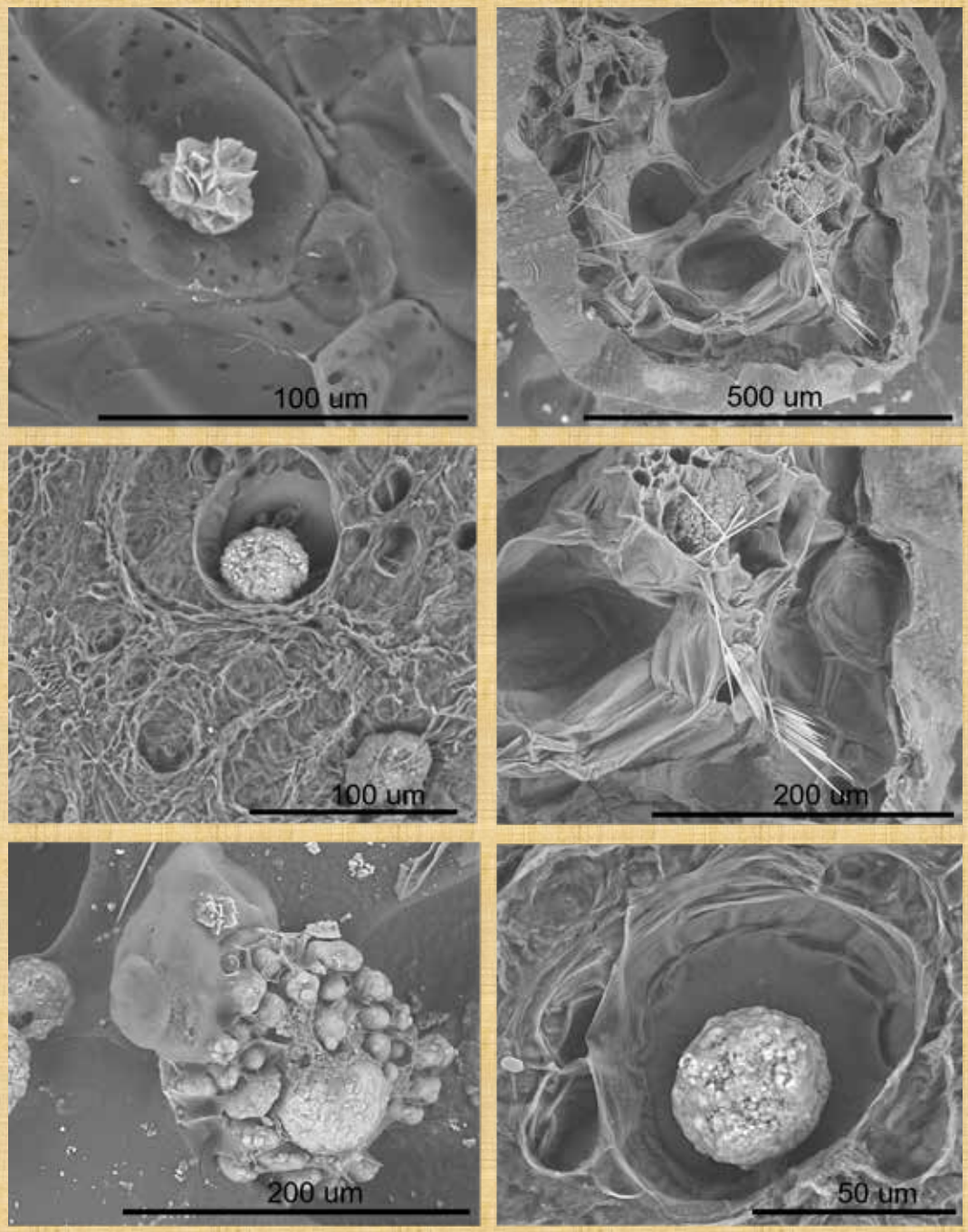
Texas Journal of Microscopy



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Bioscience Electron Microscopy Lab, University of Conn

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TSM OFFICERS 2021-2022

APPOINTED OFFICERS

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TEXAS JOURNAL OF MICROSCOPY
VOLUME 53, NUMBER 1, 2022
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Editor
Catalina Pislariu, PhD
Division of Biology, School of the Sciences,
Texas Woman's University, Denton, TX 76204

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

Collage tribute to Dr. Howard J. Arnott (1928-2022), Past President and TSM honorary member, who dedicated many years of his distinguished academic career studying mineralization and development of biological crystals in plants.

Scanning electron micrographs of calcium oxalate crystals and calcium carbonate deposits in plant tissues visualized with a Hitachi TM-1000 SEM. From left to right, top row: calcium oxalate druse and calcium oxalate raphides; middle row: calcium carbonate cystoliths and calcium oxalate raphides; bottom row: a calcium carbonate cystolith surrounded by a cluster of smaller 'neighboring' cystoliths and cystolith in idioblast. Micrographs taken by Dr. Camelia Maier and her undergraduate students at Texas Woman's University.

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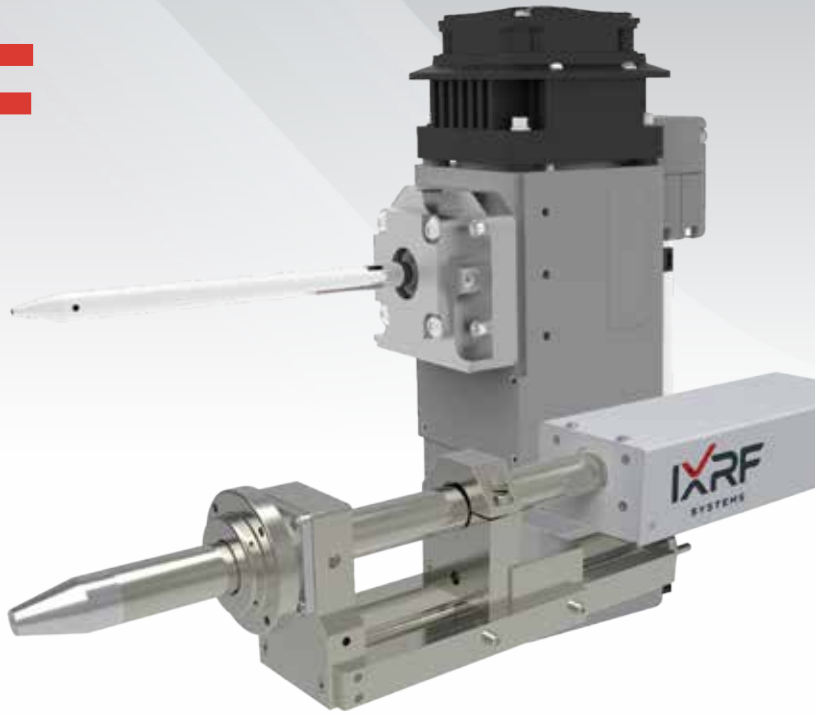

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SEM/EDS spectra

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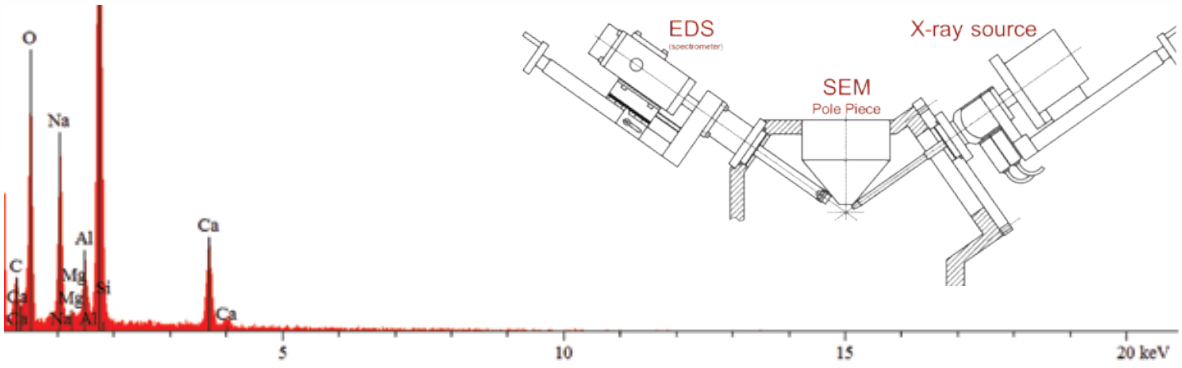
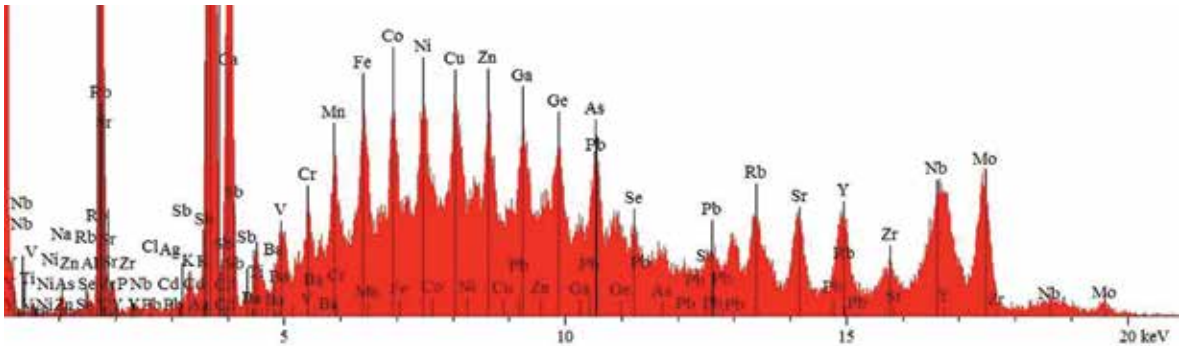


