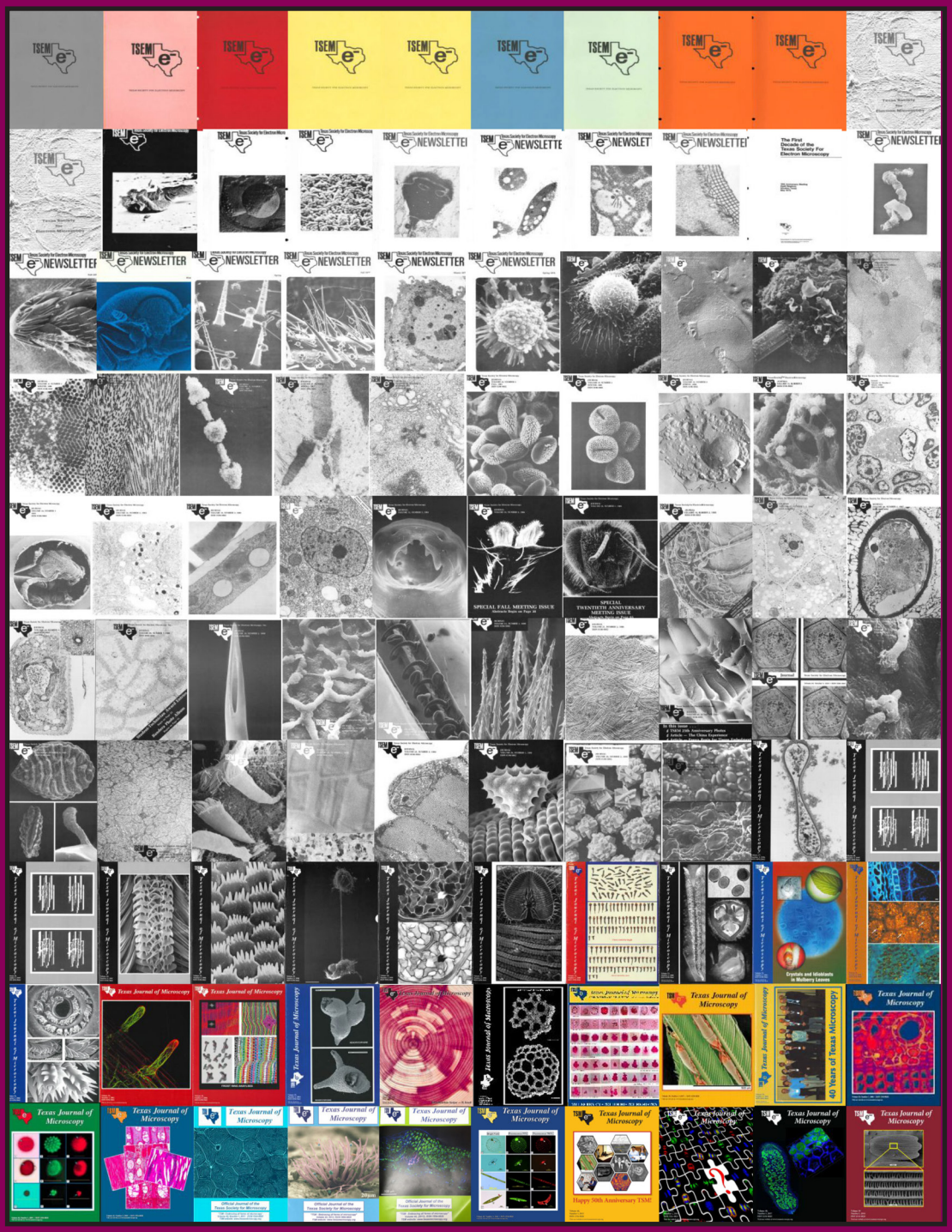
Texas Journal of Microscopy



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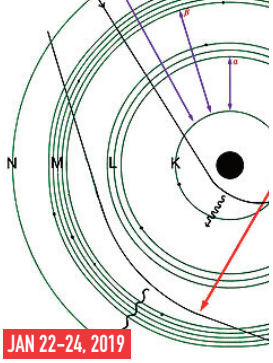
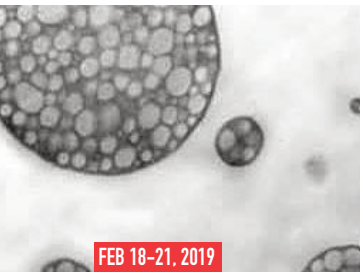
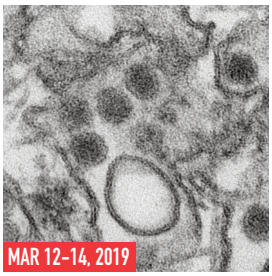


X-Ray Microanalysis Workshop: A Complete Picture

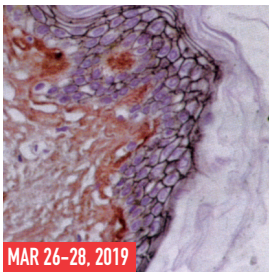
Introduction to Microscopy Techniques Workshop



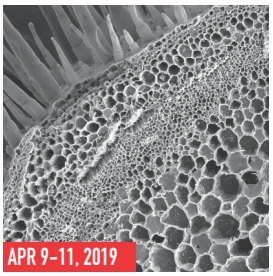
Materials Ultramicrotomy Workshop



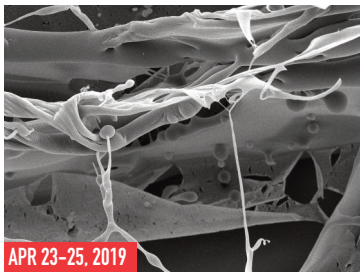
Biological TEM Workshop: A Complete Picture



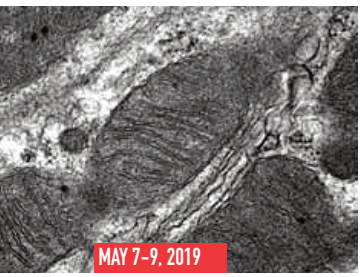
Aurion Immunogold Silver Staining



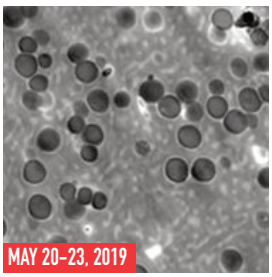
Biological SEM Workshop: A Complete Picture



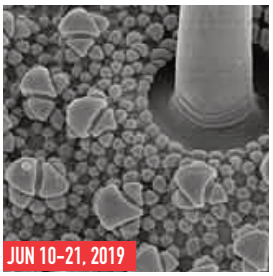
Cryo SEM Workshop



Automated and Rapid Specimen Processing for Electron Microscopy Workshop

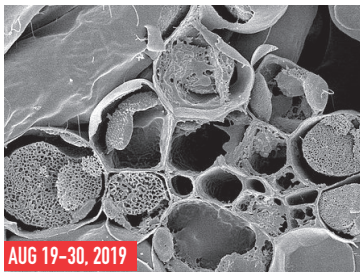


Materials Ultramicrotomy Workshop

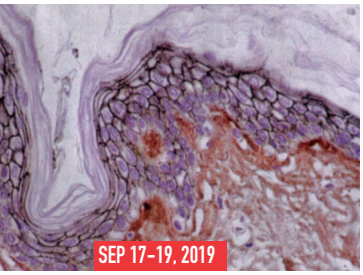


Microscopy: The Complete Image

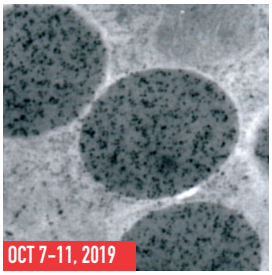
Microscopy: The Complete Image



Microscopy: The Complete Image

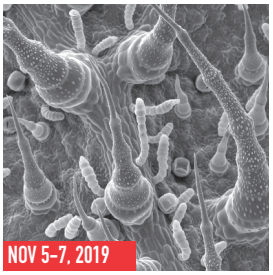


Aurion Immunogold Silver Staining

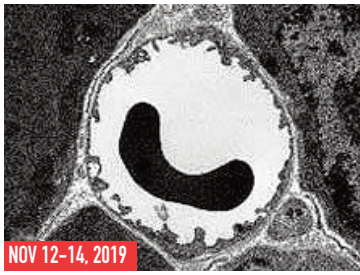


Cryosectioning/Immunogold Workshop

Introduction to Microscopy Techniques Workshop



Biological SEM Workshop: A Complete Picture



Biological TEM Workshop: A Complete Picture

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- Pharmaceutical Chemical Imaging Workshop
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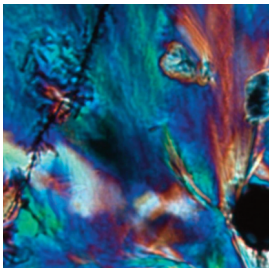

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Contents

TEXAS JOURNAL OF MICROSCOPY
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Editor
Catalina Pislariu, PhD
Department of Biology, Texas Woman's University, Denton, TX 76204

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ON THE COVER

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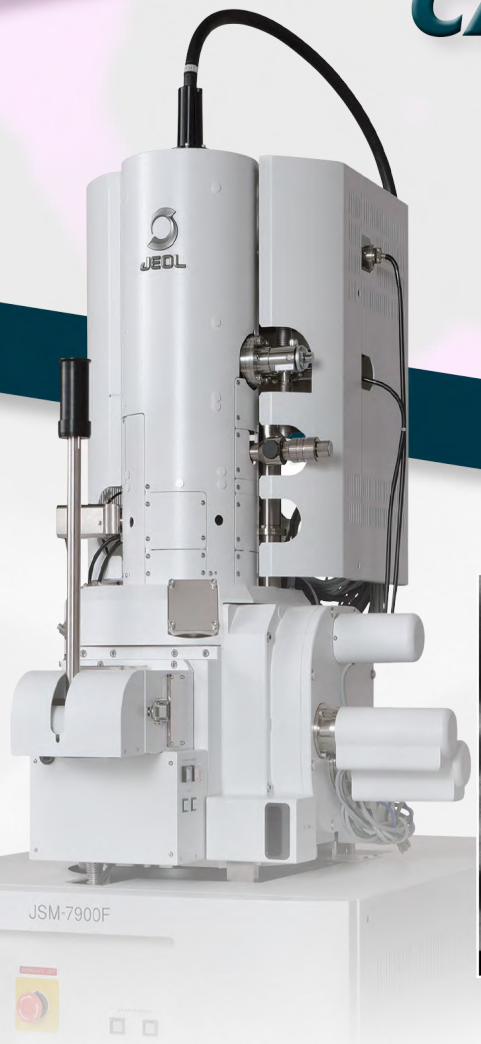

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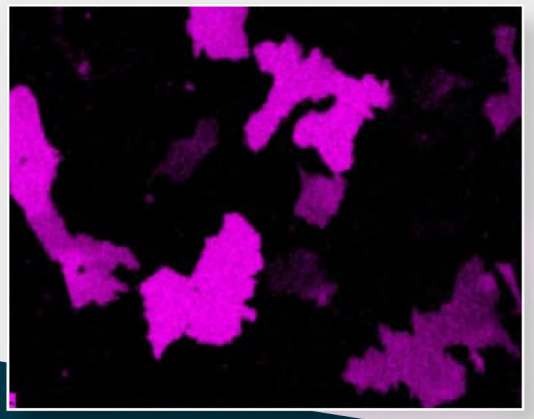

