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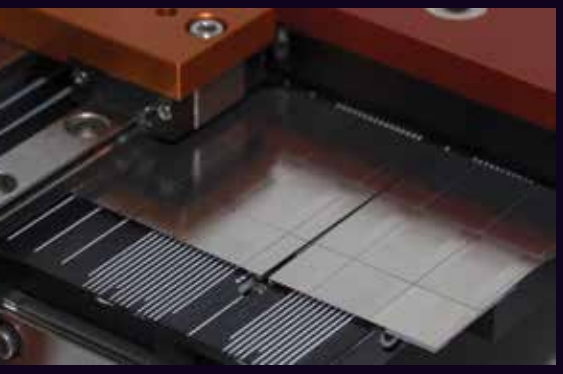

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Electron Microscopy Sciences

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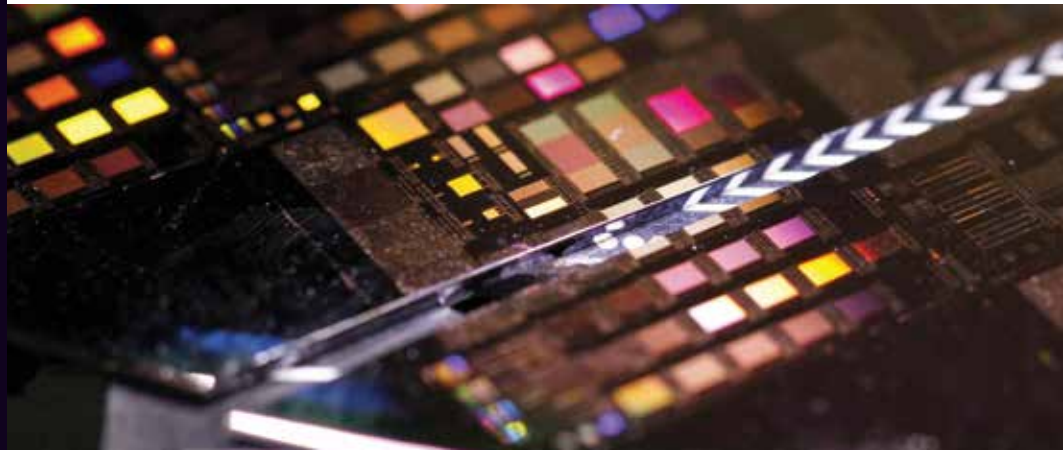

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"TSM - Embracing all forms of Microscopy"
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ON THE COVER

The pieces of puzzle are confocal microscopy images that demonstrate the co-localization of HDAC3 and H1.3 proteins to the polar microtubules and spindle poles in HeLa cells during various mitotic stages. The antibodies used for the indirect immunofluorescence staining were: anti-HDAC3 (green), anti-histone H1.3 (red), anti-Eg5 (polar microtubules motor protein - used as a positive control) (green/red), and the DNA stain - Hoechst (blue). Dr. Michael Bergel's research focuses on identifying novel chromatin-associated targets for cancer therapy and cancer prevention. Cover image by Sanil Sansar (PhD student), TWU, Dept. of Biology, Denton, Texas 76204-5799.






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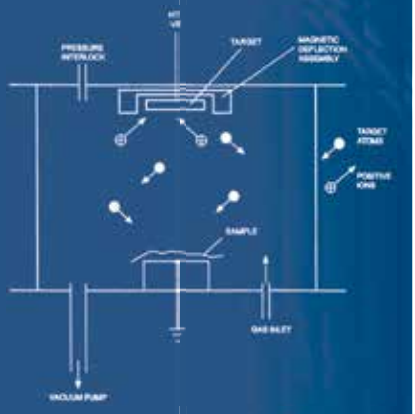

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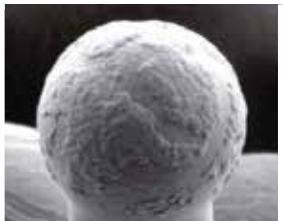
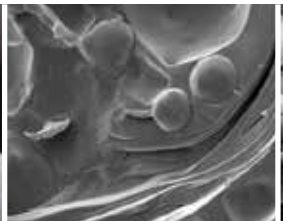
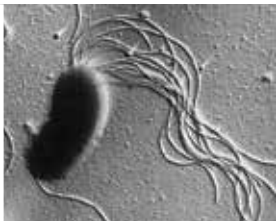



President's Message

The mission statement for the Texas Society for Microscopy (TSM) states "The purpose of this Society is to further the use, understanding, and knowledge of all aspects of microscopy and their applications as they apply to life sciences, material sciences, and industry." These words guided the TSM officers as they planned the 51st meeting of the Society. It is with great pleasure that we return for our meeting this year to Rice University in Houston, where the Texas Society for Electron Microscopy (now TSM) was founded 51 years ago.

On behalf of the Society, I would like to thank our hosts at Rice University including Emilie Ringe, Assistant Professor of Materials Science and Nanoengineering and Chemistry, and Antony Stender, Post-doctoral Researcher, and local arrangements specialist. In addition, I would like to thank the sponsors for our two workshops on Thursday, February 18. The JEOL workshop will be held at Rice University, Department of Earth Sciences and the Hitachi/Bruker workshops at the Baylor College of Medicine. Our corporate members who sponsor workshops and bring the latest research tools and technology to our meetings for display are a vital part of our membership.

We will be honored with three outstanding guest speakers at the 2016 TSM meeting. Dr. Ilke Arslan of Pacific Northwest National Laboratory will speak on her research on 3D nanoparticles. Dr. Wah Chiu of Baylor College of Medicine will share his research on cryo electron microscopy. Dr. Tom Nuhfer from Carnegie Mellon University, sponsored by FEI, will present results on his work with Plasma FIB Dual Beam and CD Backscattered Diffraction.

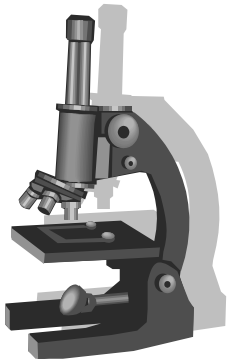
Journal Editor Camelia Maier reports that the 2016 TSM Meeting will have 24 platform and poster presentations, including those by many students. Students will have the opportunity to compete for the 'Howard Arnott' Outstanding Student Award, which is given annually in honor of Dr. Howard Arnott, TSM member emeritus. TSM has a proud history of serving as an incubator for student talent as many students attend our meetings and present their research in a supportive environment.

I would like to express thanks to the officers of TSM who have worked diligently to make this year of 2015-16 a success for the society. Stephen Mick, Program Chairman, has worked tirelessly to coordinate endless details of this year's meeting. Corporate Representative, James Long, has been our contact person for the corporate members and has assisted with workshop coordination. Secretary, David Yan, has patiently updated member records and sent numerous mailings and email communications to the membership. David Garrett has used his talents to attend to the duties of treasurer. As Journal Editor, Camelia Maier has designed and produced our 47th issue of the Texas Journal of Microscopy. We also thank our Facebook Designer and Journal Co-Editor, Nabarun Ghosh, who has kept us socially networked. Jiechao Jiang has served as both our Webmaster and Past-President. We also thank our Student Representative, Minghui Zhang, for sharing her student voice and perspective.

New officers who will be joining the Executive Council in 2016-17 include Laura Hanson as President and Bernd Zechmann as Program-Chairman. We look forward to new ideas and initiatives under their leadership. We would like to invite the TSM membership to consider nominations for TSM officers or volunteer to serve as TSM officers in the future Executive Council. Opportunities include serving in the offices of President-elect, Secretary-elect, Treasurer-elect, or Program chair-elect. Bring your talent and share it with the Society, so that it can continue to be a place of networking, growth, and learning for TSM members.

It has been an honor serving as President during the past year.

Sandra L. Westmoreland
TSM President 2015-2016



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CW	1 to 8 mm	45° 35°	50nm to 1μm	Frozen specimens sectioned wet with liquids like ethylene glycol. Set in "W" style boat.
CD	1 to 8 mm	45° 35°	50nm to 1μm	Frozen specimens sectioned dry. Set in "D" style boat.
TS	1 to 8 mm	45° 55°	50nm to 2μm	Thick sections or alternating thick and thin sections.
MT	2 to 8 mm	45° 55°	50nm to 2μm	Industrial materials sectioning. Not tested to the same ultra high standards as the types above, hence their lower price.
LC	4 to 12 mm	45° 55°	0.1μm to 5μm	Frozen specimens to be examined at light microscopy magnifications. Set in "D" or "W" style boat.
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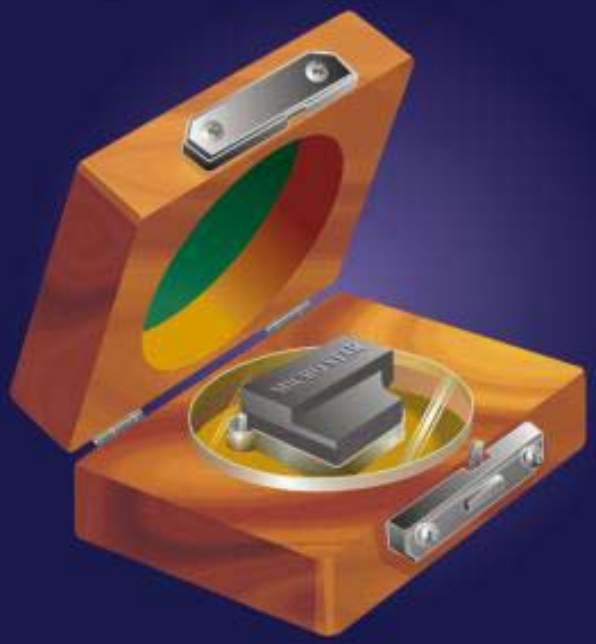
* The standard included angle of 45° is suitable for most applications. Knives with 35° reduce morphological deformation but the edge is more fragile. 55° is recommended for routine hard specimen sectioning. Custom angles and lengths available per request at no extra cost.



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Abstracts

BIOLOGICAL SCIENCES
Spring 2016

SUBCELLULAR IMPORTANCE OF GLUTATHIONE DURING ABIOTIC STRESS. ZECHMANN BERND, Baylor University, Center for Microscopy and Imaging, One Bear Place #97046, Waco, Texas 76798-7046

Glutathione, one of the most important antioxidants in plants is essential for plant defense against abiotic stress. It is involved in the detoxification of reactive oxygen species, redox signaling, modulation of gene expression and regulation of enzymatic activities. Intercellular and intracellular glutathione contents and ratios between certain cell compartments are important indicators of the plants ability to sense and fight oxidative stress providing key information about the physiological condition of the plant. Even though changes in glutathione contents have been extensively studied and its roles in plant defence are well documented, still little is known about its subcellular importance during abiotic stress.

This presentation will give an overview about the compartment-specific importance of glutathione investigated by immunogold cytohistochemistry and computer-supported transmission electron microscopy in plants during abiotic stress conditions such as high light, drought and cadmium exposure (Fig. 1). By comparing wildtype plants and mutants with altered glutathione metabolism it was possible to gain thorough knowledge about the subcellular distribution of glutathione in plants and the importance of these antioxidants in certain cell compartments in protecting against abiotic stress.