SEM-XRF spectra

With X-ray excitation added, all elements are measurable



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President's Message

The Executive Council of the Texas Society for Microscopy (TSM) is especially grateful to have the opportunity to host the 56th annual meeting at Baylor University in Waco, TX. After two years of meeting postponement, we are proud to display the hard work of our members to each other and again celebrate our shared love of microscopy. I would like to thank our program chairperson Nabarun Ghosh and our local organizer Bernd Zechmann, who did a wonderful job organizing this meeting. I would also like to thank Olympus, Bruker and Raith Corps. for their sponsorship of the incredible workshops at the Center for Microscopy and Imaging at Baylor University. This year's invited speakers are well known scientists who have contributed much to our society over time, Christie Sayes, who gave a riveting presentation at last year's Summer Seminar Series, and Bernd Zechmann both of Baylor University.

I would like to thank Catalina Pislariu, our journal editor, for all of the hard work that went into preparing two journals to be distributed at our meeting including one with peer reviewed research articles. Our society is in very good financial health according to our treasure's reports and I would like to thank David Garret for his many years of service in the position. I would also like to thank James Long for representing our corporate members with enduring enthusiasm. I am grateful to Nabarun Ghosh for his support as the social media representative. He has made great progress in making us visible to our members on Facebook and creating more of a presence on Twitter. I would like to thank Aubrey Howard, our student representative, for her many great ideas and hard work to increase the participation of students in our society. We will miss Aubrey and wish her the best as she is graduating. We hope that she remains an active member of our society for many years to come. Many thanks also go out to all of our corporate members, regular members, honorary members, and student members for your support of the TSM with your membership, ideas for engagement while we were apart, and your participation at our annual meeting.

While we continue to support our members with the ability to share our microscopy teaching and research expertise, we are planning great things for the future! Nabarun Ghosh will be planning the long-awaited meeting in Canyon, TX for next year. We hope to see you there.

Amy Jo Hammett
TSM President, 2021-2022

IN MEMORIAM HOWARD J. ARNOTT

It is with great sadness that we announce the loss of our honorary member, Dr. Howard J. Arnott, who passed away this week. Dr. Howard was president of the TSM from 1988-89 and program chairperson from 1983-1985. His service and contribution to the TSM was exceptional, and he will be dearly missed. An obituary will follow.

Amy Jo Hammett
TSM-President

IN MEMORIAM HOWARD J. ARNOTT

I know Howard since I was a graduate student at UNT in the early 1990s. As a new student presenter at the TSM meetings I was quite intimidated by his questions. All students knew that Dr. Arnott is asking tough questions. After several presentations followed by 'tough' questions, I realized that what he was doing with us, student presenters, was to make us think and go deeper in explaining our results, thus helping us plan future experiments. Later, during our research collaboration on mineral deposits in mulberry, I felt his devotion to mentoring students and young scientist first hand. He even expressed that in his laconic style in his autobiography published in the Texas Journal of Microscopy (36:1, 37:1 and 2, 38:1 and 39:1):

Since returning to Texas in 1974, my students and I have participated with some frequency in TSEM which is a great place for students "to get their act together."

The student presentation competition, named in his honor, is his legacy for our Society and will continue to inspire more students to acquire microscopy skills for their research projects. Howard's passion for microscopy and photography and his scientific curiosity are well illustrated in his enormous research publications and presentations, from the development of biological crystals in plants and fungi, virus development, seed proteins and cell walls, Euglena's eyespot, polymers, nannobacteria, plant venation, to wood development and ultrastructure among many other accomplished research projects. His scientific curiosity will continue to be an inspiration for all of us. We will miss you, Howard. Your legacy will live on.

Camelia Maier, Ph.D.
Past President, Past Journal Editor and TSM Honorary Member

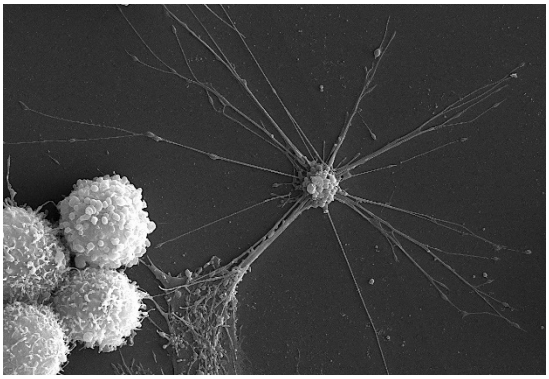
INVITED SPEAKER

CURRENT PERSPECTIVES IN CHARACTERIZATION METHODS OF ADVANCED MATERIALS ALONG PRODUCT LIFE CYCLES

CHRISTIE M. SAYES
Associate Professor
Department of Environmental Science, Baylor University, Waco, TX

Engineered materials are incorporated into composites at an increasing rate. Specific to nanomaterials, nanoparticles have unique properties and offer tailored benefits to traditional functions as compared to their macro-sized counterparts. Nanoparticles can be designed to be antimicrobial, corrosion resistant, dirt or water repellant, and UV light stable. Global revenues for nanoparticles used as coatings exceeded $1.6 billion in 2019. There are many published studies that exploit the advantages of using these novel materials in composites, however, there is little known about the structural changes that occur before versus during versus after environmental processes. Even less is known about end-of-life properties after the composites are disposed. This talk will present a framework that enables sustainability in nanotechnology while providing recent advances in method development to understand advanced material characteristics across the product life cycle.

Dr. Christie M. Sayes is a practicing research scientist in the fields of toxicology, chemistry, material science, and environmental health. She is a subject matter expert in advanced materials, human exposure & health effects and risk science. Her activities include working with partners, collaborators, and trainees in designing studies related to safety-by-design considerations of engineered materials used in drug delivery and consumer product applications.



TEXAS SOCIETY FOR MICROSCOPY 56TH ANNUAL MEETING

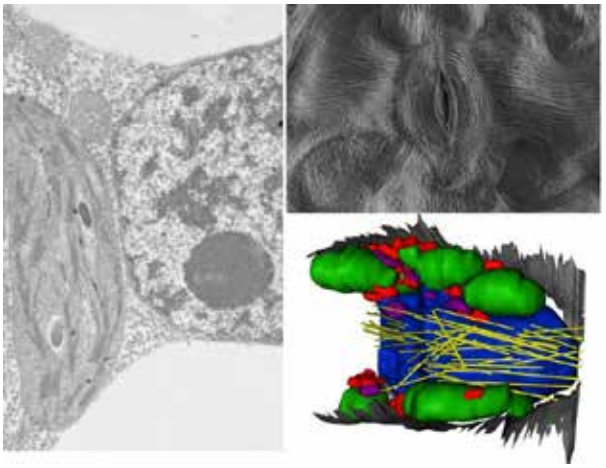
INVITED SPEAKER

SAMPLE PREPARATION OF BIOLOGICAL MATERIAL FOR TEM AND SEM INVESTIGATIONS

BERND ZECHMANN
Director and Associate Research Professor
Center for Microscopy and Imaging, Baylor University, Waco, TX

Preparation of biological samples for scanning and transmission electron microscopy is very time and labor consuming and requires extensive skills and expensive equipment. Procedures can take several days and artefacts are common. This presentation gives an overview of the most commonly used methods of sample preparation including conventional preparation at room temperature, microwave-assisted sample preparation, cryofixation and freeze substitution, and the use of surface replicas. The advantages and disadvantages of these methods will be discussed and an outlook on future development in the field will be provided.