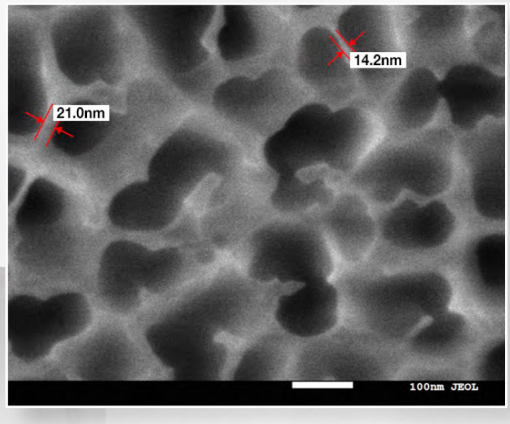

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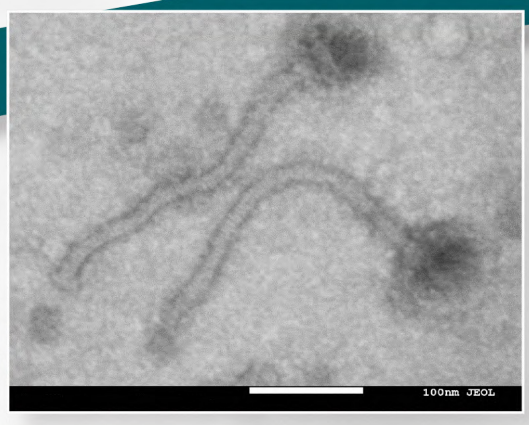

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President's Message

Another busy and successful year has gone by for the Texas Society for Microscopy (TSM). Throughout 2018, the executive council worked hard to support our members, to make the TSM more visible, and to make our organization more efficient. I am glad to report that in 2018 we have supported 18 students with travel grants to attend the meeting in Denton, gave away 2 awards for best platform presentations, and supported the research of Yasar Kasu from Texas Woman's University with our Small Grant Program. Last year the TSM also started an initiative to support grant proposals of members with a letter of support from the society. Information regarding this initiative can be found on our webpage. We also updated the webpage of our journal which publishes peer-reviewed manuscripts of our members free of charge. Members and non-members are welcome to submit manuscripts throughout the year. In August, Josefina Arellano Jimenez successfully represented the TSM at the M & M meeting in Baltimore. Our efforts paid off! In 2018 we added 2 honorary, 3 corporate and 26 regular/student memberships.

Amy Jo Hammett has done an amazing job organizing the 54th annual meeting in San Antonio. With the help of the local organizer at UT San Antonio, Josefina Arellano-Jimenez, they have organized a wonderful meeting with fantastic workshops. In this respect, I want to thank Protochips, UTSA, and Zeiss for sponsoring these workshops and for showing us the latest advances in the field of light, scanning electron, and in situ transmission electron microscopy. I also want to express my thanks to our editor, Catalina Pislariu, for preparing, designing and publishing the 50th anniversary issue of the Texas Journal of Microscopy.

Thanks to our treasurer, David Garrett, our society is in good financial health, so I also want to express my thanks to him for keeping up with our finances throughout the year. I also want to thank James Long who does a great job representing our corporate members, Aubrey Howard who does an amazing job getting students involved in the TSM. Further, I am grateful for the support of Catalina Pislariu and Shazia Ahmed in their roles as secretaries of the TSM. Finally, I want to thank Nabarun Ghosh who has done a wonderful job increasing the visibility of the TSM on our social networks.

Josefina Arellano-Jimenez, our program chairwoman elect, has already started planning for our annual meeting in 2020 which will be held at Texas A & M University in College Station. Our host and local organizer will be Stan Vitha from the Microscopy and Imaging Center at Texas A & M University. Based on their feedback so far, I am very confident that it will be another great meeting, and hope to welcome you all there again.

Finally, I want to thank all corporate, honorary, regular, and student members for supporting the TSM with their membership and for participating at our annual meetings. It has been an honor and pleasure serving as President of the TSM in 2018-2019 and I will continue to share my enthusiasm for microscopy with our members.

Bernd Zechmann
TSM president 2018-2019

INVITED PRESENTATIONS

PRECISION MODELS OF HUMAN NEURAL DEVELOPMENT AND DISEASE

JENNY HSIEH

Professor and Director of the Brain Health Consortium, Semmes Foundation Chair in Cell Biology, University of Texas at San Antonio, San Antonio, TX 78249

Dr. Jenny Hsieh is the Director of the UTSA Brain Health Consortium (BHC), which was established as a collaborative team designed to revolutionize brain health and treat disease. Starting with an individual's cells, the BHC aims to discover precision therapeutics—individualized therapy—tailored for an exact condition. The BHC uses advances in genomics and emerging methods for "disease-in-a-dish" models to accelerate discoveries. The BHC focuses on innovative new treatments for brain diseases such as mental illness, depression, epilepsy, traumatic brain injury, and Alzheimer's disease. Within the BHC, Dr. Hsieh has established a Stem Cell Core facility to facilitate reprogramming of patient induced pluripotent stem cells (iPSCs), study of organoid development and function, and CRISPR-mediated gene editing for the generation of isogenic controls. Dr. Hsieh's research has been using hiPSCs, 3D cerebral organoids, and CRISPR/Cas9 gene editing technology as approaches to understand mechanisms underlying brain disorders. Using this unique set of tools, the lab has generated important and exciting preliminary data to show that 3D cerebral organoids from human iPSCs exhibit glutamatergic and GABAergic differentiation and neuronal network activity.

MAPPING IN-SITU DYNAMIC PROCESSES AT NANOSCALE BY ELECTRON HOLOGRAPHY

ARTURO PONCE

Associate Professor

Department of Physics and Astronomy, University of Texas at San Antonio, One UTSA Circle, San Antonio, Texas 78249

The progress of transmission electron microscopy (TEM) has taken a turn in the efforts to achieve the highest spatial resolution using aberration correctors up to measuring physical phenomena within the electron column. Electron-matter interaction leads into multiple types of radiations that can be registered to analyze chemical and physical properties of materials. TEM image observation mode remains a big cornerstone for imaging the phase of the electron wave, since only the amplitude can be detected. Zernike phase contrast provided the initial idea to recover the phase of the images. Later electron holography was proposed to improve the electron microscope followed by the invention of the Möllenstedt biprism, which allowed the use of the off-axis electron holography. Electron holography provides the most direct and reliable access for dealing the problem phase in electron microscopy, but its requirements related to the coherence of the electrons are strict, and the optimized conditions due to the changes in magnification remains a significant instrumental limitation. Phase retrieval from the holograms is a complex method that requires a workflow to extract the phase in real time. Live electron holography registers the modulation of the electron wave in its phase due to the intrinsic properties of the samples or due to the external stimuli at in-situ TEM set ups. Understanding those materials' behavior under external signals in now one of the most recent challenges in TEM.

In this talk, a variety of in-situ TEM experiments performed under external stimuli and the method to recover the phase image in real time will be exposed, e.g. 1) mapping of the radiation pattern in ZnO/Ag nanoantennas, 2) direct imaging of magnetic interactions in metallic nanostructures and 3) electric and magnetic properties measurements in materials as function of temperature variations. Electric and magnetic fields shift the phase of the electron beam and produce a strong contribution in the images. In this way, electron holography is a sensitive technique to quantify the physical properties, properly separated from all the contributions present in the phase image. The example of Figure 1 shows an experimental set up of an in-situ radio frequency stimulation coupled to the TEM to study the time evolution of the radiation pattern of nanoantennas recorded at different amplitudes and frequencies in the MHz regime. The sample is mounted on an electric head chip (Fig. 1A), in which the pad serves as an electric path to introduce a modulated signal using the wave function generator G (Fig. 1C). The response of the external stimulus is registered using real time phase reconstruction off axis electron holography.

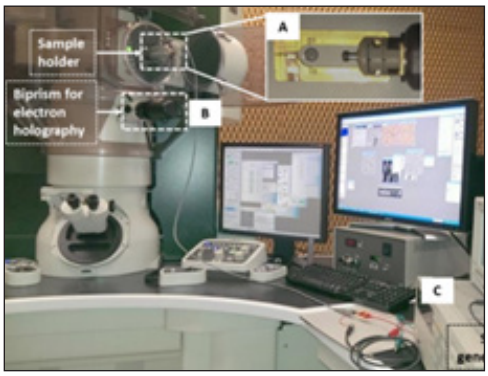

Fig. 1: Experimental set up for in-situ radio frequency stimulation in ZnO/Ag nanoantennas. (A) Sample holder, (B) Möllenstedt biprism, (C) signal generator.

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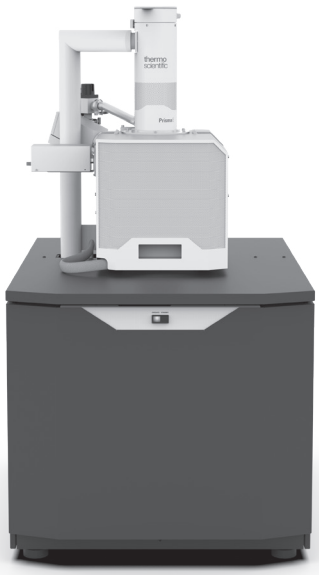

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Abstracts

LIFE SCIENCES
Spring 2019

BIMOLECULAR FLUORESCENCE COMPLEMENTATION TO EXPLORE LIAT1 INTERACTIONS. AKSHAYA ARVA and CHRISTOPHER S. BROWER, Department of Biology, Texas Woman's University, Denton TX, 76204-5799.

Arginyl-transferase 1 (ATE1) is an enzyme catalyzing the ribosome independent, post-translational arginylation of a number of proteins, including those involved in cellular stress response. Recently, a previously uncharacterized protein, LIAT1 (Ligand of ATE1), was found to interact with some isoforms of ATE1 although the function of this interaction has not yet been characterized. LIAT1 was subsequently shown to interact with a number of additional proteins including Jmjd6, a bifunctional arginine demethylase and lysyl-hydroxylase and LARP7, a component of the 7sk ribonucleoprotein (RNP) complex involved in DNA transcription. In efforts to understand LIAT1 function, we carried out bimolecular fluorescence complementation (BiFC) assays using LIAT1 fused to one-half of yellow fluorescent protein (YFP), and its binding partners fused to the complementary half of YFP. While neither fusion is fluorescent by itself, an interaction brings the complementary halves of YFP into close proximity such that fluorescence is reconstituted. These studies also provide information regarding the subcellular localization of interactions. Surprisingly, we found that ATE1 and LIAT1 interact to form punctate cytosolic structures resembling stress granules, dense aggregates of protein, and RNA stalled in translation during stress conditions. However, neither ATE1 nor LIAT1 co-localized with G3BP1, a well-characterized stress granule marker. Therefore, these LIAT1- and ATE1-containing structures are distinct from classical stress granules. These studies will provide new insights into the role of ATE1 in the cellular stress response as well as the function of LIAT1.

MICROSCOPIC CHARACTERIZATION OF THE AEROALLERGEN AND REDUCTION IN ALLERGY INDEX ON USING AIR PURIFIERS WITH AHPCO AND PLASMA NANO-TECHNOLOGY. AUBREY HOWARD[1], NABARUN GHOSH[1], JON BENNERT[2] AND JEFF BENNERT[2] [1]Department of Life, Earth & Environmental Sciences, West Texas A&M University, Canyon, Texas, 79016, [2]Air Oasis, Research and Development, Amarillo, Texas, 79118.