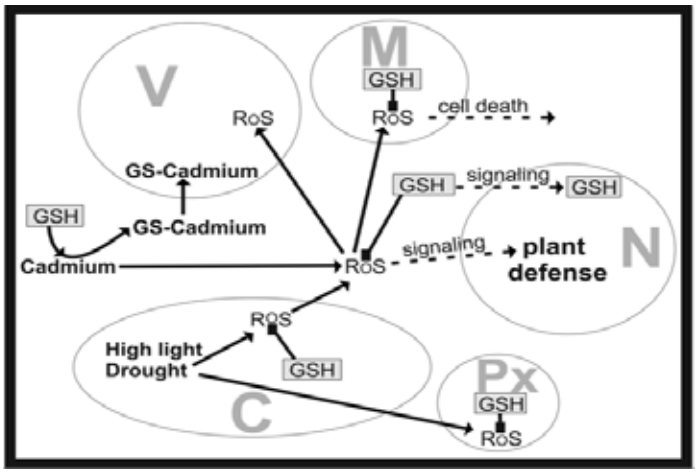

Figure 1. Compartment-specific accumulation of reactive oxygen species (ROS) induced by different abiotic stress conditions and possible detoxification and signaling pathways involving glutathione (GSH). C, chloroplast; M, mitochondrion; N, nucleus; Px, peroxisomes; V vacuoles.

PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF *MEDICAGO TRUNCATULA* TOBACCO RETROTRANSPOSON (*TNT1*)-INSERTION SYMBIOTIC MUTANTS. CATALINA PISLARIU[1,2*], SENJUTI SINHAROY[1,3], JIN NAKASHIMA[1], MICHAEL UDVARDI[1], [1]The Samuel Roberts Noble Foundation, Plant Biology, Ardmore, OK, USA; [2]Texas A&M International University, Laredo, TX; [3]University of Calcutta, Kolkata, India.

Legumes have the unique ability to establish symbiotic associations with nitrogen-fixing bacteria, collectively known as rhizobia. Bacteria convert atmospheric nitrogen into bioavailable ammonium, thus allowing legume hosts to flourish in nutrient depleted soils. A better understanding of the nitrogen-fixing symbiosis at genetic and molecular level could identify methods to make symbiosis more efficient, and reduce or eliminate the need to apply fertilizer nitrogen onto legume crops. Numerous genetic resources have been developed for the model legume *Medicago truncatula* in the last decade, including *Tnt1*-insertion and fast-neutron deletion mutant populations, the Gene Expression Atlas (MtGEA), and the genome sequence. Transcriptomic studies revealed that thousands of plant genes are involved in symbiotic nitrogen fixation (SNF); however, a small fraction of them has been functionally characterized. Uncovering new regulators of the legume-rhizobium symbiosis is crucial for a better understanding of how infection, nodule morphogenesis, and efficient nitrogen fixation take place. From a tobacco retrotransposon *Tnt1*-insertion mutant population of M. *truncatula* ecotype R108, 179 mutants impaired in nodule development and SNF during the interaction with *Sinorhizobium meliloti* were previously isolated (Pislariu *et al.*, 2012). Since only 39 mutants represent alleles of known symbiotic genes, this collection is a valuable resource for cloning novel symbiotic genes via *Tnt1* tagging. Detailed phenotypic characterization of symbiotic mutants using various histological and microscopic techniques is critical in establishing a new gene's role in symbiosis. Several mutants with blocks at different stages of symbiosis will be presented. One of the mutants lacking the activity of a PLAT (Polycystin-1; Lipoxygenase, Alpha-Toxin domain or LH2 (Lipoxygenase Homology 2) superfamily gene, will be discussed in more detail. The gene was named the Nodulation-specific PLAT-domain gene *(MtNPD1).*

MtNPD1 belongs to a cluster of 5 nodule-specific single PLAT domain-encoding genes with non-redundant putative functions. *MtNPD1* is the first single PLAT domain protein that is critical for the maintenance of a mutually beneficial symbiotic association. Using bright field, confocal, and electron microscopy, we demonstrated that the loss of *MtNPD1* function impairs nodule development, rhizobia do not fully elongate into nitrogen-fixing bacteroids, and are lysed soon after being released inside host cells. We also showed, using histochemical staining of Medicago roots expressing an *MtNPD1p-GUS* construct, that *MtNPD1* expression is induced very early during the infection process. Confocal microscopy was used to determine the intracellular localization of MtNPD1 fused to either green fluorescent protein (GFP), or red fluorescent protein (RFP), under the control of the 35S promoter (in tobacco) and the native promoter (in Medicago nodules). Co-localization with fluorescent organellar markers in tobacco epidermis revealed that MtNPD1 is processed in the endoplasmic reticulum (ER), and co-localizes with vacuoles. Confocal imaging of transgenic nodules expressing an MtNPD1p-MtNPD1-GFP fusion demonstrated that, indeed, MtNPD1 localizes to vacuole and ER-like structures, and the pattern of localization is developmentally regulated. While further biochemical assays are needed to determine the exact biological function of MtNPD1, the detailed microscopic characterization of the mutant, as well as the localization of the promoter activity and the protein, helped us determine that this gene is required for the accommodation of rhizobia inside host cells.

CHARACTERIZATION OF LIPID NANODISCS ASSEMBLY BY ELECTRON MICROSCOPY. SVETLA STOILOVA-MCPHIE, Department of Neuroscience and Cell Biology, Sealy Center for Structural Biology and Molecular Biophysics, University of Texas Medical Branch at Galveston, Texas 77555.

Nanodiscs (ND) are discoidal phospholipid bilayers stabilized by two scaffolding proteins (MSP) with a diameter in the 9-16 nm range, suitable for structural and functional studies of membrane proteins. In this study, NDs assembled from 20% galactosylceramide (GC) and 80% dioleoylphosphatidylserine (PS) lipids with two different MSP: MSP1D1 and MSP1E3D1 were characterized by electron microscopy (EM). The role of the MSP to lipids ratio and Ca^{2+} for the assembly of ordered ND stacks was investigated. Results show that ND assembled at MSP1D1 to lipids ratio of 1:40 and MSP1E3D1 to lipids ratio of 1:80 are identical in size (~12 nm diameter). Both ND populations assembled at MSP1E3D1 to lipids ratio 1:80 and 1:150 form ordered stacks at 5mM Ca^{2+}. The number and length of the ND stacks significantly increased with increasing the Ca^{2+} concentration above 5mM and decreasing the MSP1E3D1 to lipids ratio to 1:150. The stacks were formed predominantly from ND with ~16nm diameter leaving the ND with diameter of ~12 nm in a single state after 60 minutes incubation at up to 15mM Ca^{2+}, where Y-branched and side-to-side aggregates were formed. Cryo-EM studies showed that the ND bilayer structure was fully preserved in the stacks that could grow microns in length. The stacking was fully reversible by adding EDTA and could be used for segregating ND with similar size and membrane properties. The presented work gives a new perspective on stable ND assembly at close to physiological lipid and solution conditions that can be easily characterized by EM. The ND developed in this study are suitable for cryo-EM structure determination of membrane-associated proteins, such as blood coagulation factors and complexes, that require Ca^{2+} and PS for functioning.

SEM AND RAMAN SPECTROSCOPY STUDIES OF SILKWORM AND SILK CHARACTERISTICS AS DETERMINED BY MULBERRY SEXUAL DIMORPHISM. YVANTIFFANY NGUYEN[1], MICHAEL DUPLANTY[2], NASRIN MIRSALEH-KOHAN[2], and CAMELIA MAIER[1], [1]Department of Biology and [2]Department of Chemistry and Biochemistry, Texas Woman's University, Denton, TX 76204.

The silkworm, *Bombyx mori,* feeds only on White mulberry, *Morus alba (Moraceae)*, which is a dioecious species represented by individual male and female trees with different morphological, anatomical, and physiological characteristics. Prior research in our laboratory showed a correlation between the sexual dimorphism of mulberry and differences in silk morphology and structure between silkworm feeding groups (male mulberry leaf fed groups vs. female mulberry leaf fed groups). The silk fibers obtained from male-fed silkworms were smoother and thicker than those from the female-fed silkworms. In addition, silk dissolution assays showed that the male-fed silkworm cocoons had significantly more sericin and less fibroin than the female-fed silkworm cocoons (Moraru *et al.,* 2004).

This study employed SEM in observing the silkworm heads, specifically the mandible morphology, and Raman spectroscopy to determine differences in silk structure induced by the feeding method (male or female mulberry leaves). Zebra silkworms raised in spring 2015 were separated into two feeding groups immediately after hatching from eggs. Male and female cocoons were randomly chosen and weighed. In average, male-fed cocoons (including pupae inside) were lighter than those of the female-fed group, although there was no significant difference statistically. There was a significant difference between the cocoons without pupae, in that cocoons from female-fed group were heavier than those of male-fed group (Student t-test, p=0.0007). No significant difference was found between the weights of pupae in the two feeding groups. Molted silkworm heads were collected and visualized with a Hitachi

TM-1000 SEM to determine the effect of calcium deposits in mulberry leaves on the morphology of mandibles. Female mulberry leaves contain more calcium deposits than male leaves. Lengths of two teeth (of four total) per mandible of the third instar silkworms were measured and compiled measurements were compared between feeding groups. On average, the teeth on mandibles from the female-fed silkworms were longer than those on mandibles from the male-fed silkworms. However, statistical results indicated that there was no significant difference in tooth lengths between feeding groups.

Raman spectroscopy was also used to investigate differences in silk structure between the two feeding groups. Previous studies using Raman technique have indicated its usefulness in discriminating structural variance of the proteins present in silkworm silk (Shao *et al.,* 1999; Sirichaisit *et al.* 2003). In our study, preliminary data showed contrasting relative ratios between the silk from the two feeding groups around 1450 cm-1 and 1669 cm-1. These peak assignments correlate with the amino acid composition and secondary protein structure of the corresponding silks and potentially indicate differences in sericin and fibroin content.

In conclusion, it seems that mulberry sexual dimorphic characteristics affected the cocoon weight and silk structure but not the mandible tooth length. Silkworms fed with female mulberry leaves produced more and structurally different silk than the silkworms on a male leaf diet. Silk has served many purposes besides being used in the textile industry, including cosmetics and medical technology. Since mulberry is the silkworm's sole food source, it is important to study how mulberry sexual dimorphism affects the characteristics of silkworms and silk in order to obtain more silk, sericin or fibroin, depending on the desired silk application.

PHARMACOLOGICAL INHIBITION OF CLATHRIN-MEDIATED ENDOCYTOSIS AND THE EFFECT OF MAGNETIC NANOPARTICLES ON THE MORPHOLOGY OF PRIMARY NEURONS. *REMYA A. VEETTIL[1], SUMOD SEBASTIAN[1], THOMAS MCALLISTER[2], SANTANEEL GHOSH[2] and DIANNA HYNDS[1], [1]Texas Woman's University, Denton, TX 76204, [2]Southeast Missouri State University, Cape Girardeau, MO 63701.

