28TH ANNUAL SPRING SYMPOSIUM
MARINE BIOLOGICAL LABORATORY, WOODS HOLE, MA
THE NEW ENGLAND SOCIETY FOR MICROSCOPY

Friday, May 6

9:00 am **Registration:** Swope Center – Coffee and Pastries

10:00 am **Welcome (Meigs Room):** Richard Schalek, *NESM President*

Moderator: Fettah Kosar, *NESM President-elect*

10:10 am **"Facial Reanimation: A Transgenic Approach to Muscle Re-Innervation"**, Alexander Woollard, University College London, UK

10:50 am **"Paleontological Applications of Synchrotron Imaging with Insights into Human Evolution"**, Dr. Tanya Smith, Harvard University.

11:30 am **Vendor/Poster Session**

12:00 pm **Lunch**

1:15 pm **Keynote: "Wide-Field and Stereo Scanning Electron Microscopy: An Introduction to Biodiversity and Biomimetics"**, Dr. James Weaver, Wyss Institute for Biologically Inspired Engineering at Harvard University.

2:15 pm **Afternoon Break:** Coffee and Refreshments

Moderator: David Bell, *NESM Physical Sciences Director*

3:00 pm **"In Situ Experiments in X-Ray Micro Tomography"**, Dr. Eric Maire, Universite de Lyon, France.

3:40 pm **"Nanometer-sized Diamonds as Cathodoluminescent Markers"**, Dr. David Glenn, Harvard University.

4:20 pm **"TEM Sample Prep using FIB Total Release"**, Nicholas Antoniou, Harvard University.

5:00 pm **Closing Remarks:** NESM Board

28TH ANNUAL SPRING SYMPOSIUM
CORPORATE VENDOR EXHIBIT

We thank all our exhibiting vendors for their financial support. A portion of the cost for a table display is used to reduce registration costs for students and retirees.

Beockeler Instruments, RMC Products

Bruker Nano

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10:10 am - **Facial Reanimation: A transgenic approach to muscle re-innervation**

Alex CS Woollard, University College London, UK

Abstract:
There are many causes of facial palsy. Most recover but for those that don't the condition is debilitating. The paralysed side of the face drops causing difficulty with control over eye and mouth closure. Natural expression is blunted, and the loss of a symmetrical smile is what distresses patients most of all. They can become shy and introverted, avoiding social contact. The normal developmental interactions in children are particularly affected.
The gold-standard treatment to restore facial dynamism is a free-functioning muscle transfer where a nerve/muscle complex is positioned in the paralysed side of the face and the functional, contralateral facial nerve is used to power it. A smile on the good side provokes a mimic on the bad. This was pioneered in 1976, however there is still a degree of variability in the results. Research to date has focused on the nature of axonal growth through the nerve graft and the tetanic force generated by the transplanted muscle.
My research uses transgenic mice that express fluorescent proteins in their nerves to examine the patterns of re-innervation within the muscle in a facial palsy model. I hope to explain more of the discrepancies of re-innervation and thus improve the clinical outcomes.

Bio:
Alex went to school at St Paul's School, London (1985-95) and from there attended Southampton University Medical School (1996-2002). During a year's hiatus in 1999 he relocated to the Wellcome Trust to undertake a degree in the History of Medicine. Following an intern year at Southampton General Hospital he moved to University College London to complete his junior surgical rotations and became a Member of the Royal College of Surgeons of England. Since 2007 he has been a plastic surgery resident in London. Last year he began a sabbatical to pursue a higher degree and is currently a Visiting Scholar in Professor Lichtman's laboratory at Harvard University.

10:50 am - **Paleontological Applications of Synchrotron Imaging with Insights into Human Evolution**

Dr. Tanya Smith, Harvard University

Abstract:
During the past few years, synchrotron X-ray microtomographic studies have revealed the internal structure of paleontological samples with a quality far exceeding other virtual methods. The rise of phase contrast synchrotron imaging has created a revolution in non-destructive investigations of fossil samples, including the identification of insects trapped in opaque amber, imaging of microscopic fossils, and the discovery of the only known fossil brain. These techniques are so powerful that roughly 90 percent of the scans currently performed at the European Synchrotron Radiation Facility (ESRF) on fossils use the phase contrast effect. Here we briefly present recent paleontological applications of synchrotron microtomography, followed by a demonstration of paleoanthrological aspects. This includes investigations of dental enamel thickness and tooth root morphology in human and primate fossils, as well as developmental features in fossil hominin enamel and dentine. These studies have provided the

earliest evidence of the modern human life history (developmental) pattern in a 160,000 year old early Homo sapiens individual from Morocco. One of the most powerful uses of this technique is the non-destructive detection of the neonatal (birth) line, in addition to incremental feature periodicities. The lack of these parameters in previous studies has led to broad estimations of developmental timing and age at death in other fossil hominins. Given the recent finding of a rapid developmental profile in several juvenile Neanderthals, it is now possible to trace the origins of modern human life history and to resolve the long-standing debate over developmental differences between Neanderthals and our own species.