Allergies are a hypersensitivity of the immune system to normally benign substances. The Panhandle of Texas sees diagnosis rates typically double those of the state average. Seasonal allergies can have a strong impact on the quality of everyday life due to the presence of aeroallergens in the air such as pollen. In order to aid diagnosis of seasonal allergies and characterize the aeroallergens in the Texas Panhandle, research must be conducted to establish a foundation for determining the trends of data and provide a reference for the identification of allergenic weed species. Similar studies have been conducted, however they have not included native angiosperms specific to the Texas Panhandle, making it more difficult for individuals to identify plant origins of pollen. In order to address this concern, a set of observations must be made, analyzing the morphological characterization of pollen structure to aid in diagnosis, and establish a reference for researchers for linking pollen grains to the plant they originated from. We recorded the GPS location, collected the whole plant, when possible, for keeping it as herbarium specimen, and the flowers for pollen analysis. Flowering plants have been collected and their pollen analyzed and characterized. Micrographs were taken using an Olympus DP74 digital camera attached to a Olympus BX40 microscope, and were analyzed using cellSense digital software. The most significant aeroallergens recorded were the pollens such as grass pollen (Poaceae), Short Ragweed *(Ambrosia artemisiifolia)*, Common Sunflower *(Helianthus annuus)*, Hairy Sunflower *(Helianthus hirsutus)*, Silverleaf Nightshade *(Solanum elaeagnifolium)* and Lamb's Quarters *(Chenopodium album)* as well as fungal spores like *Alternaria*, ascospores from Pezizales, *Drechslera, Stachybotrys, Cladosporium* and *Curvularia.* Further analyses of the gathered data are needed for prediction on the distribution of pollen and pollen seasons. We aim to incorporate the AHPCO and Plasma Nanotechnology for developing an advanced air purification system to reduce the indoor particulate matters including all forms of aeroallergen. This data collecting stage of research will help establish a foundation for future research and is the first crucial step for the hypothetico-deductive approach.

HOW QUANTITATIVE ANALYSIS OF NANOMETER-SIZED STRUCTURES USING COMPUTER SOFTWARE CAN INFORM PHYSICOCHEMICAL CHARACTERIZATION FOR FUTURE RESEARCH. DESIRAE CARRASCO, MARINA R. MULENOS, HENRY LUJAN, and CHRISTIE M. SAYES, Department of Environmental Science, Baylor University, Waco, Texas 76706.

As the use of microscopic imaging in research expands, there is increasing need for innovative post-

processing methods to support interpretations. By measuring the length and width of nanometer sized structures with computer software specialized for custom characterization, data can be clearly presented in scientific communications. In support of these innovative methods, we used quantitative analysis to display precise length and width measurements of cellulose fibers (Fig. 1 A), nickel nanoparticles (Fig. 1 B), and lung cell mitochondria (Fig. 1 C), to record subtle changes in experimental conditions. The fibers and particles were subjected to enhanced aging over time and the lung cells were exposed to hypoxic culture conditions. Not only can the electron microscopy provide a qualitative assessment of structural changes, but also semi-quantitative analyses of size differentials. The CellSens software (Olympus, Olympus America Inc., Center Valley, PA) enabled a practical and accessible way to measure structures. The software is user-friendly, but has limitations based on the quality of the photomicrograph. Images with densely packed, overlapping structures, cannot be measured with high degree of confidence. Ultimately, we aim to use machine learning algorithms to automatically measure the nanometer-sized structures in the collected images. This preliminary dataset demonstrates how images can be quantified, and warrants further investigation in automation.

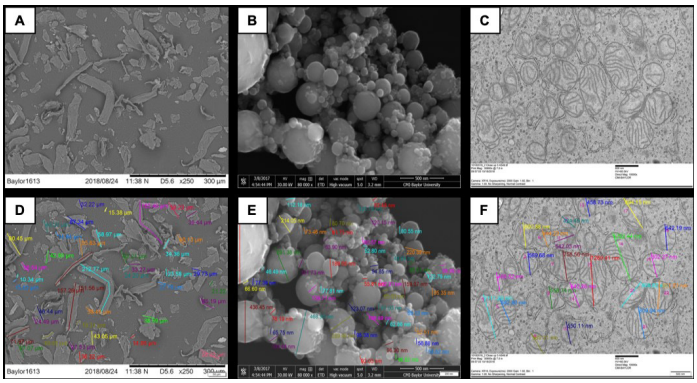

Fig. 1: Scanning electron micrograph of (A) cellulose fibers, (B) nickel nanoparticles, and Transmission Electron Micrograph of (C) human lung cell mitochondria. Length analyses of (D) cellulose fibers, (E) nickel nanoparticles, and (F) human lung cell mitochondria.

COMPARATIVE STUDY OF STOMATA FROM DIVERSE PLANT LINEAGES. URJITHA VARDHINENI, SHAZIA AHMED, and AMY JO HAMMETT, Department of Biology, Texas Woman's University, Denton TX, 76204-5799.

The presented comparative study examines the distribution and density of stomata across evolutionary lineages of plants. The principal purpose of stomata is to take in carbon dioxide and release oxygen in the environment, playing a central role in photosynthesis. The stomata density of a plant can regulate water loss rates and CO2 uptake. The lineages analyzed include: Liverwort, Moss, Lycophyte, Fern, Gymnosperm, and Angiosperm- monocot & dicot. The size and number of stomata were first determined by using a light microscope and observing 3 leaf samples from each lineage (Table 1). Detailed pictures were taken using a Scanning Electron Microscope to provide more detailed images of stomata structure. Results show differences in stomata density and abundance between different lineages (Figs.1 and 2). These differences help plants survive in a variety of habitats.

Taxonomic Group	Plant Name	Average Stomatal Density	Average Stomatal Size
Bryophtye: Liverwort	*Marchantia*	8.7 Stomata/100 μm^2	45 μm
Lycophyte	Spikemoss *(Selaginella)*	28.7 Stomata/100 μm^2	32 μm
Monilophyte	Fern (*Nephrolepis*)	25.4 Stomata/100 μm^2	40 μm
Gymnosperm	Pine (*Pinus*)	13.5 Stomata/100 μm^2	47 μm
Angiosperm: Monocot	Grass (*Zea*)	68.1 Stomata/100 μm^2	10.5 μm
Angiosperm: Dicot	Clover (*Trifolium*)	XX Stomata/100 μm^2	21.6 μm

Table 1: Average stomatal density and size in selected members from diverse phylogenetic lineages of plants. Using a Nikon Eclipse E200 microscope and a DS-Fi2 digital camera, three images were taken of each plant leaf to calculate an average stomatal density and size for each plant type.

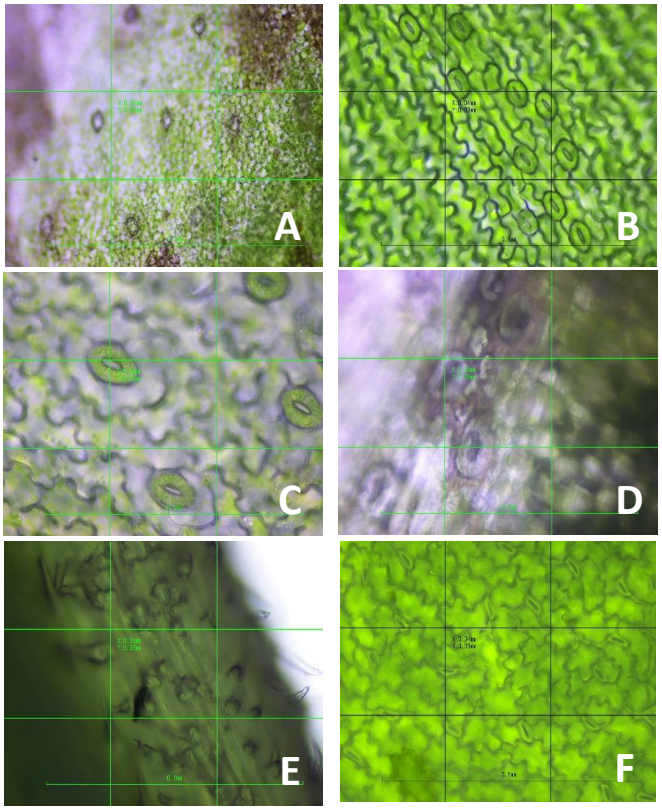

Fig. 1: Light micrographs of stomata show differences in density, arrangement, and morphology between lineages. All micrographs were taken using a Nikon Eclipse E200 Microscope at 400x. A) *Marchantia*; B) *Selaginella*; C) Fern; D) Pine; E) Grass; F) Clover.

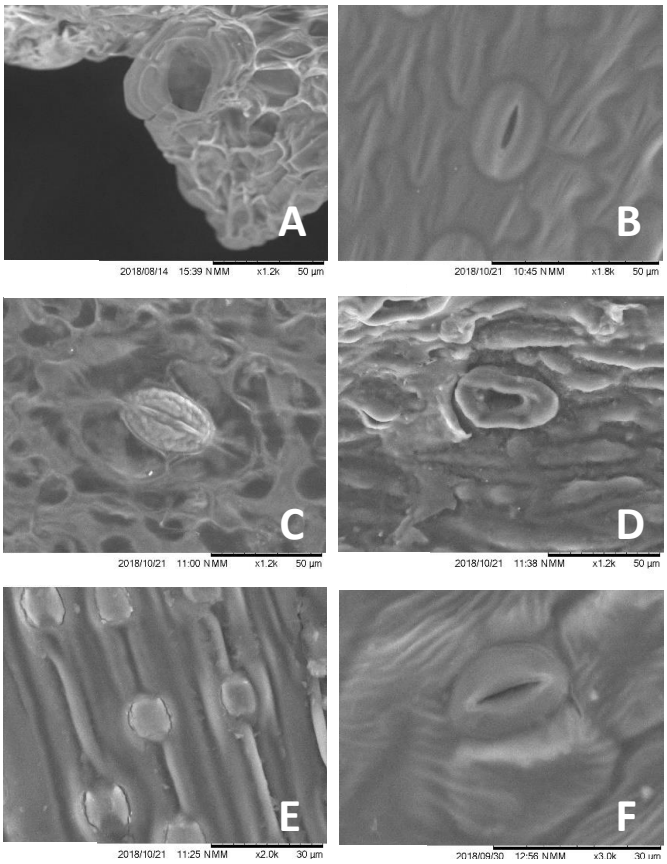

Fig. 2: Scanning electron micrographs of stomata show differences in morphology between lineages. All micrographs were taken using a Hitachi TM3030 Plus Scanning Electron Microscope. A) *Marchantia*; B) *Selaginella*; C) Fern; D) Pine; E) Grass; F) Clover. Scale bars: 50 µm (A, B, C, D); 30 µm (E, F).

CHARACTERIZATION OF SYMBIOTIC NITROGEN FIXATION MUTANTS IN *MEDICAGO TRUNCATULA* USING MOLECULAR AND MICROSCOPIC TOOLS. HALA SAMARA, JEANNE BECKWITH, LOGAN BOLING, and CATALINA PISLARIU, Department of Biology, Texas Woman's University, Denton TX, 76204-5799.

Leguminous plants can thrive in nutrient-depleted soils because they acquire fixed nitrogen in the form of bioavailable ammonium ($NH4^+$), through their symbiotic association with soil bacteria, collectively called rhizobia. Annually, symbiotic nitrogen fixation (SNF) injects around 50 million tons of N2 into the agricultural system (1). *Medicago truncatula*, a relative of alfalfa, is an important genetic model for investigating SNF. Following chemical signaling between *M. truncatula* and its symbiont, *Sinorhizobium meliloti*, specialized new organs, root nodules, develop on its root system where rhizobia convert molecular nitrogen into ammonium. Effective nitrogen fixation is indicated by the nodules' pink color (Fig.1 A). The complexity of the SNF process is reflected in the thousands of host and bacterial genes that are highly regulated (2). To facilitate the discovery and characterization of new symbiotic genes, a tobacco retrotransposon *(Tnt1)*-insertion mutant population was developed at Nobel Research Institute. Symbiotic phenotypes of putative *Tnt1*-insertion symbiotic mutants are being investigated using an array of microscopic techniques, including stereo, compound, confocal, scanning and transmission electron microscopy. Symbiotic phenotypes of *Tnt1*-insertion mutants will be presented, including those developing white or brownish ineffective nodules (Nod+Fix- phenotype) (Fig.1 B-D), pale-pink nodules (Nod+Fix+/-) indicating less efficient SNF (Fig. 1 E-I). Some mutants also display supernumerary nodulation phenotype (Fig. 1 G-I). Such phenotypes are thoroughly characterized microscopically, and results will be presented. We also engineered a range of rhizobial strains to constitutively express the *hemA::LacZ* reporter to track the bacterial infection process in symbiotic mutants, and to evaluate host-strain compatibility and nitrogen fixation efficiency.