Traumatic central nervous system (CNS) injury leads to neuronal damage and results in varying levels of functional impairment. Nanomaterial-based drug delivery systems provide potential for axon regeneration from specific neurons by crossing blood brain barrier. From our previous experiments we found that $-NH_2$ and –COOH surface functionalized nanoparticles were internalized through clathrin-mediated endocytosis. In the present study we used clathrin inhibitors, which block the clathrin-mediated endocytosis, to confirm the mechanism by which the nanoparticles are endocytosed in B35 neuroblastoma cells. Treatment with clathrin inhibitors Pitstop 1 showed a weak correlation of SFNPs (TRITC) and early endosomes (EEs) whereas, Pitstop 1-25 treatment showed a moderate correlation of SFNPs and EEs (FITC) indicating a significant reduction in clathrin mediated SFNP endocytosis. TRITC mean intensities of Pitstop 1 and Pitsop 1-25 treated cells were less than 300 and this indicates a significant reduction in the SFNPs internalized through clathrin-mediated endocytosis. FITC mean intensities of Pitstop 1 and Pitsop 1-25 treated cells were in between 300 and 600. This could be due to the formation of EEs through pathways other than clathrin-mediated endocytosis. Magnetic nanoparticles were used to study the time and dose dependant effects on neurite outgrowth in primary neurons from chick dorsal root ganglion (DRG). Dissociated DRG were treated with different concentrations of magnetic nanoparticle for 72 hours and then looked for change in the number of neurites, branches and neurite length. No effects on the morphology of neurons after magnetic nanoparticle treatment were observed. Together, these results demonstrate the feasibility of MNP nanocarriers for targeted drug delivery to encourage axon regeneration following nervous system damage.

Supported by TWU Department of Biology, The Southeast Missouri State University Department of Physics and Engineering Physics, and grants from the TWU Research Enhancement Program.

ROLE OF DYNAMIN AND CAVEOLAE IN THE ENDOCYTOSIS OF SURFACE FUNCTIONALIZED NANOSPHERES IN NEURONS AND NEURON LIKE CELLS. *SUMOD SEBASTIAN[1], REMYA A. VEETTIL[1], THOMAS MCALLISTER[2], SANTANEEL GHOSH[2] AND DIANNA HYNDS[1], [1]Texas Woman's University, Denton, TX 76204 and 2Southeast Missouri State University, Cape Girardeau, MO 63701.

Surface functionalized nanospheres (SFNPs) have the potential to target therapeutics to different subcellular destinations in damaged neurons. These SFNPs can enter the cells either through receptor-mediated endocytosis or through adsorptive-mediated endocytosis. Dynamin is a large guanosine triphosphatase (GTPase) involved in the fission of endocytic vesicles from plasma membrane in many pathways. Well-known endocytosis pathways (clathrin-mediated endocytosis, caveolae-mediated endocytosis, RhoA-dependent endocytosis and some forms of micropinocytosis) require dynamin for vesicle fission. Previously, we have demonstrated that –COOH and –NH2 SFNPs can be endocytosed through caveolae- and clathrin-mediated mechanisms in B35 neuroblastoma cells and PC12 pheochromocytoma cells. Small molecule inhibitors can be used to block dynamin and caveolae-dependent endocytic pathways to confirm whether SFNPs employ

these pathways in entering the cells. Dynamin inhibitors can rapidly and reversibly block dynamin by targeting its specific domains. In the present study, we treated B35 cells with dynamin inhibitors and tested their effect on endocytosis of SFNPs into the early endosomes (EEs). We used immunocytochemistry followed by microscopy using DAPI (nucleus), FITC (SFNP) and TRITC (EE) filters of Nikon Ti eclipse A1 confocal system for imaging. Treatment of cells with 30 μM dynamin inhibitors OcTMAB and Iminodyn-22 for 15 minutes showed a weak correlation of SFNPs and EEs, indicating a reduction in SFNP endocytosis. Moreover, TRITC-FITC mean Intensities of OcTMAB and Iminodyn-22 were less than 300 and this indicates a significant inhibition of dynamin-mediated endocytosis of SFNPs and EE formation in the treated cells. Presence of SFNPs and EEs in the cells treated with OcTMAB and Iminodyn-22 indicates the endocytosis of SFNPs and formation of EEs through dynamin-independent pathways. In the future, dynamin inhibitors and inhibitors of caveolae-mediated endocytosis will be tested in PC12 cells and rat cortical neurons to assess the mechanisms of endocytosis employed by SFNPs.

Supported by TWU Department of Biology, The Southeast Missouri State University Department of Physics and Engineering Physics, and grants from the TWU Research Enhancement Program.

MICROSCOPIC EVALUATION OF THE AHPCO NANO-TECHNOLOGY IN THE AIR OASIS AIR PURIFIERS USING AIR-O-CELL SAMPLERS. CHANDINI REVANNA[1,2], NELOFAR SHERALI[1], MITSY VELOZ[1], JEFF BENNERT[3], JIM ROGERS[1] AND NABARUN GHOSH[1], [1]Department of Life, Earth and Environmental Sciences, West Texas A&M University, Canyon, Texas 79015, [2]Department of Environmental Health & Safety, Texas Tech University, Lubbock, TX 79409 and [3]Air Oasis, Research and Development, Amarillo, Texas 79118.

The indoor air surrounding us plays an extremely important role in maintaining our health. We have assessed the Air Oasis air purifiers that utilize a new generation AHPCO (Advanced Hydrated Photo Catalytic Oxidation) nanotechnology and do not rely on filters or air passing through the air purifier. This new technology simply produces a blanket of redundant oxidizers that clean the surrounding air and sanitize surfaces by targeting the particulate matters in the air. Air sampling was conducted using two methods. First, petri plates prepared with Brain Heart Infusion Agar (Difco) were placed at 5, 10 and 20 feet distances from the air purifier. The petri plates were exposed to the air for 24, 48, 72, 96 and 120 hours with and without running the air purifier. Bacteria isolated from the room air exposure were Gram-positive bacilli such as *Bacillus* and C*oryneform* (diptheroids) sp., coagulase negative *Staphylococcus, Micrococcus,* and encapsulated Gram-negative bacilli. Fungal colonies included *Alternaria alternata, Cladosporium* sp., *Drechslera, Stachybotrys* and *Curvularia* sp. There was a gradual reduction in the number of microbial colonies formed with increased intervals on running the air purifier. In the second part, we used the *Air-O-Cell®* sampling equipment, which is designed for a rapid collection of a wide range of airborne aerosols including mold spores, pollen, insect parts, skin cells, fibers and inorganic particulates. Samples using *Air-O-Cell®* cassettes were analysed using optical microscopy and show the presence of *Aspergillus/Penicillium* at 200 counts/m^3. When Air Oasis air purifier AO3000 unit was used continuously for two weeks, the count came down to 40 counts/m^3 and after four weeks the room was completely sterilized (0 counts/m^3). This experiment using AO3000 purifiers established that the use of a negative ion purification system is an effective means of eradicating aeroallergens such as molds and microbes in the indoor air.

DETERMINIATION OF EGGSHELL MICROSTRUCTURAL CHARACTERISTICS IN EGG-LAYING CHICKENS VACCINATED WITH MYCOPLASMA GALLISEPTICUM USING SCANNING ELECTRON MICROSCOPY AND QUANTITATIVE IMAGE ANALYSIS. SANDRA WESTMORELAND[1] AND E. DAVID PEEBLES[2], [1]Department of Biology, Texas Woman's University, Denton, Texas 76204 and 2Department of Poultry Science, Mississippi State University, Mississippi State, Mississippi 39762.

Mycoplasma gallisepticum (MG) is a pathogen that causes chronic respiratory disease in chickens. The infection is easily transmitted bird-to-bird and, once infected, a bird is infected for life. Mycoplasmas are resistant to antibiotics that interfere with cell wall synthesis and they are not susceptible to lysis by detergents and alcohols. These properties have led to the development of vaccines to help prevent MG infection in commercial flocks. Vaccination for layer hens with live MG is commercially available to help control MG outbreaks. One MG vaccine, F-strain *Mycoplasma gallisepticum* (FMG), has been shown to protect flocks from field strains of MG (Peebles, 2003, *Poultry Science* 82:1397-1402). The purpose of this study was to determine the effect of vaccination of commercial layers with FMG on eggshell characteristics. Single Comb White Leghorn birds of Hy-line variety W-36 were used in this study. The experiment involved multiple variables in addition to the vaccination, including three different diets (basal diet, 2% poultry fat added diet, and 2% poultry fat plus phytase and D30 added diet), and two ages of lay (24 and 50 weeks). A time replicate of the original experiment (trial 1) was also performed (trial 2). In both trials, eggs were collected for each variable set and eggshell specimens for each egg were prepared for a radial (cross-section) view. In trial 1, each

specimen was imaged using a JOEL 35C SEM at 200X. In trial 2, each specimen was imaged using FEI Quanta 200, Mark II environmental SEM at 200X. Cross sections of eggshells were measured using Image J software. ANOVA statistical analyses were performed using SPSS to detect differences in eggshell thickness based on the variables treatment, diet, and age of lay. In trial 1, eggshell thickness was significantly thinner in shells of inoculated birds with the 2% poultry fat supplemented diet as compared to shells of control birds with same diet. Also, in trial 1, eggshells from inoculated birds that were collected at 24 weeks were significantly thinner than those collected at 50 weeks from inoculated birds. In trial 2, the eggshells from control birds at age 24 weeks with 2% poultry fat diet were significantly thinner than those of inoculated birds with the same diet. At 50 weeks, the trend was reversed with the inoculated birds with 2% poultry fat diet having significantly thinner eggshells than those of control birds with the same diet. This study demonstrates the use of SEM and image analysis for characterization of microstructural characteristics of eggshells. In addition, the study illustrates the complicated nature of the interactions of FMG inoculation with the diet and age factors in determining eggshell characteristics. More research is needed to further understand the effect of FMG inoculation with age of lay and diet on eggshell characteristics.

This project was funded by USDA-ARS research grant SCA #58-6406-4-102 to EDP.

LIVE-CELL SINGLE-MOLECULE ANALYSIS OF INTEGRIN LFA-1 AND ITS LIGAND ICAM-1.

MUNENORI ISHIBASHI[a,b,1], YUKIHIKO MIYANAGA[a,b], SATOMI MATSUOKA[a,b], JUN KOZUKA[a,b], YUICHI TOGASHI[c], TATSUO KINASHI[d] and MASAHIRO UEDA[a,b].