Dr. Bernd Zechmann is the Director of and Associate Research Professor at the Center for Microscopy and Imaging at Baylor University. He uses electron and light microscopy to study 2 and 3 dimensional ultrastructural changes in plants during abiotic and biotic stress situations.



Abstracts

LIFE SCIENCES
Spring 2020

AN ANALYSIS OF D-LIMONENE AND TRIETHYL CITRATE EFFECTS ON HUMAN LUNG CELL MODELS USING FLUORESCENCE AND ELECTRON MICROSCOPY. YANIRA BALDOVINOS AND CHRISTIE M. SAYES
Department of Environmental Science, Baylor University, Waco, TX, 76706

Vaping products are used at an increasing rate among middle to high school students due to the many options in device types and formulations available. E-cigarettes are attractive vaping device due to their affordability and variety of e-liquid flavors. In particular, cannabis e-liquids contain the active ingredient (isomers of) cannabinoids as well as diluent and terpenes in complex combinations. The purpose of this study was to analyze the potential respiratory health effects of e-liquid mixtures containing two or more ingredients because little work has been done systematically testing the toxicities of vaping product formulations. We used d-limonene as a representative terpene and triethyl citrate as a common diluent to produce dose-response curves and chemical interaction plots. Cell viability was assessed via decreasing metabolism (MTS assay) and the resulting chemical interactions were assessed in two different human lung cell models, i.e. human bronchial epithelial cells (BEAS-2B) as a surrogate for upper airway response and human alveolar epithelial cells (A549) as a surrogate for lower airway response. We hypothesized that mixtures of d-limonene and triethyl citrate would induce more cytotoxicity in lung cells as compared to individual constituent exposures. Our results showed that an equipotent mixture (97:3 % v/v triethyl citrate: limonene) resulted in an antagonistic interaction, namely the co-exposure induced less cytotoxic effects than each of the individual chemical exposures. The use of confocal laser scanning microscopy (CLSM) and transmission electron microscopy (TEM) were used to show increasing oxidative stress and detrimental mitochondrial ultrastructure changes after increasing exposure doses to lung cells. We show that the combination of microscopy and toxicology can aid in determining respiratory health effects of vaping products tested in binary combinations.

A COMPARATIVE MICROSCOPIC EVALUATION ON POLLEN AND MOLD SPORE COUNT BETWEEN TWO CITIES USING A BURKARD VOLUMETRIC SPORE TRAP. BEATRIZ BURCIAGA, MARYTRINH NGUYEN, NABARUN GHOSH, AND CONSTANTINE SAADEH[1]
Department of Life, Earth and Environmental Sciences, West Texas A&M University, Canyon, TX 79015; [1]ALLERGY ARTS, 6842 Plum Creek Dr. Amarillo, TX 79124

This investigation aims at comparing the aeroallergen counts of two adjacent areas with different topography and climatic conditions. Environmental factors contribute to a high concentration of aeroallergen that led to the increased allergy cases among the residents of Texas Panhandle. Allergy and asthma cases have doubled in the Texas Panhandle area since 2007. Pollen and mold spores are aeroallergens that are capable of provoking serious allergic asthmatic reactions characterized by sneezing, nasal itching, watery eyes and other associated symptoms. We focused on the comparative aeroallergen indices and determining the specific pollen and mold spores that are prevalent in Albuquerque, NM and the Amarillo-Canyon Metroplex of the Texas Panhandle. The purpose was to compare the daily pollen and mold spore indices with the climatological conditions and to compare features of pollination. Air analysis was preformed through the collection of pollen and mold spores using a Burkard Volumetric Spore Trap, that is located on the third floor of the Natural Science Building of West Texas A&M University. We collected the aeroallergen samples on the Sellotape that is placed on the drum, we stained them with 2% safranin, and mounted them on slides. The slides were then observed using the BX- 40 Olympus Microscope equipped with a DP-74 digital camera and the *cellSens* software. This study was conducted for two years, 2020-2021 for six months from April to September. The pollen and mold spore count demonstrated diurnal and seasonal variation. The aeroallergen indices also varied based on the meteorological conditions like wind speed, humidity and precipitation. Dry, windy weather conditions increased the production of the dry air spores like *Alternaria* and *Curvularia*. A little precipitation reflects an increased concentration of the pollen and fungal spores in the air. The most frequent pollen grains observed during the spring season are: Lamb's Quarters (*Chenopodium album*), Common Sunflower (*Helianthus annuus*), Hairy Sunflower (*Helianthus hirsutus*), Short Ragweed (*Ambrosia artemisiifolia*) and Pine (*Pinus strobus*). Rainy and humid days have an increase in mold. Seasonal trends like spring, summer and fall show an increase in pollen count of certain species of plants.

The data collected from the two decades study at our lab show a significant correlation between the number of patients suffering from allergy and asthma with the concentration of specific aeroallergen. This research would help in diagnosis of allergies and asthma, and in implementing preventative measures.

MICROSCOPIC EXAMINATION ON FIBERS, INSECT PARTS, PLANT EXUDATES AND BURNT RESIDUES IN THE AIR CAUSING ALLERGIC RHINITIS. LYANNA DELEON, AUBREY HOWARD, MARYTRINH NGUYEN AND NABARUN GHOSH
Department of Life, Earth & Environmental Sciences, West Texas A&M University, Canyon, TX 79015

Aeroallergens including pollen and fungal spores have been studied for a long time. Reports on studies correlating the presence of fibers, insect parts, plant exudates, burnt residues in the air and incidence of allergy and asthma cases is meager. In recent years, the incidence of wildfires doubled in some areas. In the state of California, wildfire encroached 2.5 million acres of land destroying forest covers, houses, and farming land. Wildfire results in increased concentration of airborne debris floating in the air, that can travel thousands of miles with heavy wind speed. The recent increase in the incident of wildfire in the Texas Panhandle area and adjacent states caused an increase of airborne PM2.5, debris and fibers, dead insect parts, plant exudates, and burnt residues that got trapped in our Burkard Volumetric Spore Trap placed on the Natural Science building of the West Texas A&M University campus. We have collected the exposed tape from the spore trap regularly and mounted them on the glass slide after dividing them in seven equidistant strips using a standard scale, and stained them with 2% Safranin-Gelvatol solution. The solution functions in a dual mode, to stain the trapped materials and a mountant. We used a BX-40 Olympus Microscope attached to a computer with a *CellSense* software to capture images from the prepared slides that were also analyzed and assessed for the trapped samples collected from the air. There is a significant increase in the concentration of PM2.5, burnt residues, and floating insect parts including whole micro-insects in the air that were trapped in our spore trap in the recent years. Comparative analysis of data collected in the last 20 years, uncovered a significant increase in the number of dead insects and insect parts and other forms of burnt debris in years when they may have traveled hundreds of miles from the source of wildfires.

MITOCHONDRIAL ULTRASTRUCTURAL DEFECTS IN HUMAN SKELETAL MUSCLE MYOBLASTS SUBJECTED TO HYPOXIC AND HYPEROXIC CONDITIONS – AN IN VITRO MODEL OF ISCHEMIA-REPERFUSION INJURY. EMMA FLETCHER[1], DYLAN WILBURN[2], AHMED ISMAEEL[1], EVLAMPIA PAPOUTSI[1], DIMITRIOS MISERLIS[3], BERND ZECHMANN[1,4], AND PANAGIOTIS KOUTAKIS[1]
[1]Department of Biology, Baylor University, Waco, TX
[2]Department of Health, Human Performance, and Recreation, Baylor University, Waco, TX
[3]Department of Surgery, University of Texas Health Science Center San Antonio, San Antonio, TX
[4]Center for Microscopy and Imaging, Baylor University, Waco, TX

Mitochondria are double-membraned organelles vital to supply the energy demands for skeletal muscle function. Beyond energy production, mitochondria also regulate many other cellular processes, including signal transduction, calcium homeostasis, oxidative stress, and cell survival and apoptotic pathways. Notably, mitochondrial structure alters in response to different cellular states, and structure is an important determinant of function (Vincent et al., 2016). Morphological deviations in response to cellular hypoxia and reoxygenation are of particular interest as these conditions are characteristic pathophysiological features of ischemia-reperfusion (I/R) events that underlie numerous pathologies, including the myopathy affecting the lower extremity muscles of individuals with peripheral artery disease (PAD) (Koutakis et al., 2015). Although abnormal skeletal muscle mitochondrial structure was previously reported in the context of aging, inherited myopathies, and several other neuromuscular disorders (Vincent et al., 2016), a depiction of ultrastructural defects in PAD myopathy is lacking.