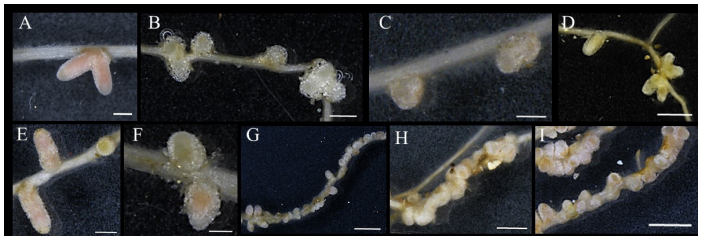

Fig. 1: Symbiotic phenotypes of nodulated root segments. A: Wild type R108. B: NF0737. C: NF0440. D: NF0063. E: NF1295. F: NF5659. G: NF19336. H: NF1526. I: NF1709. Bars = 1 mm (B, D, and G), 5 mm (A, C, E, and F), and 1 cm (H and I).

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CYTOLOGICAL ASSESSMENT OF THE GENOTOXIC EFFECTS OF ATRAZINE BASED HERBICIDE ON *ALLIUM CEPA*. LINDSEY VANDE STREEK, KATLYN MADEN, MYKALA ROBERTSON, AUBREY HOWARD AND NABARUN GHOSH, Department of Life, Earth & Environmental Sciences, West Texas A&M University, Canyon, Texas, 79016.

Atrazine is a wide-range tetrazine herbicide that is commonly used in United States agriculture. For over 50 years, atrazine has been used as a selective broadleaf herbicide in many capacities, from pre-plant to pre-emergence to post-emergence, depending on the crop and application. Currently, 96% of all atrazine used is for commercial applications in fields for the control of broadleaf and grassy weeds in crops such as sorghum, corn, sugarcane, pineapple and for the

control of undesirable weeds in rangeland. It is known to be immunotoxic and cause endocrine disruptions and reproductive issues. Present investigation focuses on atrazine and its effect on cell division in *Allium cepa* (green onions). *Allium cepa* is a widely used 'test organism', because it is highly sensitive to genotoxic materials due to its large chromosome size. *Allium cepa* was hydroponically grown in water containing differing amounts of atrazine-based herbicide. After sufficient growth, root tips were excised and pretreated with para-dichlorobenzene (p-DB), fixed with 1:3 Aceto-Ethanol and stained with 2% Aceto-Orcein stain. The prepared slides were viewed using a DM-750 Leica digital microscope attached to a ICC50-W digital camera with LAS V4.4 software to capture the images. Mitotic indices (MI) varied among the treated and the control groups with time intervals. The control group had no significant chromosomal abnormalities. We noticed a seasonal variation in terms of the MI that was higher during summer which gradually declined during winter. Results from the treated groups showed decreased MI, chromosomal clumping, sticky bridges, chromosomal breakage and low tissue quality in the treated *Allium cepa* cells. Atrazine is found in groundwater in the United States 20 times more frequently than any other herbicide; further investigation is necessary for raising public awareness about the detrimental effects of atrazine.

REFINING *IN VITRO* TOXICITY MODELS IN NEUROTOXICOLOGY: COMPARING BASELINE CHARACTERISTICS OF NEURONAL CELL-TYPES. S. PRADHAN and C.M. SAYES, Department of Environmental Science, Baylor University, Waco, TX, 76706.

The nervous system is a complex system of organs and nerve cells which is particularly vulnerable to adverse effects of toxic insult. Much of the research within the field of neurotoxicology utilizes in vivo methods of analysis but is rapidly evolving to include in vitro alternatives. This method of assessment better addresses mechanistic analysis, specifically this has been used to observe mechanisms behind neurodegenerative disorders such as Alzheimer's or Parkinson's Disease.

This study characterizes cell lines commonly used to model Parkinson's Disease, a primary (Normal Human Astrocytes; NHA) and cancer-derived cell line (SH-SY5Y). Cell lines were observed by phase microscopy for bright field images, and by Confocal Laser Scanning Microscopy (CLSM) for fluorescence images. Phase microscopy provided baseline information about cellular growth characteristics, while the fluorescence microscopy provided more detailed information on cellular morphology (Fig. 1).

The NHA and SH-SY5Y cell lines are commonly used due to their catecholaminergic expression commonly associated with Parkinson's. Proliferation data and metabolic endpoint analyses were generated, and microscopy techniques provided information about cell confluency, colony formation and morphological changes over time.

Through microscopy techniques, results yielded evidence for differences in proliferation characteristics, morphology and catecholaminergic expression between the two neuronal derivatives. Additional areas of study are mitochondrial structure through electron microscopy, whole cell analysis, and gene and protein expression pre- and post- exposure to a toxic insult.

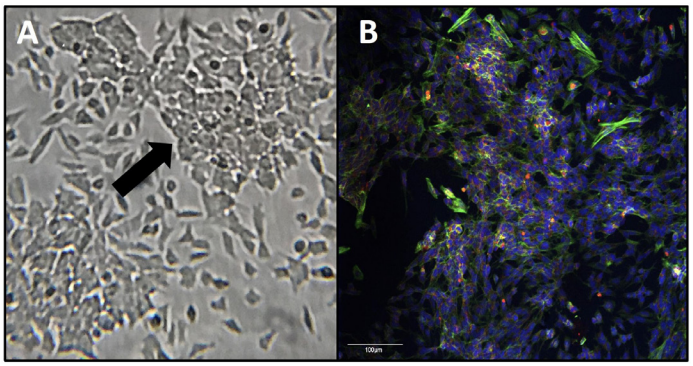

Fig. 1: (A) Phase Microscopy image of SH-SY5Y cell line at 10x magnification. Colony aggregation of catecholaminergic cells shown in microscopy image (Black Arrow). (B) Fluorescence Microscopy image of SH-SY5Y taken with CLSM at 20x magnification. Image is stained for F-actin, b-tubulin, mitochondria and the nucleus.

DEVELOPING A HIGH-THROUGHPUT SCREEN FOR MODULATORS OF THE ARGINYLATION DEPENDENT N-END RULE PATHWAY. YASAR ARFAT T KASU and CHRISTOPHER S BROWER, Department of Biology, Texas Woman's University, Denton TX, 76204-5799.

Arginyl-transferase 1 (ATE1) is an enzyme catalyzing the ribosome independent, post- translational arginylation of proteins, resulting in their degradation by the ubiquitin proteasome system through the N-end rule pathway. Mice lacking the *ATE1* gene undergo a dramatic loss of fat tissue, are resistant to high-fat diet induced obesity, and suffer from neurological perturbations indicating that ATE1 plays an important role in fat metabolism and brain function. Recently, we showed that ATE1 is required for the degradation of fragments of the TAR DNA binding protein 43 (TDP43) associated with neurodegeneration [1]. Owing to multiple cleavage sites within TDP43, a number of otherwise identical fragments were found with slightly different N-termini and altered ATE1 requirements for their degradation through the N-end rule pathway. Through these studies, we identified a polypeptide capable of functioning as an ATE1-dependent N-terminal degradation signal (Ndeg). Here, we designed an Ndeg-bearing fluorescent reporter to measure ATE1 activity. This reporter can be used in screens to identify genetic determinants or small

molecules capable of modifying ATE1 activity in cells. Ultimately, these efforts may lead to therapies useful in the treatment of neurodegeneration or obesity.

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DYSREGULATION AND MORPHOLOGICAL CHANGES OF MITOCHONDRIA WITHIN LUNG CELLS AFTER EXPOSURE TO ENGINEERED ALUMINUM NANOPARTICLES. H. LUJAN[1], M.R. MULENOS[1], D. CARRASCO[1], B. ZECHMANN[2], and C. M. SAYES[1],[1]Environmental Science, Baylor University, Waco, TX 76706; [2]Center for Microscopy and Imaging, Baylor University, Waco, TX 76706.

Aluminum (Al) is found ubiquitously in the environment, and has been extensively studied in the environmental science community. At high exposure concentrations (i.e. 1 mg/kg/day), Al functions as a deregulator of normal mitochondrial activity. [1] As advanced material processes continue to grow, aluminum nanoparticles (AlNPs) are now routinely engineered for use as a fuel, paint and cosmetic additives, as well as optical and other protective surface coatings. While the toxicity of elemental aluminum has been established, the health effects of this newly emerging nanomaterial have yet to be characterized.

Determining the mechanism of toxicity for AlNPs is warranted due to the known detrimental interactions within mitochondria (i.e. respiration, ATP production, and cellular growth rates). Furthermore, reactive oxygen species (ROS) levels within cells exposed to elemental aluminum have been shown to increase. [2, 3] Because of their size and propensity to generate ROS when suspended in aqueous suspensions, we hypothesize that the AlNPs will elicit a more profound dysregulation of mitochondrial health.

To determine the mitochondrial health in cells after exposure to AlNPs, three different types of human lung cells (i.e. cancerous, asthmatic, and normal primary) were inoculated with increasing concentrations of AlNPs ranging from 10 ppb to 100 ppm. The cell types and dosing concentrations are within the acceptable exposure level set by EPA guidelines.

Transmission electron microscopy (TEM) techniques were utilized to visualize mitochondria and determine the size, size distribution, and aspect ratio of mitochondria within each cell type before and after exposure (Fig. 1). Fluorescence microscopy and spectroscopic techniques were also employed to measure ROS levels and mitochondrial membrane potential (MMP) against control samples.

Our data shows that mitochondrial dysregulation (i.e. loss of membrane potential and average change in mitochondria size and shape) was more prominent among the asthma phenotype, than the primary or cancer cell-types.

This methodology demonstrates a novel method to determine small changes in mitochondrial morphology that has the potential to aid in the prediction of downstream adverse health outcomes after particle exposure. These findings will help guide future research using mitochondrial health as a biomarker of overall human health.

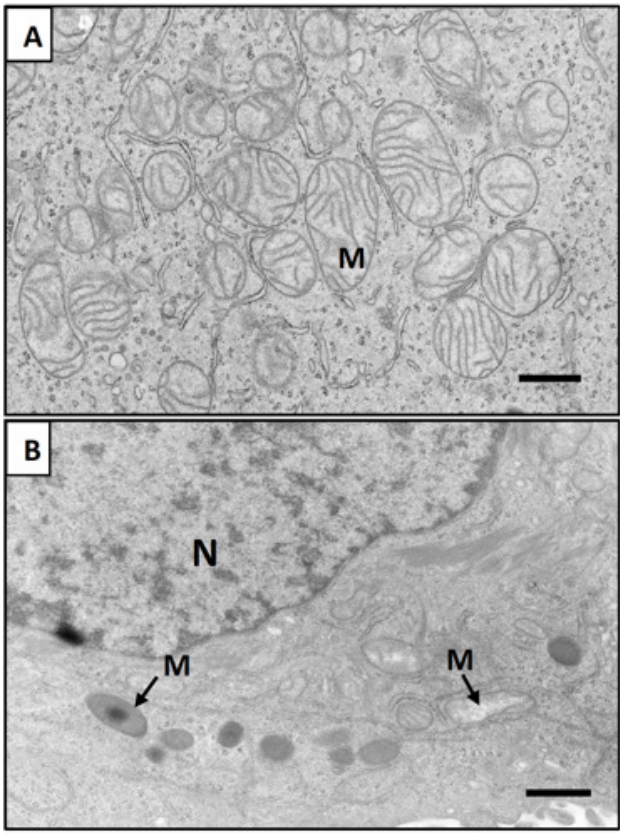

Fig. 1: Exposure to aluminum nanoparticles causes changes in mitochondrial morphology. (A) Micrograph of A549 lung cell mitochondria before exposure to aluminum nanoparticles; (B) Micrograph of A549 lung cell mitochondria after a low dose exposure to aluminum nanoparticles. The arrows in panel B show the discoloration of mitochondria, as well as the loss of cristae structure. M – mitochondria; N - nucleus. Scale bars = 600nm.

