLUS END CONFIGURATIONS TO THEIR DYNAMIC STATE IN SITU USING ELECTRON MICROSCOPY AND ELECTRON TOMOGRAPHY

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Microtubule (MT) network is one of the major components of the cytoskeleton in the eukaryotic cell. Microtubules are involved in various cellular processes including regulation of the cell division, cell motility, general cell morphology, cargo transport, etc. MTs are polymers made of  $\alpha$ - and  $\beta$  tubulin heterodimers organized in head-to-tail manner into protofilaments, which in their turn form a hollow tube with a diameter of 25nm. One of the intrinsic properties of MTs is their polarity, which manifests in the presence of a dynamic plus end and a 'stationary' minus end (in cell localized in the microtubule-organizing center). The plus end oscillates between the shrinking and growing state, a process called 'dynamic instability'. In vitro studies suggest that these phases of shrinkage and growth correspond to a specific structure of the plus end. Three major conformations of the plus end are described on the basis of this in vitro work: frayed ('rams-horn-like') end, blunt end (intermediate state), and sheet-like end, which correspond to shrinking, pausing and elongating MTs respectively. Our aim was to visualize the MT plus end conformations and to investigate the relation between these conformations and plus end dynamics in situ. For this purpose we performed electron microscopy and electron tomography on cryo-fixed, freeze-substituted, plastic embedded mouse fibroblasts. To our knowledge, this approach enabled us to be the first to visualize the MT plus end conformations in the periphery of mammalian interphase cells. Besides the three main plus end conformations (frayed, blunt and sheet-like), we were able to distinguish four other types. Exposing the cells to the MT shrinkage inducing reagent nocodazole allowed us to correlate a number of new configurations to their particular dynamic states. Applying the time-course exposure allowed us to gain insight in the chronology of events during the process of dynamic instability. As a part of 'Molecule to Cell' program, financed by Netherlands Organization for Science Research (NWO), this project is a collaboration including Department of Cell Biology and Genetics, Erasmus Medical Center Rotterdam in Rotterdam, The Netherlands, Biophysical Structural Chemistry, Leiden University in Leiden, The Netherlands and the Leiden University Medical Center for Electron Microscopy in Leiden, The Netherlands. We would like to acknowledge Prof. Dr. Jan Pieter Abrahams, Roman Koning, Jeffrey van Haren and Daniel de Geus for participating in this project.

# PLEIOMORPHIC VIRUS STRUCTURE BY CRYO-ELECTRON TOMOGRAPHY

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Cryo-electron tomography provides the only way currently available to visualize the three-dimensional structures of individual biological particles at the molecular level in a near-native state. We are using this technique to study pleiomorphic viruses whose structures vary stochastically from particle to particle while still employing the same building-blocks. Pleiomorphic viruses account for a substantial proportion of known animal viruses, including some important pathogens. Hitherto, their structural variability has precluded analysis by the conventional approach of cryo-electron microscopy followed by image reconstruction of data sets comprising thousands of particles with or without exploitation of icosahedral symmetry.

For our experimental set-up, we have installed the SerialEM [1] automated data acquisition package on our FEI Tecnai 12 transmission electron microscope. The microscope is equipped with a Gatan energy filter and 2k x 2k pixel CCD camera. Tilt series are collected over angular ranges of 120° to 140° with images acquired at 1° intervals, typically with a total dose around 60-70 e<sup>-</sup>/Å<sup>2</sup>. The tilt series are aligned and reconstructed using IMOD [2]. Denoising is accomplished by nonlinear anisotropic diffusion using the Bsoft [3] package. Resolution of the resulting tomograms is assessed with NLOO [4]. Amira is used for segmentation and visualization of the tomograms. Selected viral components with invariant structure are analyzed after extraction from the tomograms, alignment and averaging using in-house software.

As in an earlier study on Herpes Simplex Virus [5], our current work, which addresses an avian retrovirus, Rous Sarcoma Virus, and influenza virus, has revealed several novel aspects of the structure of these virions. These include information related to the glycoproteins, the matrix protein layer, viral core morphology (in the case of RSV) and ribonucleoprotein particle structure (in the case of influenza virus), as well as interactions between structural components. Future plans include extending these studies to cell entry and exit.