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Integrins are transmembrane receptors that are important for cell adhesion and migration. A key molecular mechanism for the cell adhesion is integrin-mediated mechanical linkage between extracellular ligands and the intracellular actin cytoskeleton, i.e. molecular clutch formation. It is widely accepted that this mediation requires a high affinity for ligand binding by the integrin to achieve stable clutch formation. This view, which has been established via structural and ligand binding kinetics analysis of integrin, applies well to the tight adhesion of cells with Mn^{2}+-induced high affinity integrins. However, it fails to explain cell adhesiveness under chemokine-induced physiological adhesions that show low ligand binding affinity of integrin LFA-1. In mammalian systems, LFA-1 and its partner chemokines control immune and stem cell dynamics (Fig. 1). In this meeting, I'll talk about single-molecule analysis of integrin LFA-1 (Fig. 2) and its ligand ICAM-1 in living cells, clarifying that transient, unstable clutch formation regulates the adhesion of lymphocytes undergoing dynamic migration after being stimulated with chemokines (M. Ishibashi, et al., 2015).

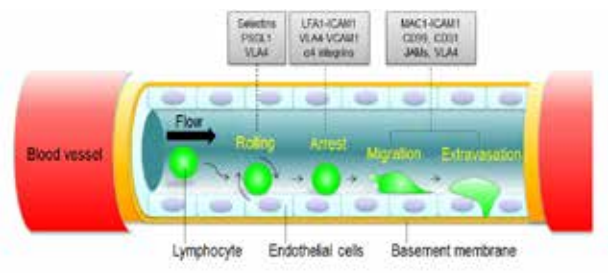

Figure 1. Immune cell dynamics controlled by some key proteins including integrin LFA-1.

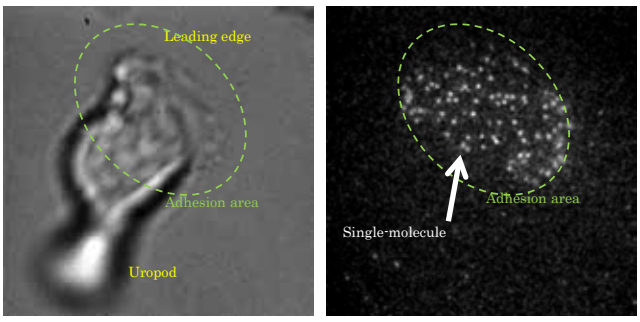

Figure 2. Single-molecule imaging of integrin LFA-1 at adhesive area of immune cell by TIRF microscopy.

Reference: M. Ishibashi et al., Integrin LFA-1 regulates cell adhesion via transient clutch formation (2015) *Biochem Biophys Res Commun* 464: 459–66, doi:10.1016/j.bbrc.2015.06.155.

MATERIAL SCIENCE
Spring 2016

SINGLE GOLD NANOPARTICLES AS LOCALIZED PLASMONIC TRANSDUCERS. EDUARDO VILLARREAL AND EMILIE RINGE, Rice University, Department of Materials Science and NanoEngineering, Houston, Texas.

Localized surface plasmon resonance (LSPR), a collective oscillation in noble nanoparticles resulting from the interaction of light, is an exceptional tool for chemical and biological sensing, surface-enhanced spectroscopies, and nanophotonic devices. The LSPR phenomenon is sensitive to changes in the environment of nanoparticles, as well as to the size, shape and metal composition of nanoparticles. In this study, a correlative analysis of size and LSPR frequency of single gold nanoparticles (NPs) was achieved by using scanning electron microscope (SEM) and dark-field optical microscopy. Statistical analysis was performed on a large number of single gold NPs, which correlated with LSPR frequency, NP size, and resonance line width. Moreover, as a proof-of-concept, specific DNA detection was achieved by monitoring single nanoparticle LSPR frequency. DNA hybridization events could be detected at $5x10^{-10}$ M, producing a 6nm LSPR frequency shift, as opposed to non-specific DNA sequences, which produced a less than 1nm LSPR frequency shift.

TEM STUDY OF HFO_2 FILMS PREPARED BY HIGH-RATE REACTIVE HIGH-POWER IMPULSE MAGNETRON SPUTTERING. NAI-WEN PI[1*], MINGHUI ZHANG[1], JIECHAO JIANG[1], J. VLČEK[2], A. BELOSLUDTSEV[2], J. REZEK[2], J. HOUŠKA[2], J. ČAPEK [2], R. ČERSTVÝ [2], S. HAVIAR [2], AND EFSTATHIOS I. MELETIS[1] [1] Department of Materials Science and Engineering, The University of Texas at Arlington Arlington, Texas 76019, USA and [2] Department of Physics and NTIS, European Centre of Excellence, University of West Bohemia, Univerzitní 8, 306 14 Plzeň, Czech Republic

Hafnium Dioxide (HfO_2) is a very important coating material for many applications (semiconductors, optical devices, nuclear industries and coating technologies) due to its numerous outstanding properties. These include a high melting point ~ 2800°C, excellent thermal stability, high refractive index and low absorption over a broad region from the near-UV to the mid-IR, high dielectric constant and high neutron absorption cross section. HfO_2-based materials can be used, for example, as thermal barrier coatings for turbine blades operating in harsh and high-temperature environments. In this work, we present the microstructure of hard and optically transparent HfO_2 films prepared by high-rate reactive high-power impulse magnetron sputtering.

HfO_2 coatings were deposited on Si substrates using a voltage pulse duration (t_1) ranged from 25 to 200 μs and an averaged target power density $\langle S_d \rangle$ ranged from 7.2 to 54 Wcm^{-2}. Effects of t_1 and $\langle S_d \rangle$ on the microstructure and property of the coatings were studied by atomic force microscopy, nano-indentation testing, X-ray diffraction, electron diffraction and high-resolution transmission electron microscopy (TEM). Five HfO_2 coatings were prepared with (1) t_1 = 25 μs & $\langle S_d \rangle$ =7.6 Wcm^{-2}, (2) t_1 = 100 μs & $\langle S_d \rangle$ =7.2 Wcm^{-2}, (3) t_1 = 200 μs & $\langle S_d \rangle$ =7.3 Wcm^{-2}, (4) t_1 = 200 μs & $\langle S_d \rangle$ =18 Wcm^{-2} and (5) t_1 = 200 μs & $\langle S_d \rangle$ =54 Wcm^{-2}. All coatings were found to possess an interlayer close to the interface followed by a nano-columnar structure layer (Fig. 1a). The coatings exhibited a mixture of high and low density regions at the nanoscale (Fig. 1b), of which the density in the interlayer is notably higher than that in the nano-columnar structure layer. The nano-columnar structure width decreases with the reduction in t_1 and $\langle S_d \rangle$. The coating prepared with t_1 = 200 μs has a monoclinic HfO_2 and that with t_1 =25 μs has an orthorhombic HfO_2 structure. The coating prepared with t_1 = 100 μs contains both monoclinic and orthorhombic phases. Variation of $\langle S_d \rangle$ doesn't change the crystal structure of the coating. The relationship between t_1, $\langle S_d \rangle$, interlayer thickness, size of nano columnar structures and the properties (mechanical and optical) were addressed.

This work is supported by the U.S. NSF under Award No. NSF/CMMI DMREF-133552.

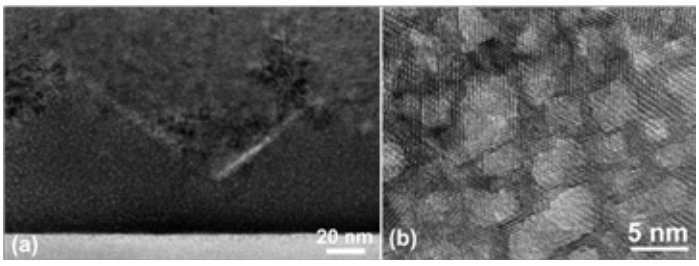

Figure 1. (a) Cross-section TEM of the HfO2 coating deposited using $t1$ = 200 μs and $\langle S_d \rangle$=7.3 Wcm^{-2}; (b) HRTEM of the interlayer in (a) showing the porous structure.

HIGHLY WATER-DISPERSED CARBON NANOTUBES AND GADONANOTUBES POLYMER-HYBRID MATERIALS. SAKINEH E MOGHADDAM[1], MAYRA HERNÁNDEZ-RIVERA[1], and LON J WILSON[1], [1]Department of Chemistry, MS-60, Rice University, P.O. Box 1892, Houston, USA

Carbon nanotubes (NTs) have captured scientists attention as nanoscale platforms for drug-delivery and medical imaging. Gadonanotubes (GNTs), ultra-short carbon nanotubes (US-tubes) containing clusters of Gd^{3+} ions, are superior contrast agents for Magnetic Resonance Imaging (MRI) which were discovered in our lab in 2005.[1] Although these linear molecular magnets are dispersible in a surfactant solution, stability of GNTs in water is a challenge for *in-vivo* studies.[2] The objective of this study

was to address the aforementioned drawback and enhance the dispensability of GNTs in water *via* leveraging the exquisite properties of biodegradable polyacrylic acid (PAA) by controlled growth of PAA chains around NTs. Highly dispersible US-tubes and GNTs were obtained by an *in-situ* polymerization of acrylic acid, using potassium persulfate as initiator. The reaction was quenched after 20 h and the polymer-wrapped NTs were purified by filtration accompanied by washing the solids with water and dispersion of the solids in a fresh portion of water to form colloidal solutions. The obtained PAA-wrapped NTs were characterized by High Resolution TEM, Energy Dispersive Spectrometry (EDS), Inductively Coupled Plasma-optical Emission Spectrometry (ICP-OES), Raman Spectroscopy and Thermal Gravimetric Analysis (TGA). We observed the NTs covered by an amorphous layer of PAA in the HRTEM image (Fig. 1). A solubility test based dispersing excess amount of NTs in minimum water was performed, following by sonication and settling for 5 h.[3] A 10:1 ratio of acrylic acid to NTs showed ~550% improvement in solubility (1.06 mg/mL for no PAA US-tubes *vs.* 5.5 mg/mL for PAA-wrapped NTs). Our study shows that the presence of thin layers of PAA enhances the dispersion of GNTs in water, while suppressing the chance of Gd^{3+} ion leakage. This new material offers a viable approach to develop NT and GNT materials for clinical applications.

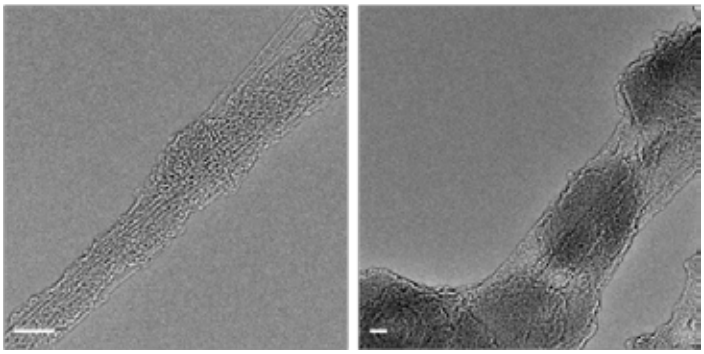

Figure 1. TEM images of GNTs (a) and PAA-wrapped GNTs (b). The scale bar is 10 nm.