Using transmission electron microscopy (TEM), we sought to characterize mitochondrial morphology in an in vitro model of I/R injury, which mimics the pathophysiology of PAD (Ismaeel et al., 2022). Briefly, primary human skeletal muscle myoblasts were cultured under cycles of normoxia (90 min at 20% O_2, 75% N_2, 5% CO_2), hypoxia (60 min at 1% O_2, 94% N_2, 5% CO_2) and hyperoxia (30 min at 30% O_2, 65% N_2, 5% CO_2) for a total of 5 days. Control cells were incubated under constant normoxia. After 5 days of the oxygen cycling conditions, the cells were collected and fixed for 48 hr in 2.5% glutaraldehyde phosphate-buffer solution at 4°C. Cells were then post-fixed in 1% osmium tetroxide for 2 h, and subsequently dehydrated in increasing concentrations of acetone (50%, 70%, 90%, and 100%). All cells were embedded in a graded series of Embed 812 (1:2, 1:1, 2:1), before complete immersion in 100% Embed 812 and polymerization at 60˚C (48 h).

TEM micrographs of cells exposed to normal oxygen conditions showed typical skeletal muscle mitochondrial ultrastructure (Figure 1) (Picard, White, & Turnbull, 2013). Specifically, mitochondria were either ovoid, rod and/or tubular in shape, and the inner (IMM) and outer (OMM) mitochondrial membranes surrounding the mitochondrial matrix and intermembrane space, respectively, were intact. Additionally, the IMM invaginations (i.e., cristae) projecting into the matrix were present and characteristically tubular in all visual fields analyzed (Figure 1). In contrast, multiple mitochondrial ultrastructural abnormalities were noted in myoblasts exposed to normoxia-hypoxia-hyperoxia (NHH).

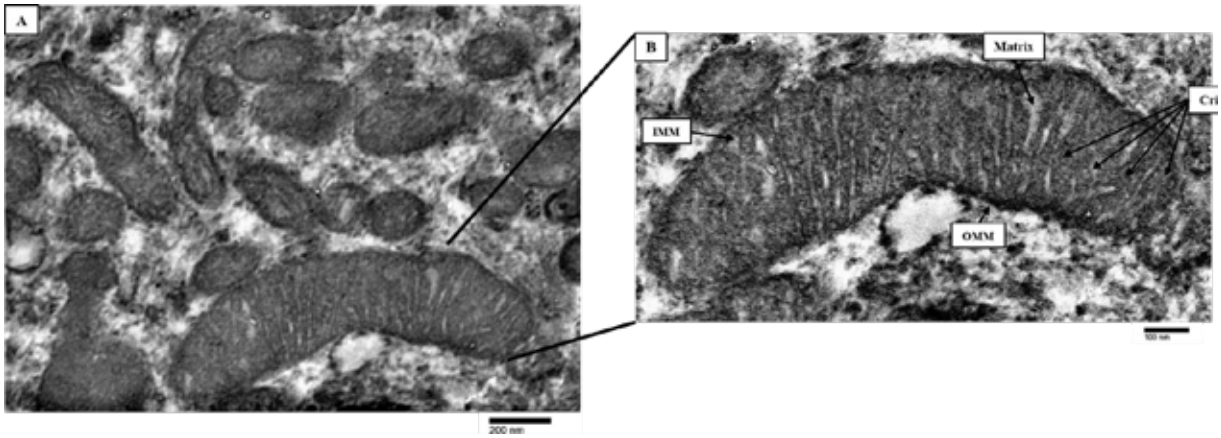


Figure 1: Myoblasts incubated under normoxia display normal mitochondrial morphology (A & B). Intact inner (IMM) and outer (OMM) mitochondrial membranes, as well as the tubular cristae (electron dense) and electron lucent mitochondrial matrix are displayed.

Many mitochondria appeared swollen, fragmented, donut- or blob-shaped, and evidence of vacuolar degeneration, consistent with I/R injury was observed (Chaanine, 2019; Picard et al., 2013) (Figure 2). Signs of degeneration include: i) an increase in the machinery responsible for mitochondrial breakdown (autophagosomes and lysosomes); ii) lysosome-mitochondrion fusion; iii) presence of mitochondria within autophagosomes; iv) focal, multifocal or complete loss and dissolution of mitochondrial cristae architecture; and v) evidence of OMM rupture.

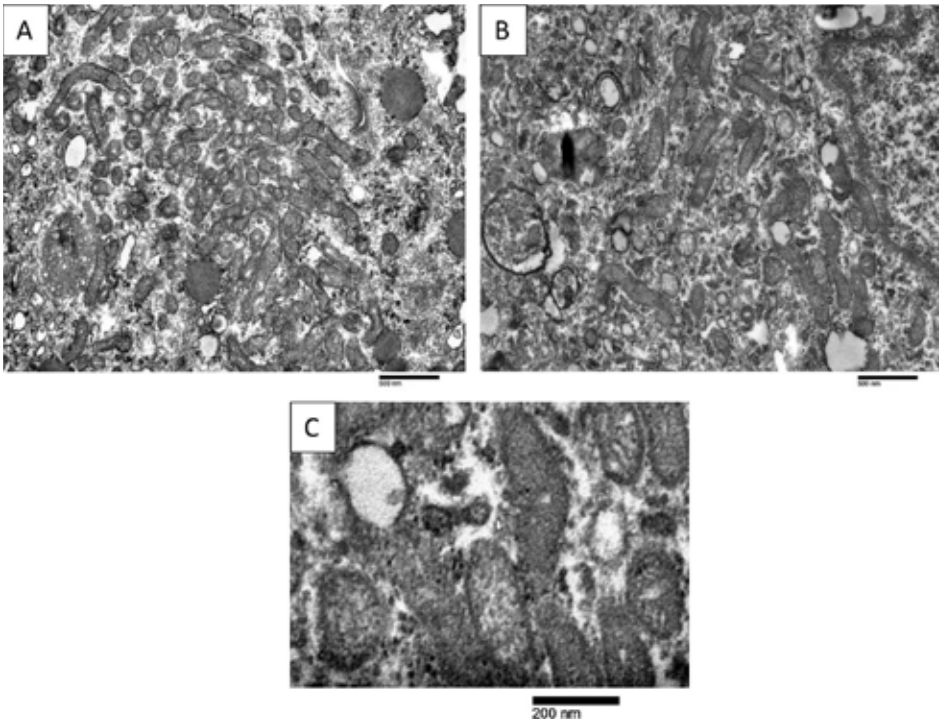


Figure 2: Myoblasts subjected to NHH show characteristic signs of mitochondrial I/R injury (A, B, & C). Many mitochondria appear either swollen (signifying necrosis), small or fragmented (i.e., apoptotic), donut- or blob-shaped (signs of oxidative stress). Evidence of mitochondrial vacuolar degeneration, including the presence of autophagosomes and lysosomes; lysosome-mitochondrion fusion; mitochondrial engulfment into autophagosomes; focal, multifocal, or complete loss and dissolution of mitochondrial cristae; and OMM rupture are shown.

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MICROSCOPIC ASSESSMENT OF REDUCTION OF PM 2.5 AND AEROALLERGEN USING PHOTO-CATALYTIC OXIDATION TECHNOLOGY APPLIED TO THE AIR PURIFICATION SYSTEM. MARYTRINH NGUYEN[1], SHAILY GOYAL[1], NABARUN GHOSH[1], AND JAY VITALE[2].

[1]Department of Life, Earth & Environmental Sciences, West Texas A&M University, Canyon, TX 79015. [2]Air for Life, UK LTD, Milton Keynes Business Centre, Foxhunter Drive, Milton Keynes, U.K.