Funded by the European Synchrotron Radiation Facility, the Max Planck Society, and Harvard University.

Bio:

Tanya M. Smith is an Assistant Professor in the Department of Human Evolutionary Biology at Harvard University, as well as an Associated Scientist to the Department of Human Evolution at the Max Planck Institute for Evolutionary Anthropology (MPI-EVA: Leipzig, Germany). She received her PhD in Anthropological Sciences from Stony Brook University, and her BS in Biology from the State University of New York at Geneseo. Following her thesis work, Dr. Smith spent four years at the MPI-EVA, where she supervised a dental hard tissue research unit. Tanya has conducted primatological and paleontological field work in Costa Rica, Madagascar, Nicaragua, and Wyoming, in addition to extensive laboratory and museum work in Europe and Africa. She is interested in human and primate evolution, particularly aspects of phylogeny, life history, and taxonomy. Tanya's current research focuses on ape and human dental development, which provides important insight into evolutionary developmental biology. Teeth - an often poorly-appreciated aspect of our anatomy - preserve permanent records of an organism's daily, near-weekly, and yearly biological rhythms. Her work has been featured in National Geographic, Science, Smithsonian, Slate, and Discovery magazines, as well as on PBS, History Channel, Voice of America and BBC broadcast media. At Harvard University she offers courses on human evolutionary anatomy, Neanderthals and evolutionary theory, and a hands-on research seminar on primate dental histology.

1:15 pm - **Wide-Field and Stereo Scanning Electron Microscopy: An Introduction to Biodiversity and Biomimetics**

Dr. James Weaver, Wyss Institute for Biologically Inspired Engineering at Harvard University

Abstract:

There has been significant progress in recent years aimed at pushing the spatial resolution limit of scanning electron microscopes. Many of these endeavors have been driven by advances in the field of nanotechnology and the need to investigate the morphological features of sub-micron size materials. While scanning electron microscopy is indeed a powerful tool for investigating objects at length-scales that are prohibitive using standard optical microscopy techniques, SEMs are also extremely useful in characterizing the micro- and macro-scale architectures of transparent, highly reflective, or morphologically complex materials. In this presentation, two scanning-electron microscopy imaging techniques (stereo and wide-field) will be introduced and applied to the imaging of a wide range of biological structural materials across length scales covering more than 5 orders of magnitude (less than 10μm to greater than 10cm). The talk is intended for a broad audience and is likely to appeal to anyone interested in microscopy, structural biology, materials science, and vertebrate and invertebrate biodiversity. 3D glasses will be provided to all attendees

Bio:

James Weaver received his Bachelor's degree in Aquatic Biology and PhD in Marine Science from the University of California, Santa Barbara. Working at the interface between zoology and materials science,

his main research interests focus on investigating structure function relationships in hierarchically ordered biological composites. He has played critical roles in the development of various model systems for the study of a wide range of biomineralization processes and is an internationally recognized scanning electron microscopist. With a strong history of national and international academic and industrial collaborations, he has coauthored more than 40 journal articles in the biological, physical, and geological sciences.

3:00 pm - ***In Situ*** **Experiments in X-Ray Micro Tomography**

Dr. Eric Maire, Universite de Lyon, France

Abstract:
X-ray imaging is useful in materials science because it allows information to be retrieved non-destructively from the interior of opaque samples. X-ray radiography has then been used for many years and more recently, X-ray computed micro tomography has emerged as a complementary and more powerful technique of visualization. Imaging is used in materials science because in order to optimize the properties of materials, a thorough understanding of the mechanisms responsible for these properties is crucial. Although post mortem observation can be a simple solution, it is more desirable to observe *in situ* the mechanisms at play. In this talk, we will recall some basic principles of X-ray imaging and then we will focus on different examples where *in situ* experiments have been carried out in X-ray radio or tomography. These examples include tensile and compression tests on ductile metals or foams and freezing of colloidal suspensions.

