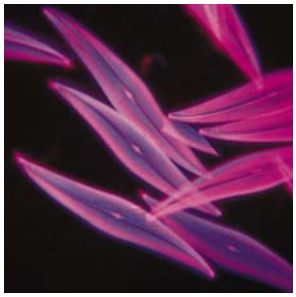
[AIMS] Arizona Imaging and Microanalysis Society

Using light, electrons, ions, electromagnetism and x-rays

Program & Abstracts

April 17, 2008
Radisson Woodlands Hotel
1175 W Route 66
Flagstaff, AZ 86001



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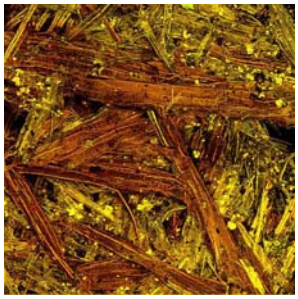

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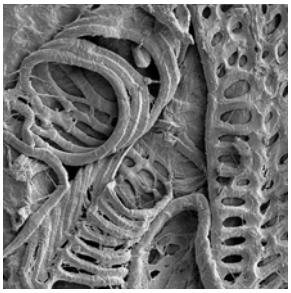

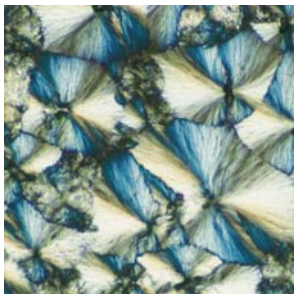

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Arizona Imaging and Microanalysis Society Meeting – April 17, 2008

Program

Check-In
| 11:30 – 12:30 |

First Session
Welcome and Opening remarks
Marilee Sellers - AIMS President
| 12:30 - 12:45 |

Presentation 1 – 60 mins
William Landis, Ph.D. Department of Microbiology, Immunology and Biochemistry, Northeastern Ohio Universities College of Medicine (NEOUCOM)
"Tissue engineering of models of human digits and ears"
| 12:45- 1:45 |

Presentation 2 – 30 mins
Steve Pfeiffer, Gatan Inc.
"*SBSFM: bridging the gap between confocal & TEM"*
| 1:30 - 2:00 |

Student Poster Presentations 3 – 45 mins
Presentations by students and judging of student posters
| 2:00 - 2:45 |

Afternoon Break – Vendor exhibits and poster viewing and judging Coffee and Pastries – 45 mins
| 2:45– 3:30|

Presentation 4 – 30 mins
Donald Speer, M.D., Professor Emeritus, Department of Orthopaedic Surgery at the University of Arizona Medical Center
"*Uses of Polarized Light for Imaging and Analysis"*
|4:00 – 4:30 |

Presentation 5 – 30 mins
Tim Vail, Ph.D., Department of Chemistry/Biochemistry, NAU
"Nanotechnology and the Diagnosis of Methicillin-Resistant /Staphylococcus/ Biofilms"
| 4:30 – 5:00 |

Presentation 6 – 30 mins
Charles Kazilek, School of Life Sciences, Arizona State University
"Through the Looking Glass in 3-D: engaging young minds in the classroom and beyond"
| 5:00 – 5:30 |

Dinner Buffet – 60 mins
| 5:30 - 6:30 |

Presentation 7 – 60 mins
Allen West, Ph.D., GeoScience Consulting
"Extraterrestrial Markers Found at Clovis Sites Across North America"
| 6:30 - 7:30 |

Closing Remarks and Student Award Announcement – 30 mins
Presentation of student awards
| 7:30 - 8:00 |

There will be a brief business meeting following the conference. Officers and members are invited to attend.

ABSTRACTS

William J. Landis, Department of Microbiology, Immunology and Biochemistry, Northeastern Ohio Universities College of Medicine (NEOUCOM), Rootstown, Ohio. (330) 325-6685; wjl@neoucom.edu
"Tissue engineering of models of human digits and ears"

Tissue engineering is a relatively new and potential powerful means of augmenting, repairing, and replacing various tissues that may be congenitally defective, injured, diseased, damaged or otherwise impaired in the human body. The approach of tissue engineering commonly involves seeding isolates of specific cells onto a biodegradable polymer scaffold to form a cell/scaffold construct. The construct is subsequently developed in vitro or in situ for ultimate use as a possible replacement tissue. Bone and cartilage structures, such as a human digit or ear, have now been modeled by tissue engineering methods. The presentation will describe by light and electron microscopy, correlated with laser capture microdissection and gene expression, the tissue engineering of current models of human phalanges and ears. Compared to bone and cartilage in vivo, these models demonstrate several similarities in structure, composition, and response to mechanical forces and they suggest great promise for further advances in clinical applications.