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THE EFFECT OF UV RADIATION ON CHROMATIN COMPACTION IN HeLa CELLS, NIH2/4 CELLS, AND HUMAN EPIDERMAL MELANOCYTES. Rituparna Sinha Roy, Mohammad Abbas, Anh Vo and Michael Bergel, Department of Biology, Texas Woman's University, Denton, TX 76204.

Chromatin, the complex of DNA associated proteins and RNA, has several levels of organization, ranging from the most unfurled fiber to the most compacted chromosomes. Gene expression, DNA replication and DNA repair are cellular functions dependent on the organization of chromatin. This study aims to understand the relationship between UVC or UVB irradiation and compaction of chromatin. A 256 tandemly repeated LacO DNA sequence integrated into the genome of NIH2/4 mouse embryonic fibroblasts was targeted by fluorescently-tagged LacR protein to visualize the hypothesized compaction of chromatin after UVC and UVB irradiation. Currently, we also study UV-induced chromatin compaction using a chromatin compaction fluorescent-dye assay kit on HeLa cells and human epidermal melanocytes (Fig. 1). By using the physiological relevant UVB wavelength we will broaden our understanding of an innate cellular mechanism that protects the DNA from damage due to exposure to solar radiation and decipher the signaling pathway that regulates this response.

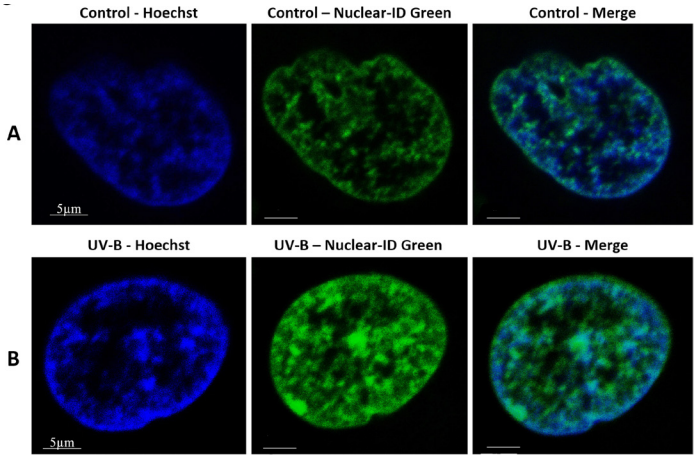

Fig. 1: Chromatin is compacted globally 5 minutes after UVB irradiation in human cervical carcinoma Hella S3 cells. Cells stained with NUCLEAR- ID Green dye A) Non-Irradiated cells B) UVB irradiated cells with 300 J/m2 dose after 5 minutes. Hoechst dye served as a counter stain. Spotty pattern was observed upon UVB irradiation.

NANOMATERIALS OF BIOMEDICAL INTEREST; POTENTIAL TOXICITY ON MICROALGAE. ANDREA CAZARES-MORALES[1]; BEATRIZ-CORDERO ESQUIVEL[2], ALEJANDRO HUERTA-SAQUERO[1], ELIZABETH SORIA CASTRO[3], ROBERTO VAZQUEZ-MUÑOZ [1,4,*], [1]Centro de Nanociencias y Nanotecnologia, Universidad Nacional Autonoma de Mexico, Mexico, [2]Centro de Educación Cientifica y de Educacion Superior de Ensenada, Mexico, [3]Instituto Nacional de Cardiologia, Mexico, [4]University of Texas at San Antonio, USA. [*]corresponding author.

Nowadays, the use of nanomaterials in health is becoming more common, for treatments, detection, and drug delivery, among others. Nanomaterials of Biomedical Interest (NBI) are designed to be safe for humans, but the risk they pose to the environment, once they are discarded, is unknown. It is highly probable that NBI will end up in marine ecosystems, where microalgae play an essential role as the base for upper trophic levels. Awareness of the hazardous effects of NBI on microalgae could help to prevent an environmental disaster. In this study, the effects arising from exposure to some NBI (Gd_2O_3: Er^{+3}/Yb^{+3}, and YAG: Pr^{+4}) were analyzed on the marine microalgae *Dunaliella* sp, *Isochrysis galbana*, and *Chaetoceros muelleri*. Stability of NBI was characterized by dynamic light scattering in the F/2 culture medium. Effects of the NBI on microalgae growth, damage on genetic material and oxidative stress were assessed. Finally, Transmission Electron Microscopy was used to evaluate the effect of NBI on algal cell ultrastructure. NBI physical-chemical properties are affected in marine culture media conditions. Gd_2O_3: Er^{+3}/Yb^{+3} and YAG: Pr^{+4} generated from mild to moderate negative reactions, with similar effects between broth nanomaterials. *Dunaliella* sp. and I. *galbana* are less sensitive to NBI than C. *muelleri*. NBI alter the structure of microalgae cells, although they are less toxic than other reported nanomaterials, such as silver nanoparticles. TEM analysis showed the effect in cell morphology and inner structural composition (Fig. 1).

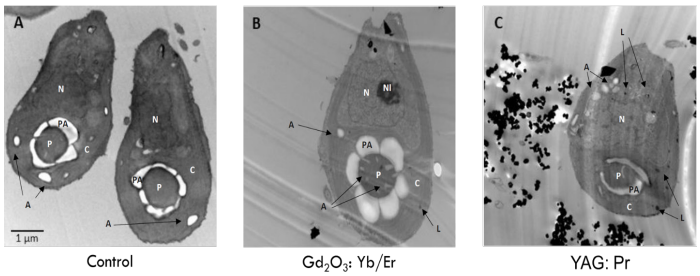

Fig. 1: Effects of NBIs on the ultrastructure of Dunaliella sp. (A) Control, (B) exposed to Gd_2O_3: Yb^{+3}/Er^{+3} and (C) YAG:Pr^{+4}. The cultures were exposed to 100 µg mL-1 of the treatments for 24 hours. *N*=nucleus; *Nl*=nucleolus; *C*=chloroplast; *P*=pyrenoid; *PA*=pyrenoid starch plaque; *A*=starch; *L*=lipid droplets.

GEOMETRICAL SYMMETRY LOSS IN GOLD DECAHEDRA PARTICLES ANALYZED BY QUASI-PARALLEL PRECESSION ELECTRON DIFFRACTION. CLEMENTE FERNANDO and ARTURO PONCE, Department of Physics and Astronomy, University of Texas at San Antonio, One UTSA Circle, San Antonio, Texas 78249.

Symmetry of non-translational crystalline structures such as metallic and semiconductor nanoparticles is normally determined by the Bravais lattices. At nanoscale level, shape and size of particles play an essential role in their physicochemical properties such as optical, catalytical, electronic and magnetic properties. For instance, noble metals exhibit different surface plasmon resonances depending on the size and shape of metallic nanoparticles. Particularly, the magneto-optical interactions have gained attention due to the potential technological applications in spintronic, electromagnetic shielding, magneto-optical data storage, and others. Nanoscale magnetic structures, and their ordered arrays, are being considered for use in several advanced technological areas such as Micro-Electro-Mechanical Systems (MEMS) and power devices as supercapacitors or batteries. In this work we have performed a systematic analysis of gold decahedra nanoparticles ranging from 10 nm up to 300 nm. The analysis was carried out using nano-beam electron diffraction on the nanoparticles oriented along the fivefold symmetry, in which five entities are identified. In order to increase the number of reflections, and to obtain quasi-kinematical diffraction patterns, we have applied precession electron diffraction in the analysis. The electron diffraction patterns oriented in the fivefold show the reflections of the five crystals separated by the multiple twins. Centrosymmetric patterns are observed in bigger nanoparticles; however, below 70 nm nanoparticles present a symmetry loss in the diffraction patterns. In this presentation we discuss the reason of the symmetry loss and how the precession angle affects the analysis.

BIMETALLIC TWO-DIMENSIONAL NANOFRAMES FOR HIGH ACTIVITY OXYGEN EVOLUTION ELECTROCATALYSTS. CHRISTOPHER P. RHODES[1,*], FERNANDO GODINEZ-SALOMON[1], YUANFANG YING[1], RUBEN MENDOZA-CRUZ[2], and JOSEFINA ARELLANO-JIMENEZ[2], [1]Department of Chemistry and Biochemistry, Materials Science, Engineering, and Commercialization Program, Texas State University, San Marcos, Texas 78666. [2]Department of Physics and Astronomy, University of Texas at San Antonio, San Antonio, Texas 78249.

Oxygen evolution electrocatalysts with high activity, extended durability, and lower costs are needed to further the development of proton-exchange membrane (PEM) electrolyzers that split water into hydrogen and oxygen. The anodic oxygen evolution reaction (OER) exhibits sluggish reaction kinetics that results in high overpotentials and significant efficiency losses. Although currently used iridium oxide (IrOx) OER electrocatalysts have shown high activity and reasonable stability in acidic conditions, iridium has very high costs and limited supply, which has motivated approaches to increase the activity and stability of iridium-based catalysts. We have explored bimetallic two-dimensional (2D) nanoframes to improve the activity and stability of OER electrocatalysts [1]. Ir-M (M=Ni, Co) alloy 2D nanoframes were synthesized by thermal reduction of iridium-decorated metal hydroxide nanosheets and subsequent chemical leaching. The bimetallic nanoframes have a porous, nanostructured carbon-free three-dimensional matrix that allows molecular access to the catalytically active surface. The interaction of iridium with nickel and cobalt was utilized to tune the surface atomic and electronic structure and increase OER activity. High Angle Annular Dark-Field Scanning Transmission Electron Microscopy (HAADF-STEM), Energy-Dispersive X-ray spectroscopy (EDX), and tomography STEM were utilized to characterize the morphology and atomic structure of bimetallic 2D nanoframes before and after electrochemical conditioning within the OER potential range. Microscopy and cyclic voltammetry measurements showed that after electrochemical conditioning, the surface is predominantly transformed from metallic to a metal oxide/hydroxide, and the metallic alloy phase is retained below the amorphous surface layer. Oxygen evolution activities were evaluated using a rotating disk electrode configuration. The OER mass activities of the iridium-metal nanoarchitectures were significantly higher than those of commercial IrO2. Different temperature treatments were determined to alter the atomic-level structure and influence the electrochemical activity and stability. Bimetallic 2D nanoframes combine highly catalytically active surfaces within a carbon-free 3D nanoarchitecture and provides the opportunity to design OER catalysts with improved activity and stability.

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UTILIZING ATOMIC FORCE MICROSCOPY FOR CHARACTERIZING CELLULOSE MATERIALS. THELMA AMEH* and CHRISTIE M. SAYES, Department of Environmental Science, Baylor University, Waco, TX 76706.

Cellulose, a low-cost, renewable, and biodegradable organic material, is the most abundant polymer in the

world. There is an increased application of cellulose fibers in several industries such as food, composites, pharmaceutics, and medical devices due to strength and biocompatibility. The characterization of these fibers is needed for product quality control purposes, as well as for assessing the potential hazards associated with its occupational exposure in industrial settings.

Microscopy is an important technique used for the physicochemical characterization of cellulose materials. Atomic Force Microscopy (AFM) was used to characterize the morphology and dimensions of nine different cellulose materials obtained from industrial manufacturers. The characteristics examined included surface structure morphology, length, width, height, and aggregation. The cellulose materials were found to be either crystalline or fibrous with heights ranging approximately from 10 to 525 nm. Of the nine samples, seven are within the nanometer size range (i.e. 1-100 nm in height) with various degrees of branching; two of the samples are highly aggregated crystalline particles.

The use of AFM enables three-dimensional characterization of microscopic structures and provides information on the external morphology. The micrographs show the unique structure of the cellulose materials examined, such as branching structures, aggregation, fray, and particulate contaminations. The physicochemical characterization of these materials is an essential component to safety analysis during manufacturing and handling processes. The data collected in this study will aid in the assessment of potential risks associated with using this material in novel applications.