Bio:
Eric Maire graduated as a materials science engineer and then obtained a PhD in the Mateis Laboratory in the INSA school in Lyon France. He then did a 1.5 years post doc at McMaster University Ontario Canada and was appointed research associated by the French CNRS in his previous laboratory Mateis. Since his appointment in 1997, Maire is developing research in two main fields: ductile fracture of metals and deformation of cellular materials. He has a world acknowledged experience in using non-destructive imaging methods (synchrotron X-ray imaging or diffraction) coupled with *in situ* thermal or mechanical loading to observe the local modification of the microstructure.

3:40 pm - **Nanometer-sized Diamonds as Cathodoluminescent Markers**

Dr. David Glenn, Harvard University

Abstract:
I will describe recent work towards the development of a nanoscale imaging technique based on cathodoluminescence (CL) emitted by color centers in nanodiamonds (NDs) under excitation by an electron beam in a scanning electron microscope (SEM). We have identified several classes of color centers that are spectrally distinct at room temperature and can be obtained with high reliability in NDs with diameters on the order of 100 nm or smaller. Compared to conventional fluorophores, ND color centers are bright and highly stable under SEM excitation. In conjunction with appropriate functionalization of the ND surfaces, ND-CL will provide nanoscale information about molecular function to augment the structural information obtained with standard SEM techniques.

Bio:
David Glenn completed his PhD in atomic physics 2009 in the group of David DeMille at Yale University. His work there focused on the development of optical and microwave tools for decelerating and trapping cryogenic beams of diatomic molecules. He is now a postdoc in Ron Walsworth's group at the Harvard-Smithsonian Center for Astrophysics. His current interests include super-resolution optical imaging, as well as the use of nitrogen-vacancy centers and other defects in diamond for magnetometry and bio-imaging.

4:20 pm - **TEM Sample Prep using FIB Total Release**

Nicholas Antoniou, Harvard University

Abstract:
Sample preparation for TEM imaging has evolved dramatically since the development of two beam (FIB and SEM) systems and associated peripherals specific to this application. Lift out probes, gas chemistries and low acceleration FIB operation have facilitated TEM sample preparation and shortened the time it takes to complete this task. Any sample preparation technique can introduce artifacts but the purpose of this presentation is to introduce a very fast process for FIB/SEM sample preparation that was developed on the CNS Crossbeam systems. The process is called Total Release and is patented by Omniprobe. We adapted and continued the development of this process at CNS on our equipment set.
It has been demonstrated that with this process and under ideal conditions a sample can be prepared in about one hour. This is a significant time savings over any other technique. The quality of the lamella is not compromised by the speed this process. The time savings is realized by removing the minimal amount of material necessary to release a lamella from the bulk sample. In addition to being very fast, this technique is more robust and has a greater margin of error and therefore higher yield.
Instead of clearing large areas in front and back of the lamella, a wedge is formed by milling 3 sides at 0 degree FIB angle of incidence and one angled cut at 54 degree FIB angle of incidence. A reference calculator can estimate the depth of the wedge based on the space between the angled and vertical cuts so that the user can create the desired size membrane.
This process will be presented in detail and is also offered as a training event at CNS.

Bio:
Nicholas Antoniou is the Principal FIB engineer at CNS/Harvard. Nicholas received his Bachelor of Science and Master of Science degrees in Electrical Engineering from Texas A&M University. He has over 20 years of work experience in the electronics field having worked in semiconductor fabrication facilities for Motorola Inc. in Austin, TX, microprocessor product engineering at Ross Technology also in Austin, and for FIB product management at FEI Company in Peabody, MA. Nicholas was the General Chair of ISTFA (International Symposium on Test and Failure Analysis) in 2009 and is currently growing his microscopy skills and knowledge.

Combinatorial Labeling and Spectral Imaging, (CLASI) to Characterize Microbial Community Structure

A.M. Valm, R. Oldenbourg, Brown University and Marine Biological Laboratory; G.G. Borisy, Marine Biological Laboratory

The ability to distinguish more than a few different labels in a single fluorescence image is severely hampered by the crosstalk and signal bleed-through of fluorophores with highly overlapping excitation and emission spectra. Here, we report the development of a fluorescence labeling, imaging, and analysis method to greatly expand the number of identifiable labels in a single image. The CLASI method involves labeling targets with specific combinations of fluorophore reporters. Spectrally recorded images are analyzed with novel linear unmixing algorithms designed to identify specific combinations of fluorophores.