The unprecedented situation with COVID-19 has drawn world's attention to the significance of air quality and air purification processes. It is necessary to develop and modify existing air purification technology to destroy airborne pathogens and purify the air completely. With increased population growth and industrial expansions, many cities are experiencing poor air quality. Global warming exerts substantial ramifications on flora and fauna all over the world. Increasing greenhouse gasses causes accelerated pollinosis and fungal spore production. The application of innovative technology producing in-demand novel products is the foundation of the new world trade and economy. Global economies are so tightly interconnected that companies, governments, and industries will soon be forced to cooperate in ways we could not have imagined a few years ago. Collaboration between the corporate worlds with academia has proved to be very important in scientific inventions. This report covers microscopic assessment and information on how a Nanotechnology research product was developed, assessed, and marketed in many countries. We tested if the Photo Catalytic Oxidation (AFLPCO) Nanotechnology could reduce the indoor aeroallergen to improve air quality. Air For Life (UK) air purifiers utilize a new generation of AFLPCO technology that does 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. We have assessed the AFL-Mini Sanifier, a unique air purifier that targets the particulate matters in the air and on circumferential surfaces. We have observed that besides pollen and spores, dusts generated from industrial areas, feedlots, and other facilities contribute to excessive air pollution. Recent fluctuations of meteorological conditions also augment air pollution with plant residues, fibers, gums, and insect parts floating in urban air. All these air-trapped materials have been identified as major aeroallergen irritants for provoking asthma, allergy, and other respiratory ailments. We also observed these materials trapped in our Burkard Volumetric Spore Trap. We used a fiberglass chamber to assess the particulate matters floating in the air and their reduction, if any when using the air purifier, AFL-Mini. We used a *LightHouse Handheld Particle Counter* to assess the PM 2.5, VOC and fungal spore concentration in the indoor air. We also prepared the glass slides coated with double-sided tape and placed them in different locations in the Aerobiology Lab to compare the indoor aeroallergen concentration before and after using the AFL-Mini air Sanifier. After staining with 2% Safranin-Gelvatol solution, the prepared slides were observed using a BX-40 Olympus Microscope. We used bright-field, and fluorescent microscopy using FITC and TRITC filters to analyze the trapped indoor aeroallergen. After testing and recording the data on PM 2.5, mold spores and pollen, we found a gradual reduction of overall aeroallergen concentration after the intervals of 24 hours, 48 hours, and 72 hours. We also used petri dishes to compare the microbial colonies formed at 48 hours and 72 hours of exposure before and after using the AFL-Mini Air Sanifier.

IMPLEMENTATION OF LIGHT MICROSCOPIC TECHNIQUES TO AID IDENTIFICATION OF AEROALLERGEN FOR DIAGNOSIS OF ALLERGY CASES. AUBREY HOWARD[1], LYANNA DE LEON[1], NABARUN GHOSH[1] AND CONSTANTINE SAADEH[2].

[1]West Texas A&M University, Department of Life, Earth & Environmental Sciences, Canyon, TX 79015 and [2]ALLERGY ARTS, 6842 Plum Creek Dr. Amarillo, TX 79124

Identification and quantification of pollen grains, mold spores, and other particulates present in the ambient air can be clinically significant when evaluating patients' symptoms, diagnosing allergy, and developing treatment. This information can also be processed and made available to the public to inform decisions of daily activities and medication behaviors for affected individuals. Light microscopy plays an important role in identifying and quantifying airborne particulates. It is the least invasive and

most cost effective when compared to other microscopic techniques. The aerobiology lab at West Texas A&M University has integrated various microscopic approaches to improve imaging of allergens. We have been using a Burkard Volumetric Spore Trap to collect, stain and observe and analyze the local aeroallergen. The database built in the last two decades helped us establish a reservoir of information and reference for identification that can help inform clinicians' diagnosis and treatment of patients with allergy and asthma at the Allergy ARTS Clinic in Amarillo. Evaluating the seasonal fluctuations of allergens, presence of specific aeroallergen at certain time in the air, and year-around aeroallergen indices are reflected in development of allergy symptom patterns seen in patient groups in the Texas Panhandle. Our record shows two major findings: a gradual increase in aeroallergen concentrations and a significant shift in flowering season with early flowering as evidence in different places of the world which could be a prominent reflection of climate change.

CHEMICAL AND CRYO-COLLECTION OF MUSCLE SAMPLES FOR TRANSMISSION ELECTRON MICROSCOPY USING METHACARN AND DIMETHYL SULFOXIDE. DYLAN WILBURN[1], EMMA FLETCHER[2], AHMED ISHMAEEL[2], DIMITRIOS MISERLIS[3], BERND ZECHMANN[2,4], AND PANOS KOUTAKIS[2]

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Muscle samples are commonly chemically fixed or frozen immediately upon collection for biochemical and morphological analysis. Fixatives such as glutaraldehyde are widely used for transmission electron microscopy (TEM) but do not preserve the molecular features of samples which are important for subsequent biochemical or immunohistochemical analysis. Methacarn has been suggested to be a preferable chemical fixative for light microscopy due to its ability to maintain immunohistological features of samples. However, the efficacy of methacarn to preserve ultrastructural features of samples as a primary chemical fixative is currently unclear. Additionally, cryo-preservation of samples for TEM analysis involves freezing processes such as plunge freezing, slam freezing, or high pressure freezing. High pressure freezing is the considered the gold standard but requires costly equipment and may not be a viable option for many labs collecting tissue samples from remote locations (Villinger et al., 2012). Previously, plunge/snap frozen muscle samples that were subjected to chemical fixation after freezing retained their striated pattern; however, considerable ice damage-induced artifacts were present (Giagnacovo, Cardani, Meola, Pellicciari, & Malatesta, 2010). The cryo-protectant dimethyl sulfoxide (DMSO) may allow for better structural preservation and decreased incidents of artifacts from plunge/snap freezing. We aimed to assess the effectiveness of methacarn as a primary chemical fixative and the effect of pre-coating samples with DMSO before plunge/snap frozen tissues to be prepared for TEM. All muscle samples were collected immediately after amputation from a patient with critical limb ischemia. Samples were then separated from adipose tissues and subjected to either chemical fixation with one of three different fixation techniques or frozen via two different freezing protocols. Three samples were chemically fixed immediately after amputation, one muscle sample was placed in 2.5% glutaraldehyde phosphate-buffer solution, while two other samples were placed in cold (4°C) methacarn solution for 48 hours. After 48 hours, one methacarn-immersed sample was washed 3 times for 10 min in phosphate buffer solution and then subsequently placed into 2.5% glutaraldehyde phosphate-buffer solution for 2 hours for secondary fixation. All chemically fixed samples were then post-fixed in 1% osmium tetroxide (OsO_4) for 2 hours, and subsequently dehydrated using increasing concentrations of acetone (50%, 70%, 90%, and 100%). Samples were embedded with increasing gradients (1:2, 1:1, 2:1) of Embed 812 before being completely immersed in 100% Embed 812 and polymerized at 60˚C for 48 hours. After sectioning 70 nm-thin sections, post-sectioning staining was completed by incubating grids with 2% uranyl acetate (15 min) and 1% lead citrate (5 min). In a similar manner, two other samples were collected and plunge/snap-frozen in liquid nitrogen with or without DMSO. These frozen samples were then subjected to the standard chemical fixation with glutaraldehyde and OsO4 outlined above.

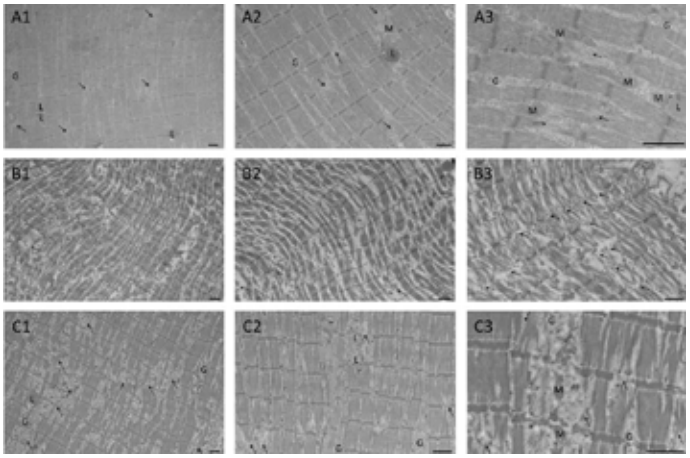

Figure 1: TEM micrographs show skeletal muscle myofibrils obtained from the samples that were initially chemically fixed in either 2.5% glutaraldehyde (A), methacarn (B), or initially fixed in methacarn and then placed into 2.5% glutaraldehyde (C). Arrows indicate areas of sarcomere damage that appear as contractile protein damage or loss of z-disc. Lipids (L) are differentially preserved between

sample preparation methods, presence of glycogen (G) granules in the intermyofibrillar space are absent with methacarn fixation, and the existing mitochondria (M) are smaller with or absent with methacarn + glutaraldehyde or methacarn fixed samples respectively. Magnification of each sample increases moving across rows from 1 to 3 for each condition and all scale bars are set to 1μm.