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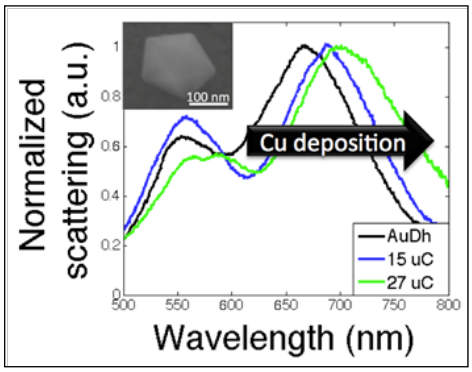
ELECTROCHEMICAL NANOPARTICLE SURFACE SYNTHESIS. ANJLI KUMAR[1], EDUARDO VILLARREAL[2], AND EMILIE RINGE[1,2], [1]Department of Chemistry, Rice University, Houston, TX, [2]Department of Materials Science and NanoEngineering, Rice University, Houston, TX.

Localized surface plasmon resonances (LSPRs) are light-driven collective oscillations of conduction electrons in nanoparticles that result in wavelength-dependent scattering, absorption, and enhancement of the electromagnetic field. LSPR-based sensing is extremely sensitive to the nanoscale environment around a particle. Molecules entering the strong electric field created by the LSPR influence the electron oscillation and thus the molecular binding event causes a frequency or color shift. Since electric fields are greatest near the tips of the nanoparticle, the sensitivity to molecules is enhanced at the tips. Limits of detection can be improved if the previously described molecular binding only occurs at the tips.



This study explores the site-dependent potential of nanoparticles surfaces by electrodepositing thin layers of metals onto the surface of nanoparticles. We demonstrate that electrochemical techniques can be used for controlled growth on nanoparticle surfaces. The proof-of-concept system studied here involves the deposition of Cu on Au decahedra. The Au nanoparticles decorated with Cu were characterized *via* dark field microscopy (DFM) and scanning electron microscopy. After electrodeposition, single-particle DFM data show a LSPR shift indicating successful electrochemical deposition. These results indicate that electrochemical surface modification is a useful, controllable post-synthesis approach with potential applications in sensing and light-driven catalysis.

SYNTHESIS OF GUANIDINIUM-BASED ANION EXCHANGE MEMBRANES AND THEIR STABILITY AND APPLICATION ASSESSMENT. HSIAO-CHIEN WU, SYED D SAJJAD and FUQIANG LIU, Electrochemical Energy Laboratory, Department of Materials Science and Engineering University of Texas at Arlington, TX, 76019.

Anion exchange membranes (AEMs) have shown promising characteristics to overcome some of the problems with their cation counterparts, such as costly catalysts and low electrochemical activity. However, most of the state-of-the-art AEMs suffer from low conductivity and fast degradation. In this work, unique guandinium-based AEMs were synthesized with ion-exchange group tethered in polymer backbones to enhance both stability and conductivity.

The guanidinium prepolymer was synthesized through a simple polycondensation process and then was used to make guanidinium–chitosan (Gu-Chi) blend membranes. A lipophilic guanidinium prepolymer, synthesized by

means of a precipitation reaction between sodium stearate and guanidinium salt, was adopted to tune solubility and mechanical properties of the blend AEMs. Results show that both ionic conductivity and methanol permeability of the AEMs can be tuned by blend composition and chemistry of the guanidinium-based prepolymer. In addition, polymer crosslinking was conducted to further reinforce the mechanical strength of the membranes and interlock the guanidinium moieties to the porous PTFE.

The selectivity (ratio of ionic conductivity to methanol permeability) of the fabricated membranes is superior to that of commercial membranes. Under full cell tests, the open circuit voltage (OCV) value for the blend AEM with guanidium polymer is much higher than that of the commercial Tokuyama A201 at room temperature. Overall, the developed membranes demonstrate superior performance and therefore pose great promise for direct methanol anion exchange fuel cell (DMAFC) applications.

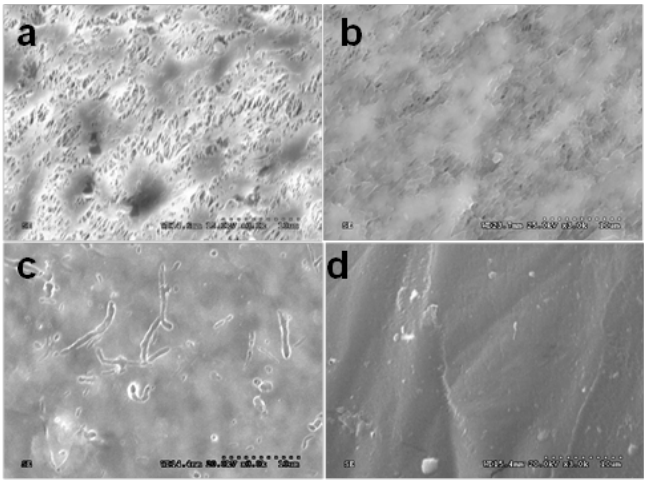


Figure 1. Different guanidium-based AEMs: a) Porous PTFE substrate; b) Partially penetrated composite membrane, AGM1, using 15% AEE-based polymer solution; c) Fully impregnated and crosslinked membrane, HGMC, using 7.5% HMDA-based polymer solution, and d) Fully impregnated and crosslinked.

TUNING AND MAPPING SURFACE PLASMONS IN Au-Ag BIMETALLIC HOLLOW NANORODS WITH AN ELECTRON BEAM. SADEGH YAZDI[*1], JOSEE R. DANIEL[2], DENIS BOUDREAU[2] AND EMILIE RINGE[1], [1]Department of Materials Science & NanoEngineering, Rice University, Houston, Texas 77005, USA, and [2]The Center for Optics, Photonics and Lasers (COPL), Department of Physics, Laval University, Québec, Canada.

Metallic nanoparticles have found their way into a wide range of applications, from photocatalysis to biomedicine, because in such nanoparticles surface plasmon can be excited and confined in a resonant manner with visible light. The frequency of localized surface plasmon resonances (LSPRs) in nanoparticles depends strongly on their shape, size, composition, the refractive index of their surrounding environment, as well as their interior structure (solid vs. hollow). A great deal of research is ongoing to understand the relationship between these parameters and the LSPR frequency in metallic nanoparticles. This study presents the effect of the hollow position within Au-Ag bimetallic nanorods on the LSPR. By using an electron beam in a transmission electron microscope we moved the material within the interior of the hollow nanorods and precisely positioned the hollow region at different places in individual nanorods. Then, by using scanning transmission electron microscopy and monochromated electron energy loss spectroscopy the surface plasmon modes were mapped with high spatial resolution. This allowed us to investigate the relation between the position of a cavity in a nanoparticle and its LSPR.

ELECTRON TOMOGRAPHY, ENERGY-LOSS SPECTROSCOPY, AND ENERGY DISPERSIVE X-RAY SPECTROSCOPY OF PALLADIUM DECORATED ALUMINUM NANOCRYSTALS. DAYNE F. SWEARER[1,*], HANGQI ZHAO[2], LINAN ZHOU[1], CHAO ZHANG[2], HOSSEIN ROBATJAZI[2], SADEGH YAZDI[3], ROWAN K. LEARY[4], PAUL A. MIDGLEY[4], PETER NORDLANDER[2,3,5], NAOMI J. HALAS[1,2,4,5], EMILIE RINGE[1,3], [1]Department of Chemistry, Rice University, Houston, TX 77005; [2]Department of Electrical and Computer Engineering, Rice University, Houston, TX 77005; [3]Department of Material Science and Nanoengineering, , Rice University, Houston, TX 77005; [4]Department of Materials Science and Metallurgy, University of Cambridge, Pembroke Street, Cambridge CB2 3QZ, UK; [5]Department of Physics and Astronomy, Rice University, Houston, TX 77005.

Plasmonic metal nanoparticles have recently shown their viability as light-driven catalysts. However, heterogeneous catalysis historically depended on other optically lossy transition metals such as Pd, Pt, Ru, Ir, etc. Through the wet-chemical synthesis of heterometallic Pd-decorated aluminum nanocrystals, the properties of a plasmonic optical antenna and catalytically active Pd nanoparticles have been combined leading to increased optical absorption in Pd and unique photocatalytic activity. Nanoparticle composition was confirmed with elemental mapping by energy dispersive X-ray spectroscopy, and further high-resolution transmission electron microscopy revealed spatial separation of Pd and Al nanoparticles. The heterogeneous 3-dimensional nature of the Pd-Al nanoparticles was revealed through electron tomography. Localized plasmonic modes on each component of the heterometallic nanoparticle system were revealed with electron energy loss plasmon mapping, performed in a monochromated, probe-corrected scanning transmission electron microscope. This study presents a novel heterometallic nanostructure that allows for the photoactivation of catalytically active metals

and shows promise for developing new selective, hot-carrier driven chemical transformations with light.

MICROSTRUCTURE AND PROPERTIES OF Hf-B-Si-C COATINGS. MINGHUI ZHANG[1,*], JIECHAO JIANG[1], EFSTATHIOS I. MELETIS[1], JIŘÍ KOHOUTL[2], JAROSLAV VLČEK[2] AND JIŘÍ HOUŠKA[2], [1]Characterization Center for Materials and Biology and Department of Materials Science and Engineering, University of Texas at Arlington, TX 76019, [2]Department of Physics and NTIS-European Center of Excellence, University of West Bohemia, Univerzitní 8, 30614 Plzeň, Czech Republic.

Nanostructured coating materials of transition metal-based diborides have attracted considerable attention due to their outstanding properties compared to the traditional binary or ternary coatings. These nanostructured coatings present a much wider potential for applications in coating technologies, such as advanced aerospace technologies, high-temperature microelectronics and optoelectronics. In this research, hard and multifunctional Hf-B-Si-C coatings ($Hf_{27}B_{57}C_8$, $Hf_{23}B_{55}Si_2C_{11}$, $Hf_{22}B_{54}Si_9C_9$ and $Hf_{21}B_{28}Si_{35}C_7$) were synthesized by magnetron sputtering. The coatings were investigated by X-ray photoelectron spectroscopy, X-ray diffractometer, high-resolution transmission electron microscopy and electron diffraction to systematically study the effect of silicon content on the microstructure and their properties.

The Si-free $Hf_{27}B_{57}C_8$ coating possesses the highest hardness of 37 GPa along with a high compressive stress of 4.9 GPa. This coating consists of hexagonal HfB_2 nano-columnar structures (~50-60 nm long, ~5-10 nm wide) strongly textured with the (001) tilted ~30° away from the coating surface. The $Hf_{23}B_{55}Si_2C_{11}$ coating is composed of smaller nano-columnar structures (~20-30 nm long and less than 5 nm wide), surrounded by ~1 nm thick amorphous boundaries. All the nano-columnar structures are well oriented with (001) parallel to the coating surface. Such a microstructure change results in a reduction of the compressive stress to 1.8 GPa while still maintaining a high hardness. The $Hf_{22}B_{54}Si_9C_9$ coating consists of refined randomly oriented HfB_2 nano-needles (~2-3 nm wide), while the $Hf_{21}B_{28}Si_{35}C_7$ coating consists of HfB_2 nanocrystalline (1-2 nm) embedded in an amorphous matrix. The results indicate that incorporation of Si into the coatings results in an increase of the volume fraction of the amorphous structure, refining of the microstructure, and also altering the orientation of the HfB_2 crystal structures, which significantly reduces the compressive stress and improves the oxidation resistance of the coating at elevated temperatures.