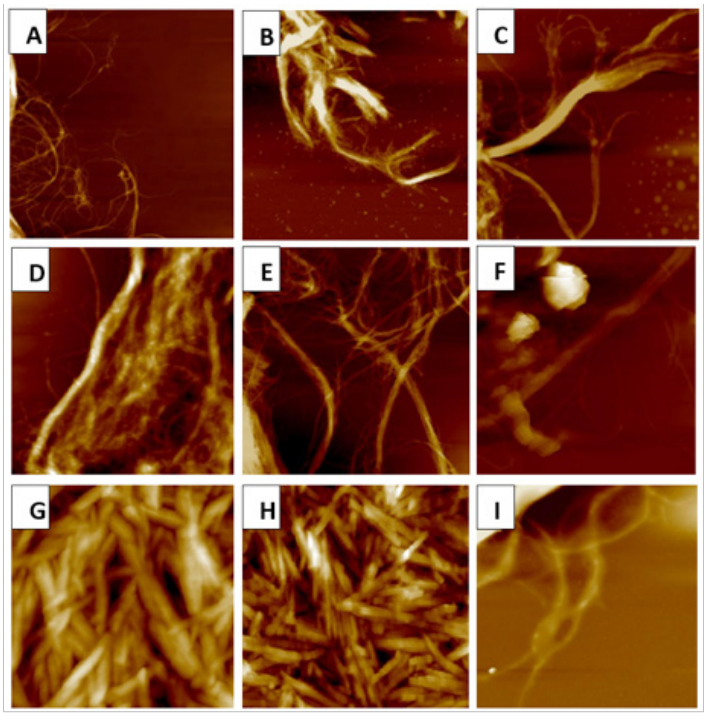

Fig. 1: AFM micrographs of cellulose fibers and crystals. Fibers show multiple branching units while crystals display high aggregation potential.

IDENTIFICATION OF ZINC PHASES WITHIN IRON ORE. MICHAEL GARDNER, M. JOSEFINA ARELLANO-JIMENEZ, and MIGUEL JOSE YACAMAN. Department of Physics & Astronomy, University of Texas at San Antonio, TX 78249.

Iron refineries process ores with diverse compositions where the presence of zinc poses a particular problem during refining and in the final product. A provided sample of raw iron ore was analyzed to characterize the chemical, physical, structural, and morphological properties of an unknown phase of zinc. Olympus DSX500/DSX-CB optical microscope was used to identify surface features. Scanning electron microscope Hitachi SEM SU1510 and AMETEK Octane Pro with EDAX was used to identify elemental composition of fragment samples. A Panalytical Empyrean XRD Diffractometer was used to identify compounds present and their structure. Fig. 1a shows the characteristic morphology of a typical fragment of 1 cm^3 in size; Backscattered electrons (BSE) image (Fig. 1b) shows regions of different composition that correspond to zinc shown by elemental map (Fig. 1c) and Energy Dispersive X-Ray Spectroscopy (EDS) spectrum (Fig. 1d). Preliminary characterization of atomic structure and spacing was carried out using a Field Emission Transmission Electron Microscope JEOL 2010F with EDAX Genesis. There was a visible distinction on micron scale, where at least one phase of zinc was present in some samples. XRD analysis showed possible presence of Zinc Oxide (ZnO-Cubic), Zinc Iron Oxide ($ZnFe_2O_4$-Cubic), and Zinc Iron ($Zn_{10}Fe_3$-Cubic). Future Transmission Electron Microscopy (TEM) analysis will be performed to confirm the presence of unique zinc phases, so that a chemical process of removal may be developed and implemented on an industrial scale.

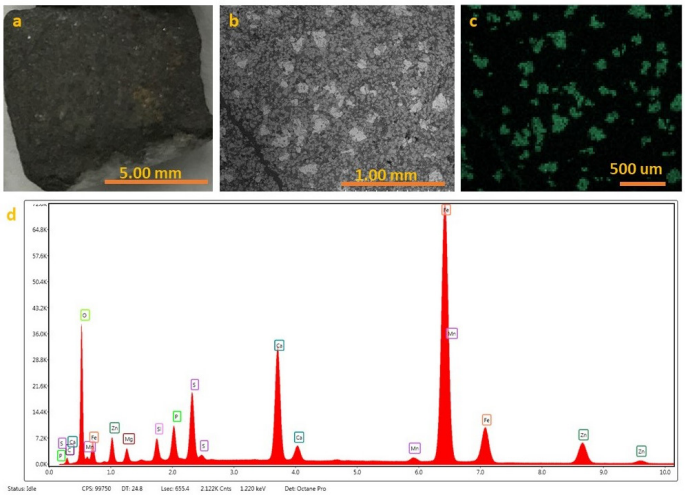

Fig.1: a) Surface analysis shows an inhomogeneity to sample composition. b) BSE image shows areas with characteristic contrast and shows correlation with zinc elemental map in (c). d) EDS spectrum of iron sample confirms presence of zinc along with other elements.

CRYSTALLINE STRUCTURE DETERMINATION OF ~ 4.5 NM PLASMONIC GOLD NANOCRYSTALS USING ATOMIC PAIR DISTRIBUTION FUNCTION FROM PRECESSION ELECTRON DIFFRACTION. M. MOZAMMEL HOQUE, ARTURO PONCE, ROBERT L. WHETTEN, and KATHRYN M. MAYER, Department of Physics & Astronomy, University of Texas at San Antonio, San Antonio, TX 78249.

Due to its dependency on atomic arrangements, the crystalline structure determination is of paramount importance to understand the basic properties of any materials. The pair distribution function (PDF) - the probability of finding neighboring two atoms from a given distance r - has been widely used to investigate disordered crystalline materials, as this method gives more detailed structural information compared with conventional X-ray powder diffraction patterns. PDF analysis can provide structural information of the complete spectrum of materials, from non-crystalline to crystalline materials, such as: the degree of crystallinity, the atomic structure and size of the nanoparticle, the atomic local environment, and the degree of internal disorder. In the present work, precession electron diffraction (PED) at liquid-nitrogen temperature has been applied to obtain the atomic pair distribution function (PDF) of ~ 4.5 nm plasmonic gold nanoparticles. PDF fit analysis has been performed with three different structure models simulated with a Python-based code; it is revealed that the structure of the ~ 4.5 nm gold nanoparticles is either a decahedron, having best fit results of R_w = 22%, or a truncated octahedron, with R_w = 24%.

STRUCTURAL AND MORPHOLOGICAL IDENTIFICATION OF FIBROUS NANOCRYSTALS USING A BATTERY OF TRANSMISSION ELECTRON MICROSCOPY SAMPLE PREPARATION METHODS. MARINA R. MULENOS and CHRISTIE M. SAYES, Department of Environmental Science, Baylor University, Waco, TX 76706.

With the advent of advanced material processing, new physicochemical characterization techniques are needed to accurately assess novel nanometer scale features in structures. Examples of advanced materials that fit into this new category are nanoparticles, microfibrillated structures, nanocrystals, to name a few. For example, cellulose is a naturally occurring and expansively abundant polymer utilized to advance many different industries [1]. It is engineered to be in many different size ranges that need to be characterized.

Since the influx of advanced materials, cellulose has been engineered into three different categories; cellulose nanofibrils (CNF), cellulose nanocrystals (CNCs), and bacterial cellulose (BC). Nanocrystals are produced in nature and are used as modern world technologies to increase conductivity, strength, and thermal stability [2, 3]. When utilizing these materials, it is important to characterize the material along all stages of the product development pipeline (i.e. raw material, intermediates, and final product) in an effort to ensure safety, while maximizing efficacy.

An important tool for understanding the safety of CNCs is microscopy, to assess size, morphology, agglomeration, and impurities. Transmission electron microscopy is the gold standard for analyzing nanomaterial characteristics but CNCs pose a challenge: due to their nanoscopic and organic nature, CNCs are difficult to image on TEM because of their low contrast. To address this problem, a battery of methods to image CNCs was used. At first, 3 different staining methods were used; lead citrate, uranyl acetate, and phosphotungstic acid. These methods worked at increasing the contrast of the CNCs vs. the background of the grid but added in many artifacts that cover the CNCs and induced artificial agglomeration. After trying this method at different concentrations, sputter coating over the sample on the grid was tested. Sputter coating with iridium, gold, and titanium was tested at 5nm film thickness. The three methods tested showed promising results where the metal coating increased overall the contrast of the sample.

Our results show that negative staining with phosphotungstic acid and sputter coating with titanium display the most details regarding CNC size and length. These are great methods that require further investigation; CNC agglomeration was apparent in all samples and with proper dispersion techniques during sample preparation, single CNCs may be seen.

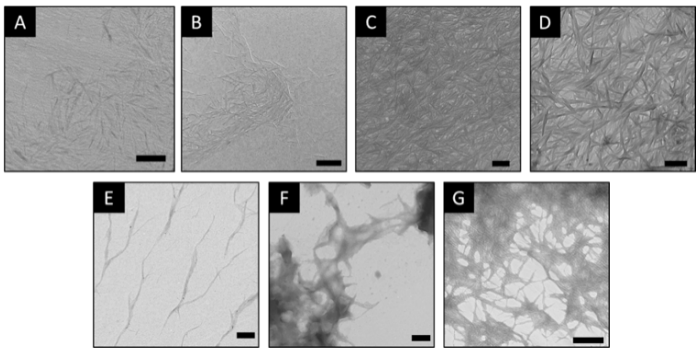

Fig. 1: Cellulose nanocrystals prepared in seven different ways for TEM. A) No preparation; B) sputter coated with iridium; C) sputter coated with gold; D) sputter coated with titanium; E) stained with uranyl acetate; F) stained with lead citrate; G) stained with PTA. Scale bar: 200 nm.

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SEMICONDUCTOR PROPERTIES IN SILVER NANOWIRES EXPLORED BY *IN SITU* TRANSMISSION ELECTRON MICROSCOPY. EDGAR OCHOA[1,2], FERNANDO MÁRQUEZ[1,2], DIEGO ALDUCIN[2] and ARTURO PONCE[2], [1]Instituto de Investigaciones en Materiales, Universidad Nacional Autónoma de México, Apdo. Postal 70-360, México D.F. 04510, México, [2]Department of Physics and Astronomy, The University of Texas at San Antonio, San Antonio, Texas 78249.

In situ transmission electron microscopy (TEM) allows the observation of the evolution and behavior of a material during a variety of tests. This method can provide live feedback of how materials react under different conditions, such as electrical probing, mechanical bending and manipulation, heating, cooling, environmental studies (gas and liquid cell) and a combination of any of these. Through electrical probing, one could obtain current-voltage curves to comprehend the electrical nature of metallic and electronic materials for diverse applications. Therefore, it is important to observe how many of these mechanisms happen and evolve as the specimen is tested in real time inside the TEM. In addition, external parameters such as mechanical deformation and heating alter the electric properties of these devices, which is commonly found in industry. In this work, we performed *in situ* TEM microscopy in pentagonal silver nanowires, prepared by wet chemistry. The electrical behavior of silver showed an unconventional I-V curve when the wires are submitted to a mechanical deformation, instead a typical semiconductor behavior. Atomic surface and internal defects are the main factors responsible for the semiconductor behavior. Structural defects have been characterized under diffraction contrast in dark field mode.

ELECTRON MICROSCOPIC CHARACTERIZATION OF POLYCRYSTALLINE THIN FILMS. PRAKASH PARAJULI, RUBÉN MENDOZA-CRUZ, MIGUEL JOSE YACAMAN, and ARTURO PONCE, Department of Physics and Astronomy, University of Texas at San Antonio, One UTSA Circle, San Antonio, Texas 78249.