Samples initially fixed with glutaraldehyde showed clearly defined sarcomeres, Z-discs, lipids, and mitochondria (Figure 1 A1-3). The micrographs of the methacarn fixed samples clearly display a loss of Z-disc integrity and indistinguishable intermyofibrillar space (Figure 1 B1-3 and C1-3). Also, there was a loss of mitochondria and lipids ultimately indicating methacarn is not a viable primary fixative for tissue sample preparation for TEM.

Similarly, freezing muscle samples in aluminum foil produced large artifacts in tissue ultrastructure (Figure 2 A1-3). There were non-uniform Z-disc alignments that appeared smeared with swollen mitochondria. The DMSO treatment before freezing appears to lessen the alterations to muscle morphological structures (Figure 2 B1-3). The key regions of the sarcomere remained distinguishable and there were fewer swollen mitochondria throughout the samples. DMSO appears to be useful for preserving the ultrastructure of sarcomeres if samples are covered before freezing.

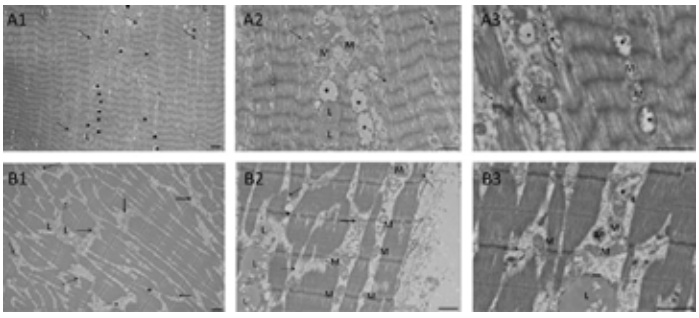

Figure 2: TEM micrographs show skeletal muscle myofibrils obtained from the samples that were initially frozen after being (A) wrapped in aluminum (B) wrapped in aluminum after being coated in DMSO. Arrows indicate areas of sarcomere damage that appear as contractile protein damage or loss of z-disc. Lipids (L) are preserved in a similar manner between freezing methods. There appears to be fewer glycogen (G) granules in the intermyofibrillar. The existing mitochondria (M) preserved better with DMSO coating before freezing in aluminum while the aluminum freezing only caused mitochondrial swelling (*). Magnification of each sample increases moving across rows from 1 to 3 for each condition and all scale bars are set to 1μm

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MICROSCOPIC ASSESSMENT OF GENOTOXICITY OF ATRAZINE ON ALLIUM CEPA AND DAPHNIA TEST SYSTEMS. MARIA LOUISA ZAVALA, AND NABARUN GHOSH.
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In recent years, there has been a significant escalation in the use of commercial weed killers in the United States to control weeds in residential and agricultural landscapes. The objective of this research is to examine the genotoxic and lethal effect of Atrazine on two test systems, *Allium cepa* L. (Green onion) and *Daphnia magna*. This study allowed us to identify the effect this chemical can have on our community health. *Allium cepa* is a standard 'test system' used very widely to test the effect of various mutagens all over the world. It is used as a model organism for chemical screening and in situ monitoring for genotoxicity of the environmental contaminants. *Daphnia* is an excellent model organism for studying multiple stressors in ecosystems. In this investigation A. cepa was chosen as a test system for the study of the chemical herbicide. *A. cepa*, can be used as a test system based upon many characteristics that are favorable for cytological studies. *A. cepa* produces abundant root growth when grown hydroponically. They possess large, well-defined chromosomes when properly prepared; the chromosomes are few in number (2n=16) and can be easily analyzed from metaphase plates. Two areas were emphasized in this study. The first area emphasized was the standard plant test system, *Allium cepa*. Bulbs of *Allium cepa* were submerged in chemical treatments containing the herbicide at various concentrations. At 24, 48, and 72 hours after the treatment, the root tips were excised for pretreatment, fixation and staining. The excised root tips from the control and treated sets of bulbs were pretreated with a saturated solution of para-Dichlorobenzene (p-DB) for 3 hours in two separate labelled vials. After the pre-treatment, the root tips were washed with distilled water three times and fixed with 1:3 Aceto-ethanol overnight. The fixed root tips were stained with 2% Aceto-Orcein-1N HCl solution. The stained root tips were squashed in 45% acetic acid. After the squash preparation, the prepared slides were observed and analyzed with a BX-40 Olympus Microscope attached to a computer with a *CellSense* software. We examined the slides to determine the mitotic index (MI), active mitotic index (AMI) and if any chromosomal anomalies were induced. The cytological assessment and comparative study were done for the treated and control set of the *A. cepa*

root tips to determine the effect of the herbicide. The MI was calculated by dividing the number of cells that were undergoing mitosis by the total number of cells present, then multiplying the quotient by 100 to give the percentage. After 48 hours, it was observed that there was a steady decline in the MI after the cells were under the herbicide treatment. However, at 72 hours, the MI began to recover. The AMI was calculated by dividing the number of cells in anaphase and telophase and dividing it by the total number of cells present, then multiplying the quotient by 100 to give the percentage. Results show that the AMI increased at each concentration and longer durations of herbicide exposure. We observed the best chromosome morphology revealed at the metaphase plate that was used for the karyotyping. While using the digital microscopy system, we opened the image with MS Photo Editor. We, then, clicked on the picture that we wanted to edit. Next, we focused and cropped the image to an area that we wanted to enhance. The captured image was modified to improve its visual quality. From this study, it can be concluded that within 24, 48, and 72 hours of the herbicide treatment, the MI and AMI have been significantly affected in the treated set when compared to the control set. *Daphnia* reflects a high sensitivity to environmental pollutants. Exposures to environmental stressors, *Daphnida* demonstrate reproductive decline, aberrant vertical mobility and phenoplasticity to name a few. Abiotic and biotic stressors include chemical substances, synthetic hormones, acidity, salinity, etc. We tested the survival rate of *Daphnia* at different concentrations of the herbicide and recorded the concentration for the LD50 (Lethal Dose 50). We captured the micrographs screening the slide at various magnifications under a BX-40 Olympus microscope equipped with *CellSens* software. We found that *Daphnia* exhibited lethality even with a very low dose of the herbicide proving thereby the herbicide subjected to this experiment is both genotoxic and lethal to the plant and other organisms.

MATERIAL SCIENCES
Spring 2022

SPATIALLY-RESOLVED PHOTOLUMINESCENCE OF MONOLAYER MOS2 UNDER CONTROLLED ENVIRONMENT. BLAKE BIRMINGHAM[1], JIANTAN YUAN[2], MATTHIAS FILEZ[2], DONGLONG FU[2], JONATHAN HU[3], JUN LOU[4], MARLAN O. SCULLY[5], BURT M. WECKHUYSEN[2], ZHENRONG ZHANG[1]
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One photonically and chemically interesting material, monolayer (ML) MoS2, has a direct bandgap that is strongly dependent on its nanoscale surface structure. The direct bandgap can be easily modulated by a small number of adsorbed molecules. Raman microspectroscopy in a reaction cell was used to perform in situ characterization of the ML photoluminescence (PL) in response to the presence of common atmospheric gasses (Figures 1A and 1B). We demonstrate that laser irradiation in ambient atmospheric conditions has little effect on the basal plane PL (Fig. 1C), electron-hole pair recombination is not hindered, and the material remains constantly emissive. Conversly, edge sites in gaseous environments begin with low PL emission that can increase or decrease with continuous photoirradiation. Photoirradiation in controlled gas environments reveals that O_2 is necessary to increase the PL intensity at the MoS2 flake edges, attributed to the charge transfer of chemisorbed O_2 (Fig. 1D). N_2 or H_2O and N_2 environments induce decreasing PL intensity upon repetitive laser irradiation. However, the H_2O and O_2 gas mixture, a combination designed to mimic ambient conditions, is necessary to maintain the PL intensity at the interior of the ML MoS_2 flakes. Our study demonstrates that photoreactions with the gaseous environment on the MoS_2 ML flakes should be taken into consideration even upon mild photoirradiation because they strongly impact the flakes' optical properties. We also showed that reactant phase can have a dramatic impact on the PL modulation: gaseous pyridine and thiophene nearly completely quench flake PL but liquid exposure reduced emission by only one-half (Birmingham et al., 2019).