Steve Pfeiffer, Gatan, Inc.
"SBSFM: bridging the gap between confocal & TEM."

Serial Block Face Scanning Electron Microscopy (SBFSM) is a new technique allowing the automatic acquisition of serial images at the ultrastructural level in an SEM. This technique was developed by Denk & Horstmann at the MPI, Heidelberg. Using an automated ultramicrotome mounted within the SEM chamber, a 20 to 50nm layer is shaved off a block of standardly embedded TEM sample. The freshly exposed block face is then imaged in back scatter mode. This is repeated over a distance of up to 400 microns. Images are acquired in perfect serial section registration, with no subsequent image realignment necessary. Large block faces (500 micron by 500 micron) have been imaged with this technique.

This technique is meant to bridge the gap between confocal microscopy & TEM serial section reconstruction. While not having quite the resolution of a TEM image, resolution is more than adequate to reconstruction neurons over large volumes. A number of serial image series will be shown.

Donald P. Speer, MD, Department of Orthopaedic Surgery University of Arizona
"Uses of Polarized Light for Imaging and Analysis"

Polarized light microscopy (PLM) is a venerable but generally underutilized method for assessing organic (biological) materials. Most commonly, birefringent properties of crystalline or anisotropic materials are exploited using PLM. Other optical properties of materials and, significantly, of common histological stains are considered less often, or not at all, in biological applications. Study of serial sections prepared (1) unstained, (2) with H&E staining and (3) with metachromatic staining (typically Mason trichrome or toluidine blue) permits correlation of several properties of target tissues and/or materials. Rotation of histological specimens between the polarizer and analyzer affords a "cinematic" image of broad areas, allowing two- and three-dimensional reconstruction of tissues and/or materials. Introduction of various filters or wave plates is a useful adjunct, not only for analysis but for enhancement of photomicrographs.

Several applications of these protocols helpful in our studies will be reviewed, including:

1. Reconstruction of normal collagen microarchitecture of growth plate and articular cartilage;
2. Comparison of chemical crosslinking methods in the biodegradation of an extruded collagen filament implant material;
3. Assessment of biological fixation of a collagen implant to bone.

Timothy Vail, Ph.D., Department of Chemistry/Biochemistry Northern Arizona University
****Nanotechnology and the Diagnosis of Methicillin-Resistant /Staphylococcus/ Biofilms****

Infectious biofilms result from bacterial communities encased within an exopolysaccharide matrix. They are resistant to antibiotics and the host's immune defenses. Biofilms are often associated with indwelling medical devices, including cathers, prosthetic heart valves, and prosthetic joints. There are currently no approved biofilm-specific point-of-care diagnostic devices or therapeutics. This presentation will focus on the development of a biofilm-specific rapid assay that utilizes nanoparticle colloidal gold coupled with the host's own immune response as a probe-reporter pair. Implications for further development of nanoparticle-based therapeutics will also be discussed.

Charles Kazilek, Director of Technology Integration and Outreach, School of Life Sciences, Arizona State University
"Through the Looking Glass in 3-D: engaging young minds in the classroom and beyond"

Capturing the attention of young minds in the sciences can be a challenge. Often we place the details of science first and leave the fun things to the last. For microscopy this need not be the case and in fact microscopic images can be some of the most exciting content students come in contact with in the classroom and on the Web. The Paper Project chronicles handmade and mould made paper images produced by a scanning-laser confocal microscope. As a scientific tool confocal microscopy has clearly shined. Beyond its use in scientific research this instrument has also provided a wealth of images and content that can be used in the classroom and at home to engage people of all ages. In its ninth year, the Paper Project has grown from a small web-based project, to an extensive program that includes a touring gallery exhibit, a 3-D immersive room installation, and a dance performance. Educators are able to use multiple content areas and activities from the Paper Project web site to capture the imagination of their students and stimulate their interest in science.

Allen West
GeoScience Consulting, Dewey, AZ 86327
Email: *allen7633@aol.com*
"Extraterrestrial Markers Found at Clovis Sites Across North America"

There is substantial evidence on Earth for an extraterrestrial (ET) impact event about 12,900 years ago. We propose that it triggered severe changes in climate and led to abrupt environmental changes that contributed to broad-scale extinctions and rapid human behavioral changes at the end of the Clovis Paleoindian Period. Twenty-five 12,900-year-old sites in North America are marked by a thin, discrete layer, containing varying peak abundances of many ET markers, including nanodiamonds, magnetic microspherules, carbon spherules, soot, fullerenes with ET helium, and iridium. The talk will include data from a number of well- known Clovis sites, including Murray Springs, AZ, near Sierra Vista, which is one of the best known Clovis mammoth kill-sites. There, a distinctive carbon-rich impact-related layer, called a "black mat," lies above extinct mammoth bones, and a thin layer, containing the impact event markers, lies just under the black mat and drapes over the mammoth bones and Clovis artifacts.