Materials used in technological applications are polycrystalline containing a substantial number of grains and a complex network of various types of grain boundaries. A thorough study of texture and structure-chemistry of grain boundaries of the films remains a fundamental prerequisite for the proper and efficient use of them, since most of the properties of films such as those mechanical, functional and kinetic depend on these parameters. Herein, we present the exploration of microstructural features and grain boundary segregation phenomena (structure and chemistry) of thin films, carried out by using advanced electron microscopic techniques, namely, scanning precession electron diffraction and aberration-corrected electron microscopy (STEM-HAADF imaging and EDS/EELS spectroscopy). Experimental results on the analysis of microstructural features, their evolution due to annealing and alloying, and atomic-level grain boundary segregation of metallic alloy thin films are explored in detail.

EDUCATION/LIFE SCIENCES ABSTRACT
Spring 2019

CAMPUS TO COMMUNITY: OUTDOOR EDUCATION AT THE CETA CANYON HELPING THE ELEMENTARY AND MIDDLE SCHOOL STUDENTS IN CHARACTERIZING BIOLOGICAL SPECIMENS USING DIGITAL MICROSCOPES. YANETH CHAVEZ, ANNA CENICEROS, SANDY BABITZKE and NABARUN GHOSH, Life, Earth & Environmental Sciences, West Texas A&M University, Canyon, Texas, 79016

Ceta Canyon was founded in 1918; it is a retreat center hosting many events such as church camps and school trips. It is a great site for camping and excursions. Two faculty members from the Department of Life, Earth and Environmental Sciences took some research students and the pre-service student teachers and other research students with the objective of outdoor education. Recent nationwide surveys reveal significant decline in students' interest in Math and Sciences [1]. Present day students prefer digital media and involvement of technology in their instructions. We developed the concept of applying more inquiry-based approaches in the classroom, using more hands on, direct observation, and field techniques. For this purpose we developed a partnership and collaborative research project entitled: "Environmental Inquiry by the Science Students: Improve the scientific communication with the community". As part of this project, we carried out a survey on more than 80 students who performed experiments in the laboratories based on Environmental Inquiry [2]. This research project was aimed at inspiring the young minds in using various techniques involved in Biology, including Digital Microscopy. This Project involved some pre-service student teachers (Biology-Education Majors) who aimed at improving the classroom teaching and gaining the learning experience by bringing the university educational experience to the local community. We initiated the activity for the school students with a brief introductory lecture on the importance of conserving our ecosystems, the impact of reforestation and reducing pollution by recycling everyday disposable objects. The activity followed the collection of zoo- and phytoplankton and small insects by using various types of collection nets and tools. We helped the students in their observation on collected specimens under dissecting and compound microscopes. Using the Olympus BX-40 Microscope, we captured

micrographs of collected microscopic organisms as well as anatomical sections. The micrographs were captured with DP Manager and were analyzed using the Image Pro 6.0 software. We used those micrographs for identification and characterization of the specimens. Twenty-five organisms were viewed and photographed using bright field setting. The micro-arthropods were viewed and photographed with an Olympus SZ-40 stereomicroscope attached to a DVC camera that helped us to identify the specimens. We identified the algal specimens as: *Spirogyra, Cladophora*, Pinnate Diatoms, Cyanobacteria like *Oscillatoria, Gloeocapsa* etc. Besides a rich planktonic diversity, we found common freshwater insects like dragonflies, damselflies *(Odonata)*, stoneflies *(Plecoptera)*, caddisflies *(Trichoptera)* and mayflies *(Ephemeroptera)*. The organisms varied in compositions and concentrations in the three parts of the stream namely riffle, run and pool. We helped the students also in capturing video clips of the moving live specimens using the digital microscopes and discussed the importance of those organisms in our environments. Characterizing these organisms in the laboratory helped us in using them for teaching, conference presentation and publications. This Complete 2 Compete (C2C) grant-supported project is aimed at improving the classroom teaching and learning experiences, and at the same time, introducing the name of WTAMU to the local community, thus helping increase student recruitment.

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TECHNICAL ABSTRACTS
Spring 2019

UNIQUE FLATQUAD SDD… THE ULTIMATE ANALYTICAL APPROACH. TED JUZWAK and JOHN MASTOVICH, Bruker AXS Inc., 5465 East Cheryl Parkway, Madison, WI 53711.

The Silicon Drift Diode (SDD) Energy-Dispersive (EDS) detector has becomes the industry standard, as it has superior throughput while maintaining resolution and light element sensitivity, and holds calibration from low to high count rates. Today, larger active areas and multiple-detector configurations for Scanning Electron Microscopes (SEMs) and Scanning Transmission Electron Microscopes (STEMs) are available, providing very high collection efficiency and rapid analysis. An alternative and unique approach is the implementation of an SDD array between the pole piece and the sample (like a backscatter detector), providing the highest solid angle possible (> 1sr) and the ultimate throughput.

Numerous applications ranging from soft materials [polymers & biologicals] to minerals [geological & clay fillers] and materials [metals & composites] will be discussed to illustrate the analytical benefits of the Flat Quad SDD technology. Significant advantage is the enabling of low dose/low voltage applications, as depicted in Figures 1A and 1B. Clear data are generated from highly topographical samples, that typically produce extreme x-ray shadowing, from depths of many microns from within the crater, as shown in Fig. 1C. Simultaneous EDS - Electron Backscatter Diffraction (EBSD) is possible from thinned samples, gathering the utmost spatial resolution as illustrated in Fig. 1D & E. Overall, due to the proximity of the detector to the sample, the FlatQUAD provides superior collection efficiency regardless whether the SEM or Scanning Transmission Electron Microscopy (STEM) applications are carried out.

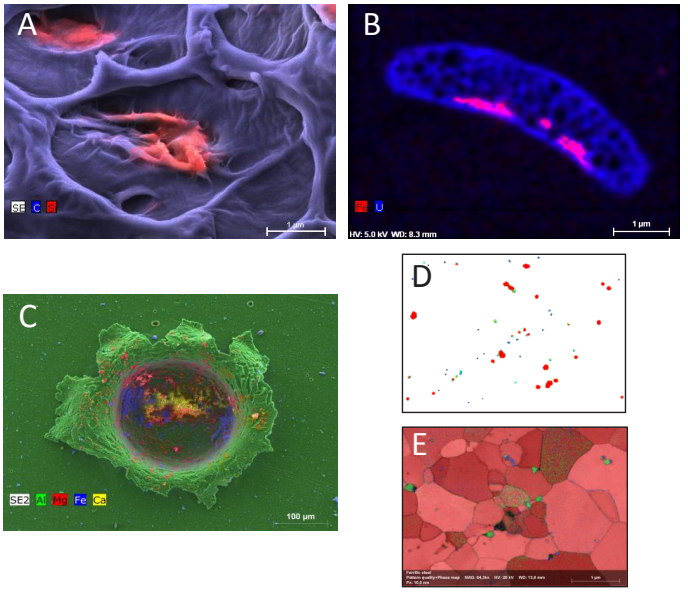


Fig. 1: Representative examples of Flat Quad SDD technology-enabled scanning electron microscopy. A) Scanning electron micrograph of a polymer with organo-clay filler (3 kV, 220 pA, 10 kcps, 320 s, 1024 x 768 pixel); B) Scanning transmitted electron micrograph of a magnetotactic bacterium (5kV, 300pA, 800s, 600 x 450 pixel); C) Scanning electron micrograph of stardust collected in gold foil crater from a NASA experiment (12kV, 2nA, 210s, 4096x3072 pixels); D) and E) Scanning transmitted electron micrograph of ferritic steel. EDS: map top – EBSD phase: map bottom. Ferrite – Red, Titanium – Green, Yttrium – Blue.

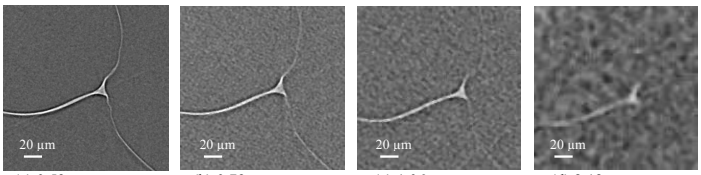
HIGH RESOLUTION X-RAY COMPUTED TOMOGRAPHY FOR FOAM CHARACTERIZATION. AYA TAKASE[1*] and THOMAS FITZGIBBONS[2, 1]. Rigaku Americas Corporation, The Woodlands, TX 7738[1, 2]. The Dow Chemical Company, Lake Jackson, TX 77566.

X-ray microscopes can visualize the solid walls and cellular structure in foam materials in 3D without having to slice and potentially alter the cell structure. This allows us to study cell morphology in detail, and calculate parameters such as porosity and cell volume distribution. Some foam materials, however, have less than a few micron thick walls to achieve high porosity and low thermal conductivity; and they require true submicron resolution for accurate characterization. However, resolution of conventional laboratory source X-ray microscopes is often limited to a few microns. This is mainly because the cone beam geometry is used, and the X-ray focus size, vibration and drift cause blurring of the image and limit the achievable resolution.

In this study, a high-resolution, high-contrast and high-speed laboratory X-ray microscope with true submicron resolution was used to characterize foam materials. A polyisocyanurate foam used as general building envelope insulator was characterized at various resolutions, and their image quality and accuracy of porosity calculation were compared. While the high-resolution image revealed details of the cell morphology and provided an accurate porosity value, the lower resolution images caused thin walls to become blurred and disappear from the images, resulting in an overestimation of the porosity.

Rigaku nano3DX X-ray microscope was used for the measurements. To achieve high contrast and high resolution for low X-ray absorbing samples such as foam materials, the nano3DX mainly uses the X-ray target's characteristic radiation in the quasi-parallel beam geometry. The MicroMax-007 is used as the X-ray source and operated at 1200 W. The X-ray energy is selectable by choosing different target material to optimize the contrast for different absorption rates and sizes of the samples. The quasi-parallel beam geometry does not use the X-ray divergence for magnification and is immune to image blurring caused by X-ray focus size, vibration and drift.

The Rmax R-Matte® Plus-3 was used as the sample. The nano3DX was configured with Cu target with 8.0 keV operating at 40 kV – 30 mA. The 270 nm lens was used at pixel binning 2, 3, 4 and 8 for effectively 0.53, 0.79, 1.06, and 2.12-micron pixel size. The scan duration varied from 10 minutes at binning 8 to 4 hours at binning 2. The computed tomographic cross sections are shown in Fig. 1. The wall thickness varied from 1.5 to 5 microns and as the resolution approached the wall thickness, they started to disappear and were missed in the porosity calculation. This demonstrates the importance of the true high-resolution capability when analysing thin walled foam materials.



(a) 0.53 µm (b) 0.79 µm (c) 1.06 µm (d) 2.12 µm

Fig. 1: Tomographic cross sections of Rmax foam with (a) 0.53 µm, (b) 0.79 µm, (c) 1.06 µm and (d) 2.12 µm effective pixel resolution.