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## ELECTRON TOMOGRAPHY OF THE IMMUNOLOGICAL AND VIROLOGICAL SYNAPSES

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A lymphocyte creates a well-defined contact area called the immunological synapse with its target cell during immune response. It enables the transduction of signals initiating either activation or killing of the target. During synapse formation the cytotoxic T lymphocyte (CTL) is polarized towards the infected target cell. The microtubule organization centre (MTOC) moves to the synapse and lytic granules are transported along the microtubules to release their content into the synaptic cleft thereby inducing the death of the target cell. Certain viruses such as HTLV-1 and HIV-1 utilize the mechanism of synapse formation to spread from an infected to a new host cell. Upon cell-cell contact these viruses trigger the formation of a virological synapse with the infected cell polarized towards the target cell. Viral protein and genome is thought to be transported along microtubules to the synapse, bud into the synaptic cleft and infect the target cell.

Electron tomography of Epon embedded 100-400 nm thick sections of Cytotoxic T lymphocytes with target cells and of HTLV-1 infected cells was used to visualize the 3-D structure of the immunological and virological synapse, respectively. Single and double axis tilt series were recorded with 200 and 300 kV FEG-TEMs on CCD using the TVIPS and the FEI tomography software packages. Tomographic reconstructions were calculated with the software IMOD and data modeling was performed using IMOD and AMIRA.

The tomograms and models obtained allow observing the organization of the synapse, of the cytoskeleton and the organelles within the cells at different stages of synapse formation. Both synapses have membrane-membrane contacts separated by 10-40 nm and pocket-like structured areas. However, the virological synapse is less structured. Whereas lytic granules are selectively released into a sealed degranulation pocket within the immunological synapse, virus buds both into the synaptic cleft and into the extracellular space next to the virological synapse. Electron tomography therefore provides a powerful tool for the understanding of the functionality of immunology and virology related synapses.

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# FOURTH INTERNATIONAL CONGRESS ON ELECTRON TOMOGRAPHY

## Program Committee:

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Bruce McEwen  
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Daniela Nicastro  
Gina Sosinsky  
Niels Volkman  
Grace Osborne (Congress Coordinator)

## Keynote Speaker:

**Dr. Roger Tsien**, Professor of Pharmacology and Chemistry & Biochemistry, Howard Hughes Medical Institute, University of California, San Diego. Dr. Tsien is one of the world's leaders in the field of fluorescent probe design and implementation for biological systems. His research has developed tools to allow pulse-chase determination of the age of individual protein molecules, localization by electron microscopy, and rapid photochemically induced inactivation. His keynote speech will be focused on specific labeling of proteins with applications in light and electron microscopic tomography.

## History of the ICET

The International Congress on Electron Tomography (ICET) provides a forum for structural and cell biologists, materials and computational scientists, and others to exchange information about how electron tomography (ET) elucidates structure-function in cellular arrays and larger assemblies. ET provides a link between high-resolution structural determination and visualization in the native cellular context. Collecting data from a single specimen is the key strength responsible for electron tomography's wide applicability, but this requirement also presents many unique technical and computational challenges.

Prompted by these challenges, Drs. Abraham Koster and David Agard organized the International Congress on Electron Tomography over March 16-19, 1997. Forty-nine attendees convened at Ringberg Castle in Southern Germany for the 1st ICET. In recognition of the importance of electron tomography, meeting manuscripts were published as a special issue of the *Journal of Structural Biology* in December 1997. A second meeting was held four years later in Amsterdam in 2001, and the resulting manuscripts appeared as a special issue of the *Journal of Structural Biology* in 2002. Three years later, attendance at the 3rd ICET in Rensselaerville, New York had tripled and included many international participants new to the electron tomography.

Today, electron tomography is well-established, and has a broad range of applications well beyond those envisioned at the time of the first conference. Electron tomography is in the forefront of current electron microscopic techniques due in part to its practitioners who are among the leading innovators in technology development for electron microscopy.

## NOTES

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