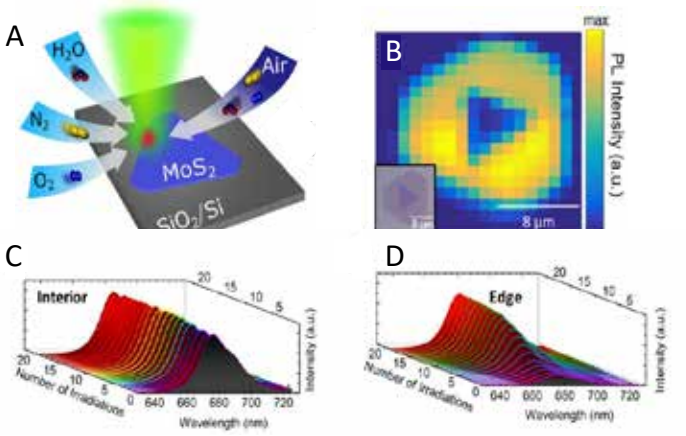

Figure 1. A) Optical microspectroscopy study of photoluminescence (PL) modulation of ML MoS2 by ambient gasses in a reaction controlled environment (Birmingham et al., 2018); B) Scanning microscale PL map of a typical ML MoS_2 flake on SiO_2/Si with a bilayer center. Inset: Optical image of the flake mapped in part A. Evolution of PL spectra upon photoirradiation in an ambient environment. PL spectra taken on the interior (C) and edge (D) of a ML MoS_2 flake upon consecutive photoirradiations in an ambient environment.

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$ZnTiO_3$ NANOPARTICLES FOR APPLICATION AS PHOTOANODE IN DYE-SENSITIZED SOLAR CELLS (DSSC). SUSANA BORBÓN[1,2], SHADAI LUGO[3], NAYELY PINEDA[4], ISRAEL LÓPEZ[1,2].

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The synthesis of $ZnTiO_3$ monodispersed nanoparticles has been carried out by sol-gel method in air atmosphere and room temperature. The synthesized nanoparticles exhibited hexagonal crystalline structure as confirmed by XRD analysis and Raman spectroscopy. FE-SEM showed a monodispersed particle size distribution, with a particle size distribution between 39 and 62 nm, and a homogenous quasi-spherical morphology through the sample. The optoelectronic properties were studied by DRS, obtaining an indirect band gap between 3.08 and 3.20 eV. The specific surface area and pore size distribution were determined by a N_2 physisorption analysis, as 13.96 m_2/g and 2-10 nm, respectively. The synthesized $ZnTiO_3$ nanoparticles are a promising alternative for dye-sensitized solar cells (DSSC) photoanodes since they yield an outstanding dye loading capacity.

ULTRAFAST SYNTHESIS OF HKUST-1 NANOPARTICLES BY SOLVOTHERMAL METHOD: PROPERTIES AND POSSIBLE APPLICATIONS. EDER M. CEDEÑO-MORALES[1], MIGUEL A. MÉNDEZ-ROJAS[2], LETICIA M. TORRES-MARTÍNEZ[3,4], LUIS F. GARAY-RODRÍGUEZ[3], ISRAEL LÓPEZ[1,5], IGOR E. UFLYAND[6], BORIS I. KHARISOV[1].

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Although an astonishing plethora of Metal-Organic Frameworks (MOFs) has been developed to date, the quest for superior, greener methods that yield compelling crystalline structures is still a challenge. For the first time, a non-conventional solvothermal method has been employed to synthesize the Hong Kong University of Science and Technology (HKUST-1) MOF. HKUST-1 nanoparticles have been synthesized by a Monowave 50 (Anton Paar) reactor at 5, 10, 15, and 30 min instead of staggering times of 10 to 24 h. XRD and FTIR analysis confirmed the obtention of these crystalline structures. Moreover, a quasi-spherical morphology and a statistical particle size distribution of 83 $\pm$ 29 nm were estimated from the scanning electron microscopical characterization for the sample synthesized in 10 min. The alleged obtention of monocrystalline structures with a band gap energy of 3.5 eV makes them promising nanostructures for a variety of applications.

DETECTION AND REMOVAL OF ALIZARIN RED S FROM AQUEOUS SOLUTIONS BY OPTICAL FIBER WITH FUNCTIONALIZED CARBON NANOTUBES. OXANA VASILIEVNA KHARISSOVA, GERARDO ALEJANDRO MONTANO GONZÁLEZ, BEATRIZ ORTEGA, EDER M. CEDEÑO-MORALES

Universidad Autónoma de Nuevo León, Monterrey, Mexico.

The treatment and reuse of wastewater from textile printing and dyeing has always been a major concern in the field of wastewater treatment. Anthraquinone dyes are the second largest group of synthetic dyes that are widely used to dye different textiles due to

their bright color, high fixing rate, and strong color fastness. As a typical anthraquinone dye, Alizarin Red S (ARS) has been widely used in the textile industry, but unfortunately ARS is extremely resistant to degradation due to its fused aromatic structure, which may cause serious threat to aquatic ecosystem and human health. Therefore, considerable interest has been focused on the removal of ARS from dyeing wastewater prior to discharge.

In the present work, the functionalization of sensors based on optical fiber and multilayer carbon nanotubes was analyzed. Fiber optic sensors (FOS), possessing such properties as small size, absence of interference with electromagnetic radiation, high sensitivity and the ability to design multiplexed detection systems, have found several applications. Carbon nanotubes (CNT) found application in electronic equipment, hydrogen storage and even adsorption and separation processes. This development could solve the problem of alizarin red dye contamination of water. Efficient removal of Alizarin Red S from aqueous solutions by unnationalized carbon nanotubes was noted.

A SMF-28e single-mode fiber with a core diameter of 8.2 μm and a cladding diameter of 125 μm was used as the basis for the tapered fiber optic detector. An array of MWCNTs with Fe nanoparticles on the conical fiber optic detector surface was synthesized by spray pyrolysis chemical vapor deposition (CVD) method at 800°C for 20 min inside a tube furnace, using ferrocene solution in toluene. as a catalyst precursor. The structure formed was applied for the detection of alizarin in water. The morphologies of MWCNT-Fe were analyzed by scanning electron microscope (SEM) and transmission electron microscope (TEM). The X-ray powder diffraction (XRD) pattern was recorded on a Bruker Advance D8 X-ray diffractometer (D8 ADVANCE, Germany) at room temperature.

COLLECTION OF RAMAN SIGNAL IN LIQUID USING PLASMONIC VORTEX FIBER. ROHIL KAYASTHA, BLAKE BIRMINGHAM, AND ZHENRONG ZHANG
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Tip-enhanced Raman spectroscopy (TERS) can achieve nanoscale resolution which overcomes the diffraction limit of light, by nanofocusing the light beam using surface plasmon polariton (SPP) in a conical Kretschmann configuration. The nano-focused light creates an enhance electromagnetic field (EM) called hotspot at the apex of the probe. Here, we use plasmonically coated tapered optical fiber to excite and collect the Raman signal from the liquid sample.

Vortex fiber with a M-shaped refractive index profile is used because it maintains the polarization state of the propagated beam. The tapered fiber is gold (Au) coated to use it as the plasmonic tip for TERS. The vortex fiber is necessary to maintain the radially polarized light throughout the fiber. Because only a radially polarized light can plasmonically excite into SPP mode at the tapered region that will nano-focus the light at the tip apex. We experimentally study the plasmonic excitation of vortex fiber with internal illumination of the radially polarized beam to demonstrate the back collection of Raman signal from the liquid sample. The tapered fiber tip is created by chemically etching the fiber. We demonstrate concentration dependence of the plasmonic coupling in the liquid sample; the refractive index of the liquid changes as the concentration changes therefore stronger Raman signal is observed at certain liquid refractive index. We performed a z-scan experiment where the fiber tip is gradually submerged into the liquid from the air and vice versa. Stronger Raman signal at the air-liquid interface were observed in comparison to when the fiber tip was fully submerged into the liquid (Figure 1). The stronger signal at the interface could represent coupling of SPP mode or the waveguided mode to a tapered liquid layer that forms over the tip. The air-liquid interface experiment also demonstrates that most of the signal is obtained near the tip apex than from the shaft of the probe.