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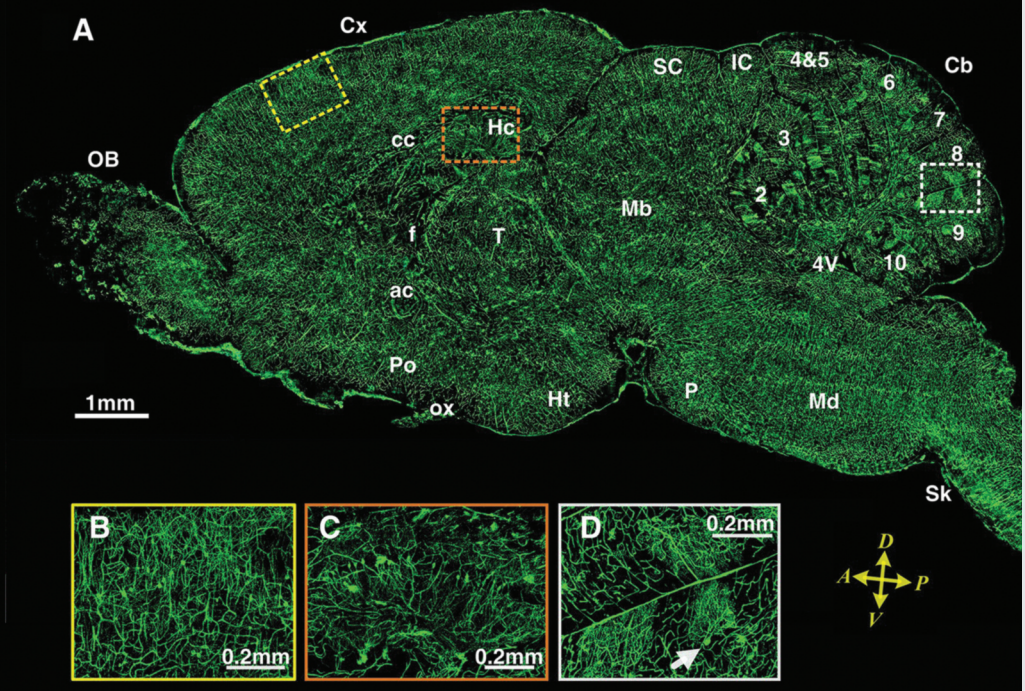

Creating a High Resolution Atlas of the Mouse Brain...

(A) A sagittal image reconstructed from a stack of 100 virtual sagittal sections (total thickness of 0.1 mm). These sections were transformed from the original coronal sections. The sagittal image was located in the right hemisphere about 0.4 mm lateral to the middle. Almost all major regions of the brain can be seen in this image, e.g., the Olfactory Bulb (OB), Cerebral Cortex (Cx), Hippocampus (Hc), Fornix(f), Anterior Commissure (ac), Thalamus (T), Cerebellum (Cb), Midbrain (Mb), Pons (P), Medulla (Md), Corpus Callosum (cc), Superior Colliculus (SC), Inferior Colliculus (IC), Hypothalamus (Ht), Preoptic Area (Po), Optic Chiasm (ox), 4th ventricle (4V) and nine lobules of the cerebellum (Arabic numerals, 2 to 10). The three regions inside the different colored rectangle in (A) are the positions of (B), (C) and (D), which illustrate the cerebral cortex, hippocampus and cerebellum, respectively. In the reconstruction of sagittal image, no dislocation was observed along the D-V axis, i.e., the coronal sections are inherently aligned along the A-P axis.

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A 3D structural dataset of a Golgi-stained whole mouse brain at the neurite level was obtained. The morphology and spatial locations of neurons and traces of neurites were clearly distinguished. Researchers found that neighboring Purkinje cells were sticking to each other.

Acknowledgement

Micro-Optical Sectioning Tomography to Obtain a High-Resolution Atlas of the Mouse Brain Anan Li, Hui Gong, Bin Zhang, Qingdi Wang, Cheng Yan, Jingpeng Wu, Qian Liu, Shaoqun Zeng, Qingming Luo

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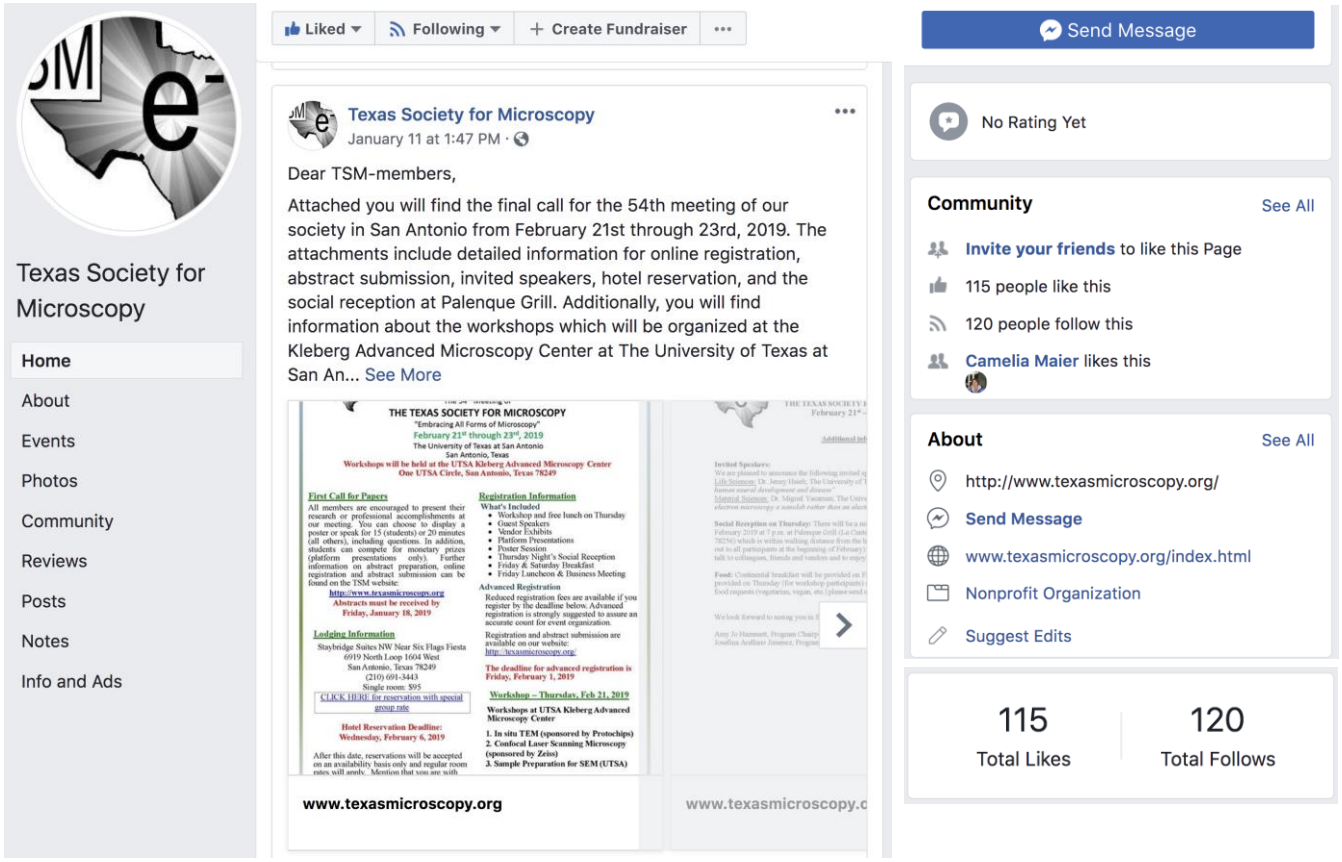

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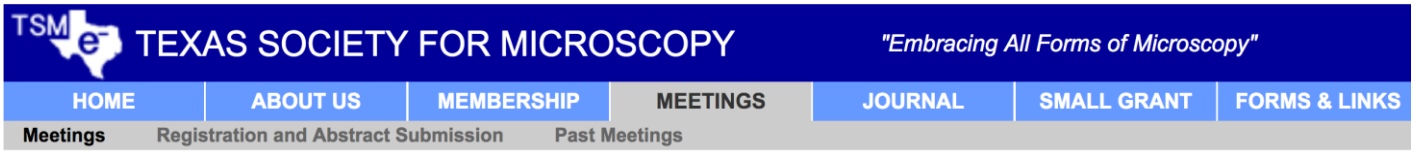

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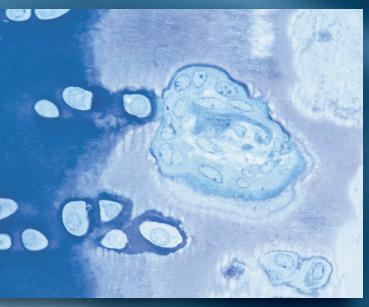

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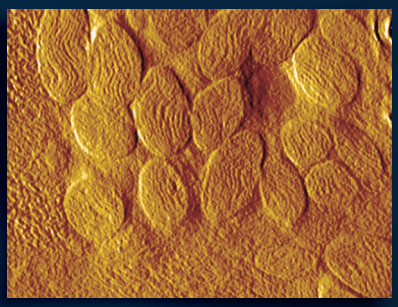

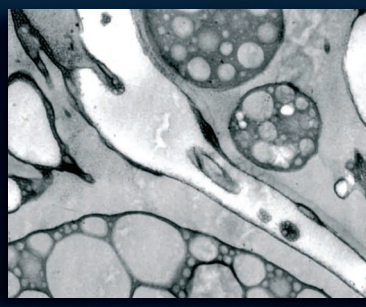

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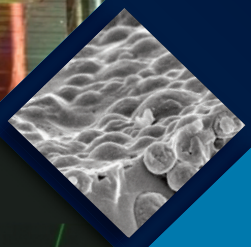

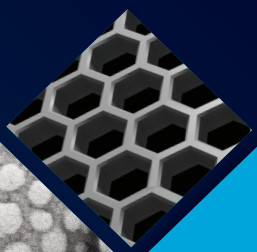

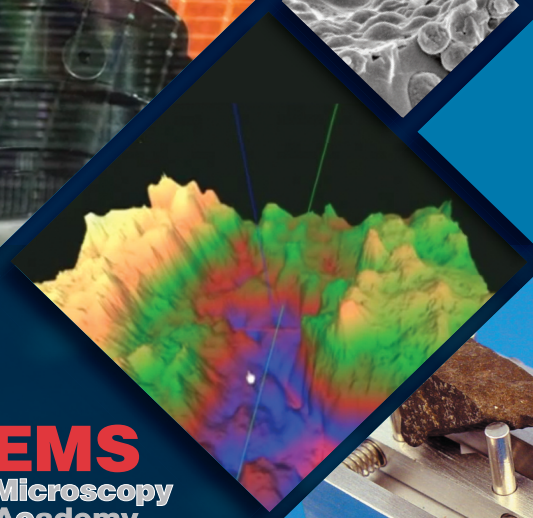

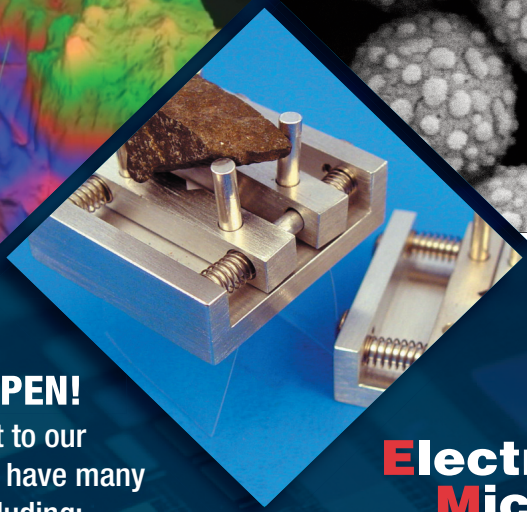

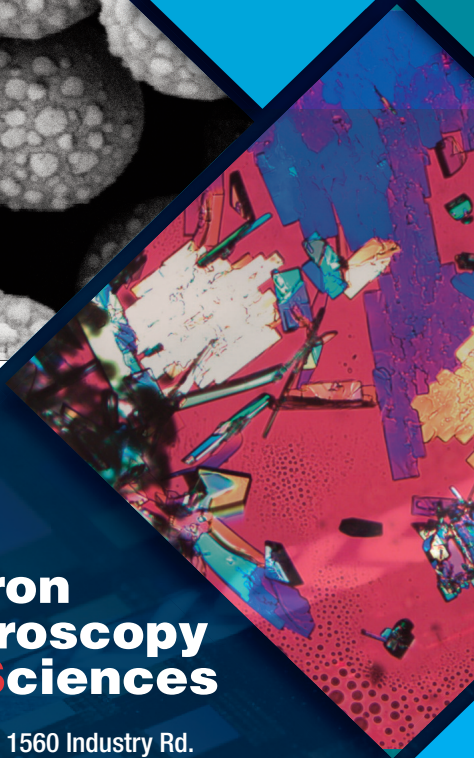

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