STUDENT ABSTRACTS

Remote Microscopy for Education and Outreach

K E Dorame, Steven Hernandez, Supapan Seraphin, Gary Chandler, David Bentley

Scanning electron microscopy and X-ray analysis are standard tools in the nanotechnology world, but access to them by students is limited by the high cost and limited number of instruments. The Quartz PCI-Taipan system allows students to access the state-of-the-art microscopes, acquire data, and interact with the instrument operator in real time from their computer in any remote setting with an internet connection. Using this technology, we have developed an on-demand remote access system to the existing University Spectroscopy and Imaging Facilities (USIF) of the University of Arizona (UA). We have interfaced the system to our new Hitachi S-4800 FESEM along with EDS and S-3400N variable-pressure SEM with a new Raman/CL spectrometer and EDS, providing a teaching package unequaled anywhere. We continue to test the system and develop procedures that will work with various computer networks. One of the goals is to test collaboration curriculum for use in the classroom at the UA and NAU. Examples of classroom activities with step-by-step procedure will be presented and challenges with our solutions will be discussed.

Reference:

[1] Quartz PCI Taipan brochure, http://www.marinereef.com/pdfs/taipan.pdf

Simultaneous Raman Spectroscopy and SEM Analysis of Carbon Nanotubes

Binh Duong*, Yitian Peng**, Margo Ellis*, Supapan Seraphin*, and Hao Xin**

*Dept. Materials Science and Engineering, University of Arizona, Tucson, AZ 85721
**Dept. Electrical and Computer Engineering, University of Arizona, Tucson, AZ 85721

Both SEM and Raman spectroscopy have been used extensively to determine morphology and optical properties of carbon nanotubes (CNTs), respectively. Each technique was used separately. Only recently that it is possible to analyze samples with both techniques simultaneously using Hitachi S3400 equipped with the Renishaw Structural and Chemical Analyzer. The major advantage of combining Raman with the SEM includes the unique ability to obtain critical information on morphological, physical and electronic structure from the same area at a high-spatial resolution. In this work, SEM images show CNTs of about 20 nm diameter. On the other hand, Raman spectra show that the tubes are bundles of single-walled CNTs which have diameter of about 1.5 nm. Correlations of morphology and processing conditions will be presented.

A SEM-Structural Chemical Analyzer Study of Multi-Walled Carbon Nanotubes Grown by CVD

M. Ellis,* T. Jutarosaga,** S.M. Smith,*** Y. Wei,*** and S. Seraphin*

* Department of Materials Science and Engineering, University of Arizona, Tucson, Arizona
** Department of Physics, King Mongkut's University of Technology Thonburi, Bangkok, Thailand
*** Physical and Digital Realization Research Laboratory, Motorola Labs, Tempe, Arizona

Carbon nanotubes (CNTs) are popular due to their unique electrical, optical and structural properties that are desirable for many types of applications. These properties are greatly affected by CNT structure which can be altered by applying various growing conditions. In this study, CNTs are grown by a chemical vapor deposition (CVD) process using two different catalysts. A combined SEM and Structural Chemical Analyzer instrument (SEM-SCA) and TEM are used to compare the optical (Raman) and structural properties of the CNTs. The SEM-SCA is a relatively new instrument configuration, therefore, in order to optimize Raman acquisition parameters, this study examines sample preparation methods for SEM-SCA analysis and how laser power setting and neutral density (ND) filter attenuation affect the Raman responses of the CNTs. Results for the optimization of Raman acquisition parameters and the optical and structural properties of the CNTs will be discussed.

Abnormal spore wall formation in a Chlamydomonas mutant

Timothy R. McBride
Department of Biology, Northern Arizona University, Flagstaff, Arizona.

Chlamydomonas monoica Strehlow is a model for analysis of algal zygospore genetics and morphology. Preliminary ultrastructural analyses of the wildtype zygospore have focused on zygospore morphogenesis and have provided evidence for the role of wall components in conferring abiotic stress resistance in zygospores. To further understand zygospore wall assembly, and to determine the composition of the stratified wall, a bumpy zygospore (buz) mutant with abnormal zygospore wall formation was investigated. Light microscopy (LM) and scanning electron microscopy (SEM) revealed zygospores with unusual surface projections of various sizes and shapes and considerable variability in phenotype, ranging from zygospores with large bulbous projections to those with a near-wildtype phenotype. LM showed that bump frequency increased over time during zygospore mophogenesis. Transmission electron microscopy (TEM) was used to compare buz mutant and wildtype ultrastructure at several time points during zygospore development and revealed abnormalities in wall assembly. Fluorescence microscopy revealed a blue autofluorescent ring around the periphery of mutant and wild type zygospores when exposed to ultraviolet (UV) light. Although this blue autofluorescence is absent from the buz bumps, they stain with the fluorochrome Auromine O suggesting the presence of a cutin or suberin-like component. The buz mutation, which is controlled by a single, recessive nuclear gene, also results in chloroform vapor sensitivity in buz zygospores. However, even in the absence of chloroform exposure, germination

of buz zygospores is less than 50% of wildtype. The decreased germination efficiency may be due to excess wall material that prevents degradation of the zygospore wall and release of the progeny cells produced by meiotic germination.