This work is supported by the U.S. NSF under Award No. NSF/CMMI DMREF-133552.

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IMPROVED SOLAR ENERGY STORAGE EFFICIENCY OF ALL VANADIUM PHOTOELECTROCHEMICAL CELL USING GEOMETRY-ENHANCED MATERIALS.

ZI WEI, YI SHEN, DONG LIU and FUQIANG LIU*

Electrochemical Energy Laboratory, Department of Materials Science and Engineering, University of Texas at Arlington, Arlington, TX 76019,USA

As renewable energy becomes more prevalent, there is a pressing need for large-scale and high-efficiency solar energy storage as a sustainable solution to the problem of energy shortage. To this end, we herein describe an all-vanadium photoelectrochemical storage cell (all-V PESC, Fig. 1) [1-3] based on geometry-enhanced anatase TiO_2 nanobelts (TNBs) to significantly improve storage efficiency. The TNBs, consisting of both highly crystalline type I belts with thermodynamically stable {101} facets, and heterostructured type II belts with exposed high-energy {001} and {100} facets, were obtained by stirring-assisted hydrothermal synthesis. The feasibility of solar energy storage using the geometry-enhanced TNBs by generating energy-rich vanadium redox species in the all-V PESC is demonstrated. The obtained incident photon-to-current efficiency (IPCE) was ~22% at 350 nm without any external bias, double that of commercial P25 TiO_2 (~11%). Besides, a continuous flow reactor (CFR) was integrated with the all-V PESC and it is discovered that the introduction of flow during photoelectrochemical conversion greatly enhanced photocurrent by 5 times comparing to non-flux condition. The enhanced photocatalytic performance is largely attributed to improved charge separation efficiency implemented by TNBs and enhanced charge transport boosted by vanadium species flow. This concept may be extended to other nanostructured semiconductor materials and photoelectrochemical systems, and potentially offers significant ramifications to sustainable and renewable energy.

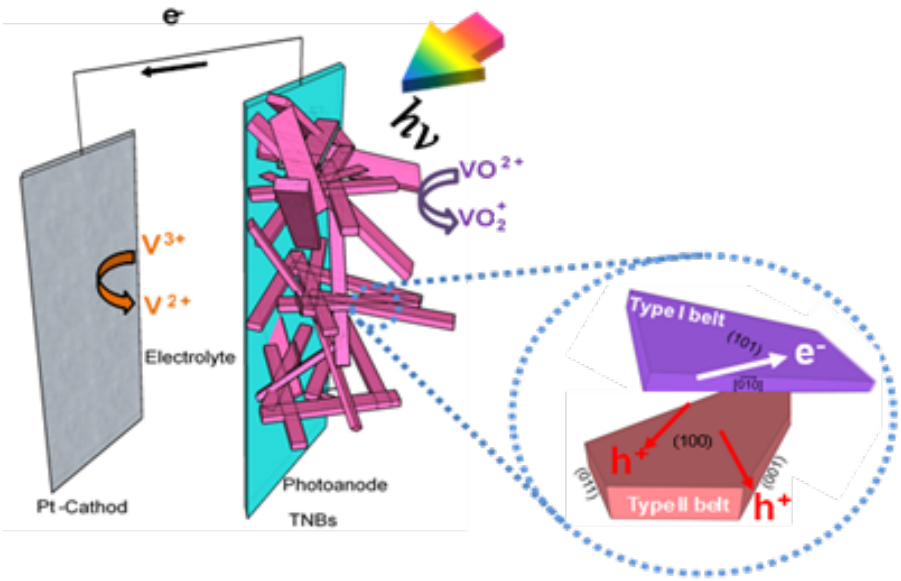

Fig. 1. Schematic illustration of an all-V PESC. Two different vanadium redox electrolytes, i.e., VO^{2}+ and 0.01 M V^{3}+ (separated by a Nafion 117 membrane) were used as the anolyte (in contact with the photoanode) and catholyte, respectively. Electrode reactions at the photoanode and Pt cathode follow: VO^{2}+ + H_2O → VO^{2}+ + e- + 2H+ and V^{3}+ + e- → V^{2}+, respectively.

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EDUCATION AND CLASS PROJECTS
Spring 2016

SEM STUDY OF TEA PRODUCTS FROM FOUR MEDICINAL PLANTS. PABLO MIRANDA and CAMELIA MAIER. Department of Biology, Texas Woman's University, Denton, TX 76204.

In the plant biology course at Texas Woman's University, students have the possibility to work on hands-on individual projects employing microscopy. The objective of this project was to study the morphology and anatomy of four medicinal plants commercially available as tea products: Guava, *Psidium guajava (Mytaceae)*, whose brewed leaves are used to treat intestinal issues; Tasmanian bluegum, *Eucalyptus globulus (Mytaceae)*, whose steam-distilled leaves serve as a source of essential oils; Chamomile, *Matricaria chamomilla (Asteraceae)*, whose upper stems and inflorescences are used as bactericidal and for intestinal issues, and Quinine bark tree, *Cinchona officinalis (Rubiaceae),* whose quinine is an anti-fever agent used to prevent and treat malaria and quinidine to treat arrhythmia.

Herbal tea specimens of each plant were used as found in their bags or sectioned, placed on stubs, and viewed with a Hitachi TM-1000 SEM. Calcium oxalate crystals were observed in *P. guajava* stem pith (Fig. 1A). Secondary cell wall were observed in *E. globulus* stem xylem (Fig. 1B). Stigma and pollen were observed in *M. chamomilla* disc flower (Fig. 1C) and the bark structure in *C. officinalis* specimens (Fig. 1D). Very few studies on the morphology and anatomy of the above plant species are published and even fewer to none on the tea products obtained from these plants. SEM studies can determine the quality of herbal products. Guava tea product in this study showed fungal and bacterial growth on the specimens, which could have colonized the plants in their natural environment or the herbal products during the preparation process and storage.

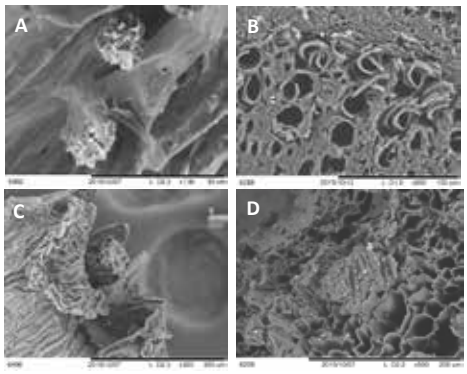

Figure 1. Representative scanning electron micrographs of tea specimens from four medicinal plants. (A) *P. guajava* calcium oxalate crystals in longitudinal stem; (B) *E. globulus* primary and secondary xylem cell walls; (C) Stigma with pollen and corolla of *M. chamomilla* disc flower; (D) *C. officinalis* bark.

MORPHO-ANATOMICAL STUDY OF THE NATIVE PLANT SNOW-ON-THE-PRIARIE, *EUPHORBIA BICOLOR (EUPHORBIACEAE)* NKEMJIKA UKE AND CAMELIA MAIER. Department of Biology, Texas Woman's University, Denton, Texas 76204.

Snow-on-the-prairie, *Euphorbia bicolor (Euphorbiaceae)* is a native spurge found only in five south-central USA states that thrives in hot dry climate conditions, although it seems a bit ironic considering its name. The name comes from the fact that, when in bloom, the white inflorescence bracts in a densely population of this species give the impression of snow cover from a distance. There is limited research on *E. bicolor.* Plants of *Euphorbia* genus have been shown to possess cytotoxic, antiviral, antimicrobial, and antiproliferative activities. *E. bicolor* is an annual plant, contains phytoestrogens and an irritant milky sap that oozes off any broken plant parts and is used by American natives as a medicinal plant in treating pain. The objectives of this project were to learn plant morphology and anatomy on a real plant specimen and to contribute microscopy images to the description of this native plant. Specimens of different plant organs were dissected, placed on stubs and viewed with a Hitachi tabletop SEM (TM-100). Figure 1 presents a few of *E. bicolor* morpho-anatomical characteristics, such as epicuticular wax crystals, papillae and stomata on the leaf upper epidermis (Fig. 1A), nectary on a petal sprinkled and milky sap droplets (Fig. 1B), stem structure in cross section (Fig. 1C) and amyloplasts in stem pith (Fig. 1D). One characteristic not previously described for *E. bicolor* is the presence of stomata on both upper and lower leaf epidermises. Snow-on-the-prairie, similar to some plants in the *Euphorbiaceae* family possess excessive hairs on leaves, bracts and stems as a defense mechanism against herbivory and for reducing water loss. This study contributes to enhancing our knowledge of the morpho-anatomy of *E. bicolor.*

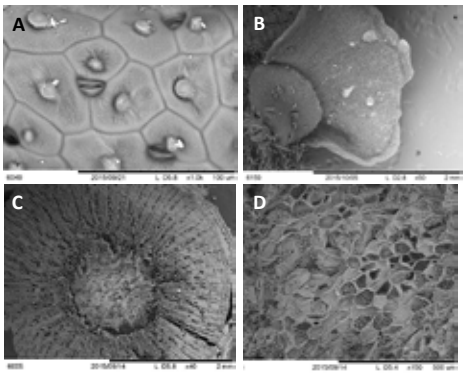

Figure 1. SEM images of some morpho-anatomical characteristics of *E. bicolor (Euphorbiaceae)*. A) Leaf upper epidermis with stomata, papillae, and epicuticular wax; B) Nectary (with landed pollen) at the base of a petal (with milky sap droplets); C) Cross section through stem showing pith and secondary xylem; D) Details of pith parenchymatic tissue containing numerous amyloplats.

TECHNICAL ABSTRACTS
Spring 2016

APPROACHES FOR DETECTING AMINES WITH FLUORESCENCE MICROSCOPY. ANTHONY S. STENDER* and EMILIE RINGE. Rice University, Department of Materials Science and NanoEngineering, Houston, TX 77005.

The emphasis on nanomaterials research, multi-modal microscopy, and miniaturized chemical reaction chambers over the past decade led to a deeper understanding of chemistry and materials at the single-particle (and single-molecule) level. Optical darkfield and electron microscopy have been the primary techniques for characterizing materials on the nanoscale. However, when plasmonic or scattering intensity data are uninformative for an experiment, optical darkfield loses its appeal as an imaging technique. Likewise, it is not always plausible to perform real time electron microscopy experiments.

Fluorescence microscopy is a mature technique in the biological sciences, but its application to questions from materials science and organic chemistry has remained quite limited to date. Its primary missions in nanomaterials research have been related to the study of organic dyes and inorganic quantum dots on the single molecule/particle level, in a manner akin to use of darkfield with metallic nanoparticles. Its limitation is that most reactions do not involve a chemical that generates a detectable fluorescent signal. Moreover, fluorescent dyes are often designed with biological experiments in mind; they simply act as stains or tags that highlight features of interest.