As proof-of-principle, we have imaged mixtures of E. coli labeled with combinations of organic fluorophores and demonstrate that we can distinguish 120 differently labeled microbes in a mixture using binary combinations of 16 fluorophores. Application of the combinatorial strategy using fluorescent in situ hybridization (FISH) with 15 taxon-specific probes enabled the analysis of the spatial structure of human dental plaque, a multi-species microbial biofilm. We quantified the frequency of inter-and intra-taxon cell-to-cell associations and identified a total of 36 statistically significant inter-taxon pairings among 12 taxa in the extracted biofilm.

Application of our CLASI-FISH method resulted in the first quantitative analysis of the microscopic spatial relationships of microbes of fifteen different taxa in a complex microbial community. Development of this technology will allow a comprehensive understanding of the normal human oral flora and foster the development of new and effective strategies to study, monitor, and control infection. In future, the CLASI approach will be useful for the systems level analysis of many complex microscopic biological structures, including the simultaneous visualization and identification of multiple phenotypic markers on single cells or tissues.

Protein Expression in Mouse Salivary Glands: Effects of Spaceflight

M.I. Mednieks, M. Tsesis, D. Manz, N. Larson, A.R. Hand
University of Connecticut School of Dental Medicine, Farmington, CT, USA

Exposure to zero gravity (ØG) during spaceflight alters physiology and protein expression in many tissues. Rats flown on SpaceLab 3 exhibited changes in heart and salivary gland ultrastructure and expression of the type II regulatory subunit (RII) of protein kinase A (PKA) (Mednieks and Hand, Am J Physiol 252:233, 1987).

Objective: To determine changes in salivary gland structure and protein expression of mice flown on Space Shuttle Discovery.
Methods: Adult female C57Bl/6J mice housed in Animal Enclosure Modules (AEMs) were flown on the 15-day STS-131 mission. Ground control mice were housed in AEMs for the same length of time. Tissue collection occurred within 5 hr of landing. Salivary glands were fixed and processed for ultrastructural analysis and immunogold labeling, or frozen at -80oC for determining protein expression using gel electrophoresis and Western blotting.
Results: Autophagic vacuoles and apoptotic cells were observed more frequently in parotid glands of flight animals, otherwise the morphology was similar to that of controls. Quantitative immunogold labeling showed a significant decrease of PKA RII in acinar secretory granules and the cytoplasm ($p<0.01$). No differences were seen in labeling for parotid secretory protein (PSP). Electrophoretic analysis showed several changes in protein banding patterns, and Western blotting confirmed the substantial decrease in RII, as well as amylase, in parotid tissue extracts of flight animals compared to controls. In the submandibular gland no significant differences in RII were found between flight and control mice and neither reacted with anti-amylase antibody. Thus, ØG modifications in protein expression are not universal, but are cell and tissue dependent indicating functional specificity.
Conclusions: The results show that protein expression in salivary glands is altered by travel in space. These findings may form a basis for developing analyses using saliva for testing the effects of spaceflight on astronauts.
Support: NASA grant NNX09AP13G

Spatial distribution of human gut bacteria in a gnotobiotic mouse model

Y. Hasegawa, Brown University and Marine Biological Laboratory; J. Mark-Welch, G.G. Borisy, Marine Biological Laboratory

Activities of intestinal bacterial communities are known to be crucial for maintaining human health. The goal of this project is the development of a novel imaging-based assay to study spatial arrangements of bacteria in intestines of gnotobiotic mice inoculated with a synthetic human intestinal bacterial community. The gnotobiotic mice were created by inoculating fifteen human intestinal bacteria, including Bacteroidetes, Firmicutes and Actinobacteria, into germ-free mice. In this study, we (1) explored protocols to preserve spatial distribution of bacteria in intestinal sections and (2) demonstrated the ability to simultaneously distinguish multiple bacterial species using fluorescence in situ hybridization (FISH) combined with spectral imaging. Our experiments showed that spatial arrangements of intestinal contents, including bacterial cells and food particles, were successfully preserved in sections of plastic (glycol methacrylate) embedded intestines. Furthermore, six or more bacterial species labeled with FISH probes were simultaneously distinguished by spectral imaging analysis, and our preliminary analysis of micron-scale bacterial spatial distribution revealed enrichment patterns of certain bacterial species in certain areas of the intestinal sections.