Very Low Energy TEM: Diffraction from Nanostructures and Holography of Small Electric Fields

B. McMorran and A. Cronin

Department of Physics, University of Arizona, 1118 E 4th St, Tucson, AZ 85721

We built a very low energy (500 eV to 5 keV) TEM. We use this device to analyze nanoscale gratings and very small electric fields around charged tips. The gratings can be thought of as artificial crystals - large arrays of closely-spaced slits etched all the way through material membranes. Using them to create low energy electron interference fringes, we are able to image electric fields of roughly 0.1 V per 100 µm. We find that low energy electrons are coherently transmitted through the slits of the grating, but there is a non-uniform refractive index within the free space of each slit. This refractive index is mediated by the self-induced image potential of the electrons as they pass through the slits. Here we discuss the microscope, and the imaging modes used to analyze the gratings and the charged tips.

In Situ Synthesis of Fe Catalyst and Carbon Nanotubes by Chemical Vapor Deposition

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[1] School of Materials, Arizona State University, Tempe, AZ 85287
[2] Department of Physics, Arizona State University, Tempe, AZ 85287

Carbon nanotubes (CNT) have many advantageous properties and potential for diverse materials applications if they can be grown in a controlled manner. In-situ observations of their synthesis by chemical vapor deposition (CVD) can reveal insights into their possible growth mechanisms. Using an environmental TEM, we deposited Fe nanoparticles on SiO2 support by the thermal decomposition of Fe2(CO)9 vapours. Subsequently, these particles catalyzed the growth of multiwalled CNT from an acetylene precursor. We were able to observe the nucleation and growth of CNTs from the catalyst particles as well as the deactivation of catalyst particles by encapsulation with graphene. We will present images and video clips of these reactions as well as a growth rate analysis of a video sequence in which carbon nanotubes repeatedly grow and detach from the same catalyst particle.

Cytotoxicity, mutagenicity and cellular localization of insoluble depleted uranium in CHO AA8 and human lung 16HBE14o cells

Michelle R. Romanotto[1], Virginia H. Coryell[1], Monica Yellowhair[2], R. Clark Lantz[3], Diane M. Stearns[1]

[1]Department of Chemistry and Biochemistry, Northern Arizona University, Flagstaff, AZ;
[2]Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ;
[3]Department of Cell Biology & Anatomy, University of Arizona, Tucson, AZ

The heavy metal uranium is a suspected human carcinogen with both chemical and radiological effects. Superimposed on its possible modes of action is the influence of its solubility. We have previously shown that DU as soluble uranyl acetate (UA) is mutagenic in repair-deficient Chinese hamster ovary (CHO) EM9 cells through direct DNA damage that includes strand breaks and uranium-DNA adducts. The aim of the current study was to compare the toxicity of depleted uranium (DU) in its soluble and insoluble forms. Repair proficient CHO AA8 and repair deficient CHO EM9 cells were exposed to DU as soluble UA or as particulate UO_2, UO_3, or U_3O_8 for 24 hr. Cytotoxicity was measured by clonogenic survival, mutagenicity was measured at the hypoxanthine (guanine) phosphoribosyltransferase (*hprt*) locus, and localization of uranium particulates was visualized by transmission electron microscopy (TEM). All three particulate DU species exhibited a dose-related decrease in cell survival with cytotoxicity of $UO_3 > UO_2 > U_3O_8 >> UA$ on a micromol/cm^3 basis. Similar cytotoxicity results were also observed in the human bronchial epithelial cell line 16HBE14o; however, the human cell line appears to be more sensitive to particulate uranium than the CHO line. The particulate U compounds were only weakly mutagenic at the *hprt* locus in CHO cells. TEM detected differing distributions of UO_2, UO_3 and U_3O_8 particulates within CHO cells. These high valent particulate depleted uranium oxide complexes model those formed upon burning of depleted uranium metal. Therefore, their enhanced toxicity relative to soluble DU necessitates further investigation to assess the possible risks in humans exposed to insoluble DU. (Supported by NIH Grants CA96320 (DMS), CA096281 (RCL), F31ES014971 (MY), and The Alfred P. Sloan Foundation (MY))