The development of "turn-on" fluorescent probes has provided an opportunity to study non-fluorescent materials and reactions in real time. Turn-on probes remain relatively non-fluorescent until they react with a specific organic functional group, such as an amine. These probes have been used in bulk-scale analytical chemistry experiments and for biological staining, and as such, they are often optimized for biological conditions. We are currently developing methods and sample chambers to incorporate these probes into our single particle studies of nanoparticles and chemical reactions in aqueous environments. In a bulk aqueous environment, turn-on fluorophores behave as advertised and can be monitored with a fluorometer. When attempting to image the surface of a glass slide or a nanoparticle that is coated with amines, the best approach is to follow a typical staining protocol. The ultimate goal is to witness the production of amines during chemical reactions, but such reactions are typically run under harsh conditions that conflict with the optimal working range for the fluorophores. Reaction conditions have also proven more complicated to maintain when the reaction matrix is reduced from 100 mL to less than 1 mL. Thus traditional methodologies do not work for detecting amines in that setting. To observe these reactions, specialized reaction chambers have been produced that sit atop of a cover slip. The chambers are covered with a seal and can hold several milliliters of liquid or gas. The main challenge yet to be overcome is in finding a balance between the conditions required by the reaction and those required by the fluorophore (e.g. pH, concentrations, etc.) at the reduced scale.

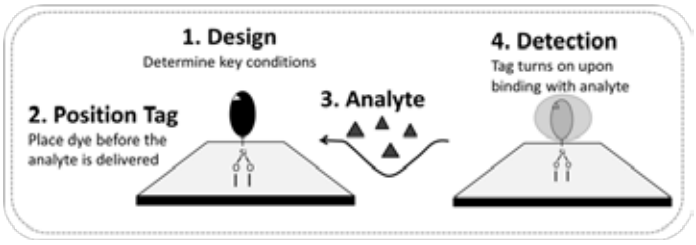

Figure 1. The four steps required to detect an analyte with a turn-on fluorophore in a materials science application. First, a tag must be found that will bind to the analyte under the proper chemical conditions. Next, the tag must be positioned where it can come into contact with the analyte. Third, analyte is introduced to the still non-fluorescent tag. Finally, a binding event occurs, and the tag fluoresces. If desired, the location of the event can be resolved with super-resolution techniques.

THREE-DIMENSIONAL MICROSTRUCTURE STUDIES OF METALS AND CERAMICS BY DUAL BEAM XE-PLASMA FIB SEM. NOEL T. NUHFER, MARC DE GRAEF and GREGORY S. ROHRER, Department of Materials Science and Engineering, Carnegie Mellon University, Pittsburgh, PA 15213.

While liquid metal source FIB SEMs have revolutionized three-dimensional microstructural characterization, the rate at which they can mill material is a significant limitation. Material removal rates in an Xe-ion plasma FIB SEM can be as much as 50 times greater and therefore enable experiments that are impossible using the conventional liquid metal source FIB technology. In this study, the performance of plasma-FIB (FEI Helios PFIB) installed at Carnegie Mellon University in 3D orientation mapping experiments and in grain boundary mobility experiments is described. Faster milling rates increased both the area that can be sectioned and the rate of sectioning, making it possible to create 3D reconstructions of larger volumes. The variance of microstructural characteristics within these larger volumes and the use of the FIB to create microfabricated bicrystals that can be used to measure a large number of grain boundary mobilities are presented.

IN-SITU TEM TECHNIQUES FOR CHARACTERIZATION OF ENERGY-RELATED COMPOSITES.

ARBARA ARMBRUSTER[1] and TOSHIE YAGUCHI[2 1]

Hitachi High Technologies America, Inc., Pleasanton, CA, and [2]Hitachi High-Technologies Corp., Ibaraki, Japan

The importance and versatility of *in-situ* transmission electron microscopy has expanded exponentially in the last 15 years, providing observation of the physical behavior of materials to external stimuli including temperature, environment, stress and applied fields. Catalysis is a dynamic process and information about morphological changes during reactions is critical to understanding and improving catalytic materials and processes at the nanoscale.

Keys to the expansion of this technology are the development of 120 – 300kV TEM capabilities and of a new class of *in-situ* side-entry specimen holders that support heating experiments up to 1500°C (Yaguchi et al., 2015). A 120kV TEM equipped with a high-speed turbo molecular pump, an oil-free scroll pump and safety equipment such as a gun-airlock valve enabled high temperature *in situ* TEM observation of solid-gas reactions routinely and safely. Gas pressure in the specimen area can be raised up to 0.1 Pa when operating the microscope with the gas-injection heating holder and a tungsten or LaB6 filament.

In Figure 1, a binder-coated Pt/graphitized carbon (GC) electrocatalyst was used for the heating experiments. When the sample was heated to 220°C, rapid morphological change of the carbon support occurred immediately after air introduction. Some of the Pt particles grew and agglomerated as a consequence of the morphological change in the carbon support. The results revealed that the system can be routinely applied to *in-situ* TEM observation of electron beam sensitive nanomaterials in gaseous atmospheres.

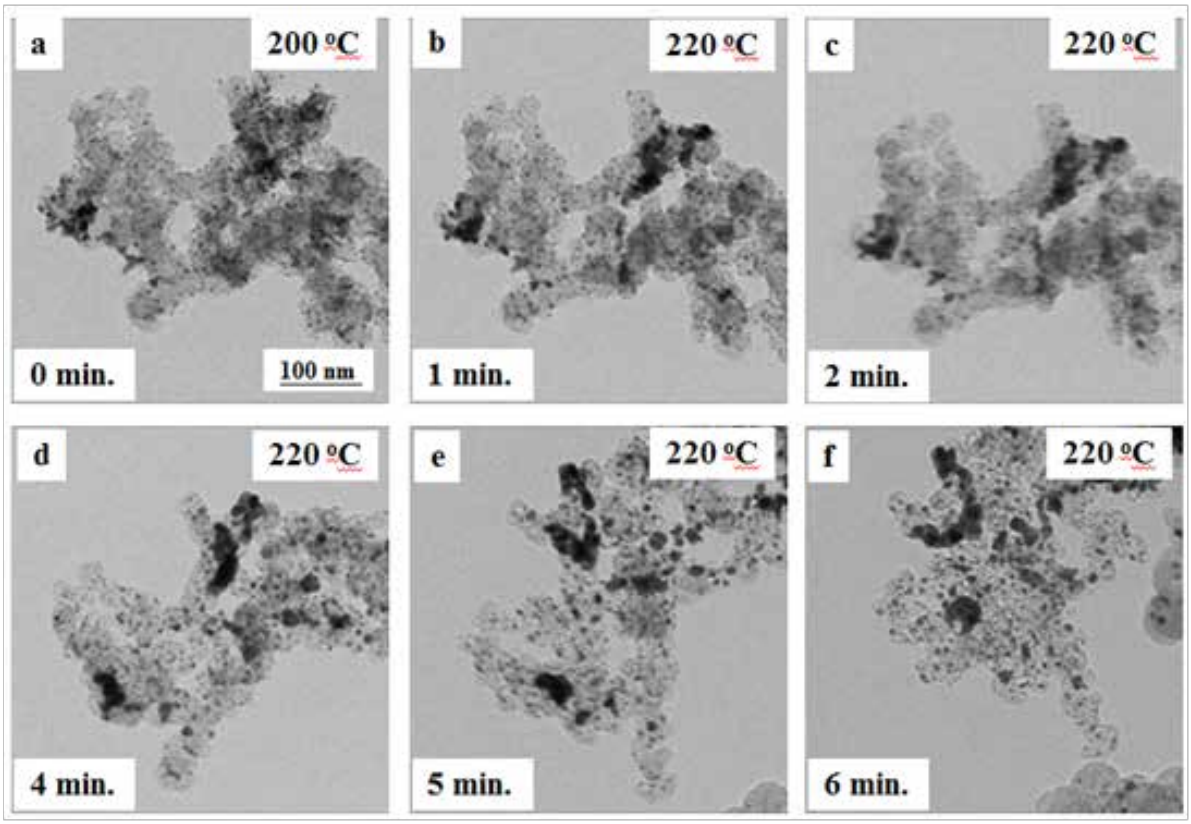

Figure 1. Video *in-situ* sequence images representing the degradation of a Pt/GC electrocatalyst of a fuel cell.

Reference: Yaguchi T., Tamura K., Kubo T., Kondo M., Matsumoto H., Shimizu T. and Kamino T. (2015) Development of *in-situ* TEM techniques dedicated for characterization of energy related composites and its application. *Microsc Microanal* 21 (Suppl 3): 1817.

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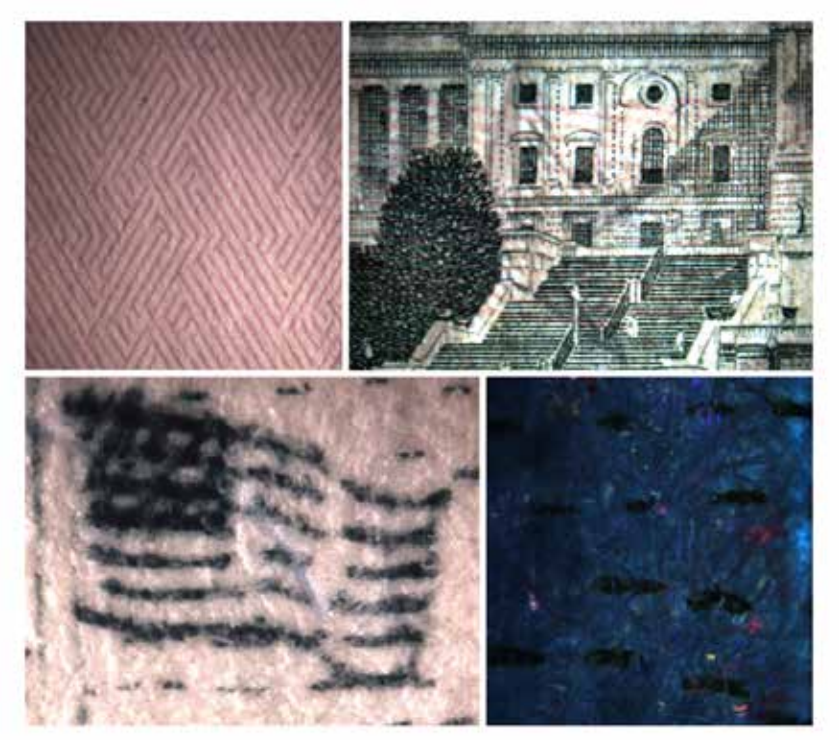
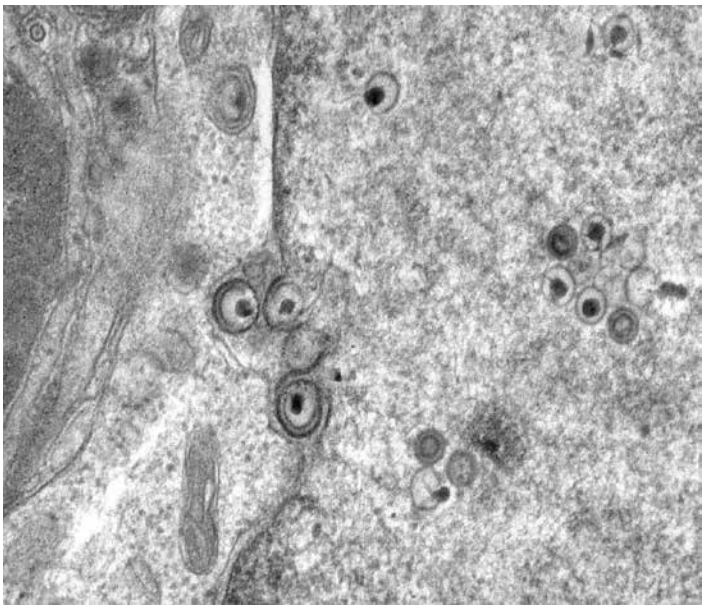


Light microscopy-fluorescence micrograph collage by
Dr. Tina Gumienny, TWU, Dept. of Biology, Denton, Texas 76204-5799



Transmission electron micrograph by
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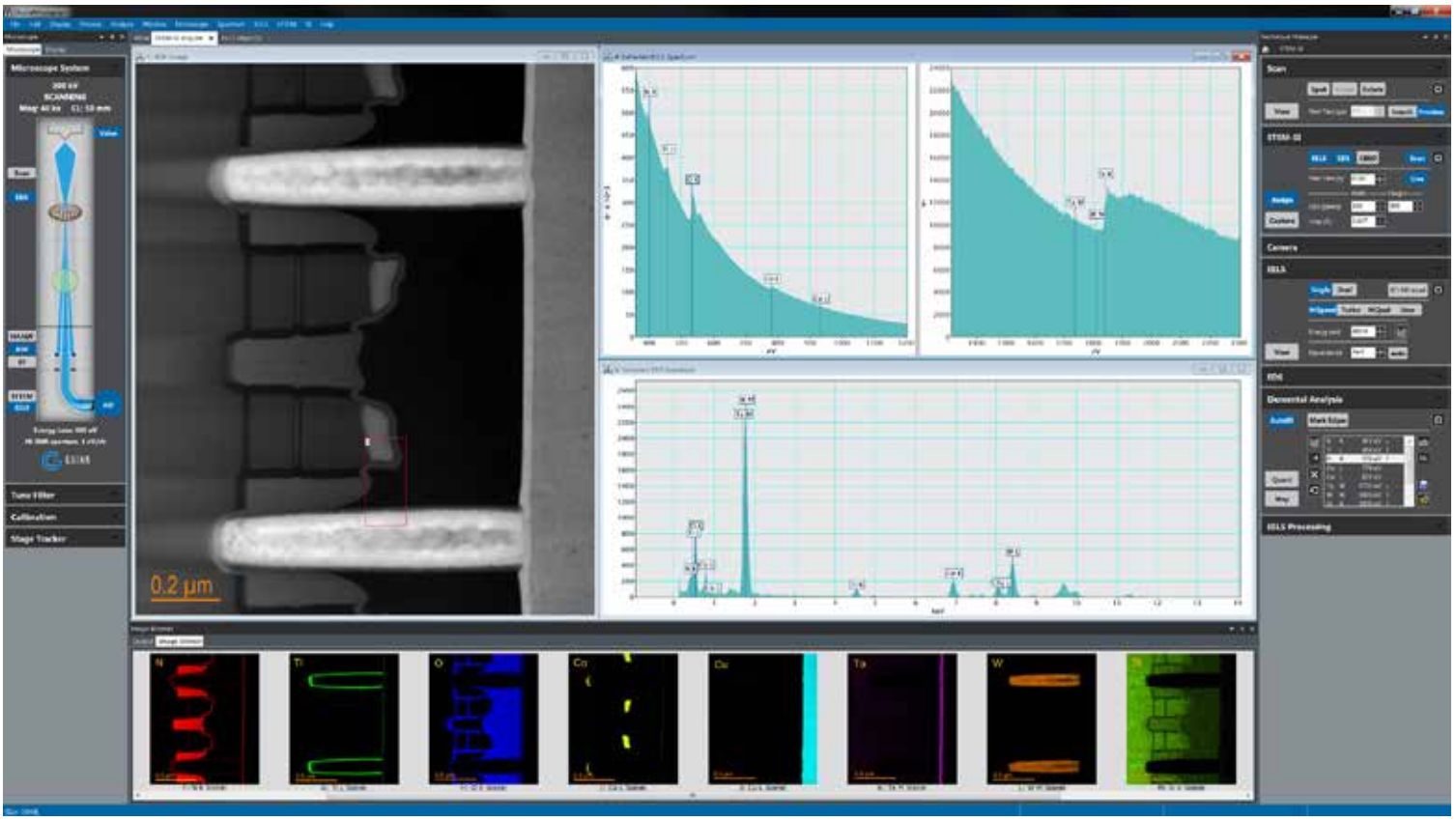

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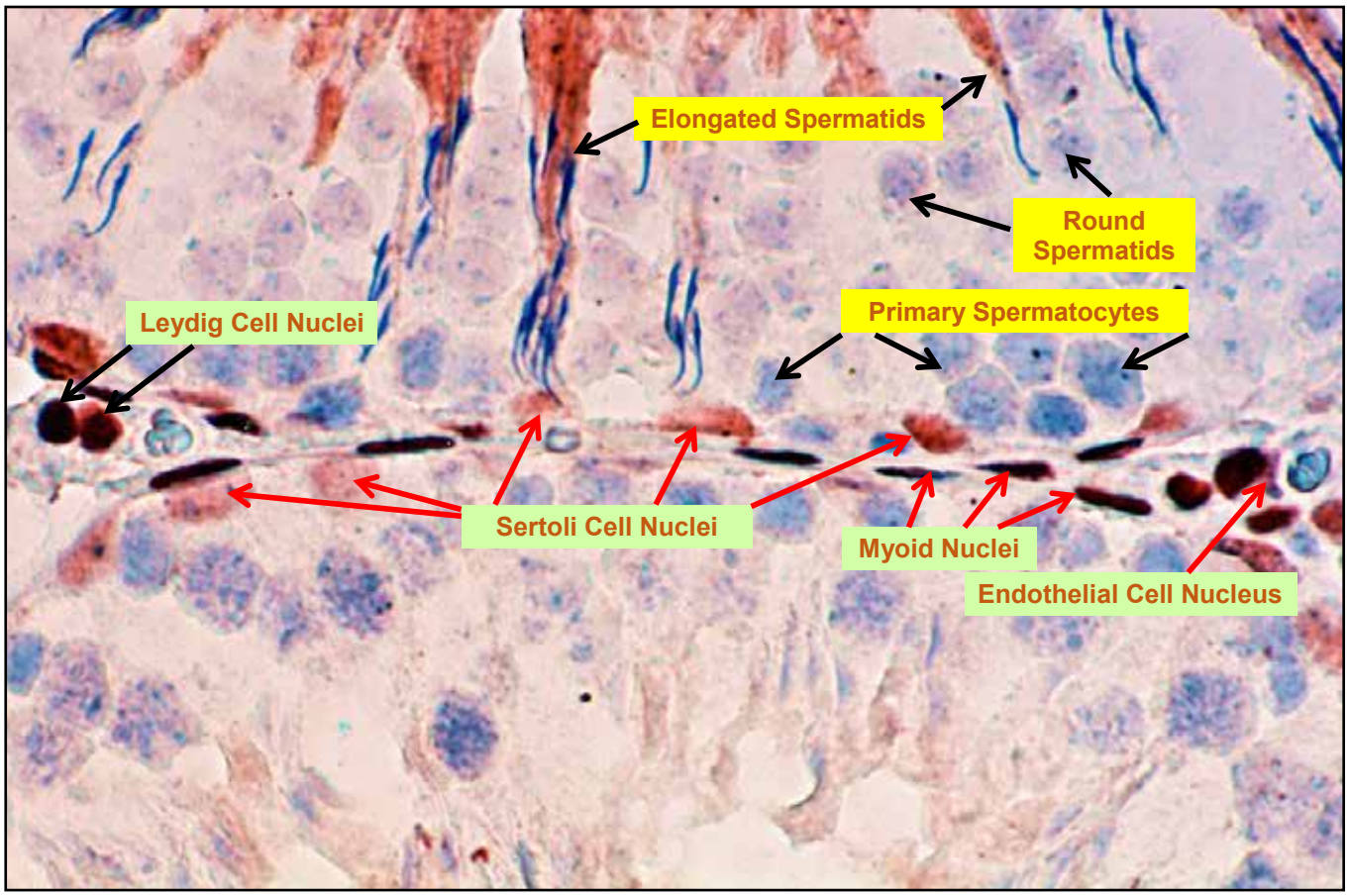

Dr. Nathaniel Mills is a professor in the Department of Biology at Texas Woman's University in Denton, Texas 76204-5799. He mentors graduate MS and PhD students as well as undergraduate research students. Dr. Mills lab uses light and confocal microscopes in studying hormonal regulation of gene expression in male reproductive tissues. The light micrograph (300X) shows the immunolocalization (Nova-red) of androgen receptors in nuclei of Sertoli, Leydig, Myoid, endothelial cells and in stripped cytoplasm of elongated spermatids. The DNA is stained with hematoxylin.

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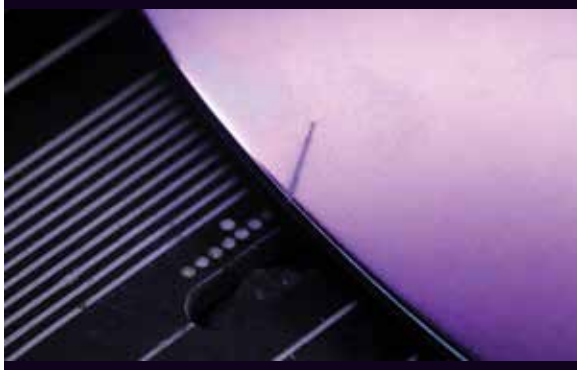

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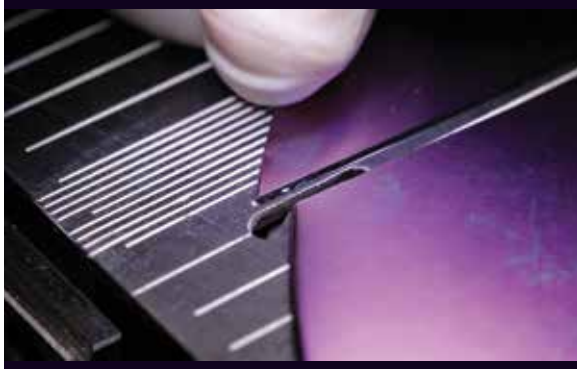

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