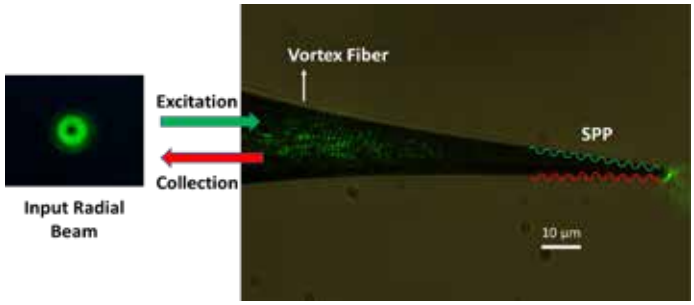

Figure 1: Optical image of the side emission from the tip apex of a gold coated tapered vortex fiber showing excitation and collection of radial beam using the SPP.

FROM ALUMINUM FOIL TO TWO-DIMENSIONAL NANOCRYSTALS USING ULTRASONIC EXFOLIATION. WEIGANG LU[1,2], BLAKE BIRMINGHAM[1], DMITRI V. VORONINE[3,4], DREW, STOLPMAN[2], SHARAD AMBARDAR[4], DENIZ ALTUNOZ ERDOGAN[5], EMRAH OZENSOY[5], ZHENRONG ZHANG[1], TOURADJ SOLOUKI[2]
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Aluminum (Al) nanostructures have unique optical properties such as widely tunable surface plasmon resonances from deep UV to NIR that can be used for label-free fluorescence enhancement and surface-enhanced Raman scattering (Lu et al. 2021). Various Al nanostructures have been fabricated using sophisticated "top-down" lithographic and "bottom-up" colloidal methods. Here, we developed a simple and efficient method of synthesizing two-dimensional (2D) Al nanocrystals from commercially available Al foil using ultrasonic exfoliation under ambient environment. 2D Al nanocrystals with sizes from a few hundred nanometers to several micrometers and thickness in the tens of nanometers were isolated through centrifugation separation (Figure 1). The exfoliated 2D Al nanocrystals are covered with a passivated Al_2O_3 nanolayer. The determined exfoliation mechanism is a combination of the preferred cleavage along the (111) surface planes and layer-by-layer Al_2O_3 exfoliation from the surface of the 2D Al nanocrystals. We demonstrate that the 2D Al nanocrystals can be assembled at water/air interface and transferred to different substrates to form Al nanocrystal films. These 2D Al nanocrystal films exhibit surface plasmon resonance in the visible spectral range and show enhanced Raman signals of adenine using a 532 nm excitation. These 2D Al nanocrystal films could be further developed for new optical and sensing applications.

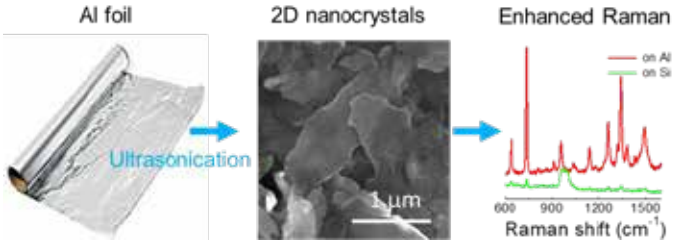

Figure 1 Synthesis of Al 2D nanocrystals which show enhanced Raman scattering of adenine.

Reference

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OPTICAL AND MORPHOLOGICAL APPROACHES IN THE FERROELECTRIC $BIFEO_3$ THIN FILMS CHARACTERIZATION HÉCTOR MIRANDA[1], E. DE OBALDÍA[1], A. WATSON[1], D. VILLARREAL[2] AND E. CHING-PRADO[1].

[1]Pierre and Marie Curie Laboratory, Faculty of Science and Technology, Technological University of Panama, Panama City; [2]The University of Texas at Dallas, Richardson, TX.

An $BiFeO_3$ thin film was fabricated on FTO/glass substrate by sol-gel method with a sequential layer deposition process. The raw materials using in the starting solution were, bismuth nitrate pentahydrate ($Bi(NO_3)3{\cdot}5H_2O$), iron(III) nitrate nonahydrate ($Fe(NO_3)3{\cdot}9H_2O$). All of these previous reagents were mixed together using ethylene glycol ($HOCH_2CH_2OH$) as solvent. A 5 mol% excess of bismuth was used to compensate for its volatility during high temperature annealing, which was 550°C for 15 minutes through rapid thermal annealing (RTA). In order to contrast the corresponding value of the film thickness, Scanning Electron Microscopy (SEM) and UV-visible Spectroscopy, respectively, were used for characterization. SEM micrographs showed a homogeneous surface with spherical and irregular shape grains and intergranular spaces almost zero (Fig. 1A). Also, the well-defined grain boundary formations suggest that the film has good adherence to the substrate. The grain size histogram obtained through an image analysis process revealed a grain size of 45 to 398 nm under the growth condition used. In addition, cross-section image analysis revealed that the thickness of the film was 185.9 nm. On the other hand, the transmittance spectrum was fitted, on 190 to 1100 nm optical range, using the classical dispersion theory of Drude-Lorentz to approximate interest optical parameters such as: the complex dielectric function, the complex refractive index, the optical band gap, etc. Furthermore, the optical spectrum modeling allows approximating the value of the film thickness through the multiple reflections that occur at the interfaces of the glass/FTO/$BiFeO_3$ array. Thus, the thickness obtained from the optical analysis was 167.8 nm, revealing that SEM and UV-visible Spectroscopy techniques provide close results, which contributes to the reliability of the thickness value found in this work (Fig. 1B)

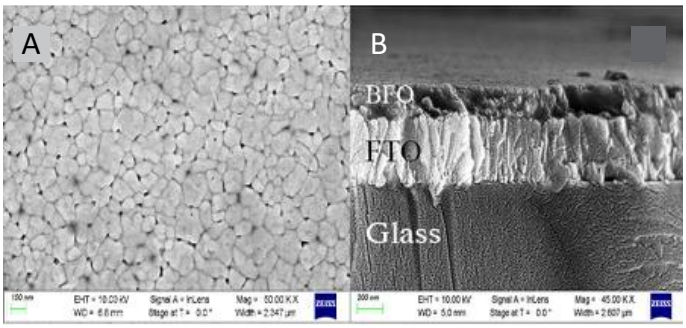

Figure 1. SEM images of the A) surface and B) the cross-section micrograph of the $BiFeO_3$ thin film.

PHASE TRANSITION OF ANATASE TIO2 SINGLE CRYSTALS WITH LARGE PERCENTAGE OF (001) FACETS: A RAMAN MAPPING AND SEM STUDY. HAO ZHU, WEIGANG LU, BLAKE BIRMINGHAM, NOLAN CRAFT, KENNETH PARK, ZHENRONG ZHANG

Department of Physics, Baylor University, Waco, Texas 76706, USA

TiO_2 has been extensively studied in the fields of photo-catalysis, electrochemistry, etc. Rutile and anatase are the two main polymorphs of TiO_2. Many studies show that mixed-phase TiO_2 particles have higher photoactivity than single-phase particles due to the synergistic effect between anatase and rutile. It is known that anatase-rutile phase transition (ART) is an irreversible process in photoreactions, but the detailed phase transition process is not clear. Understanding the mechanism of the ART process is critical for the design of TiO_2-based high activity photocatalysts.

In this work, the ART process of anatase micro-particles with a large percentage of (001) facets was studied using a lab-built Raman microscope and scanning electron microscope (SEM). Our results show that in the process of ART, phase concentration evolution obtained via Raman microscope matches the morphological evolution observed in SEM images (Figure 1).

The ART processes of each anatase particle are distinctive depending on the various defects which serve as rutile nucleation sites. Two types of transition pathways are observed. In one pathway of growth, rutile nucleation formed at a corner of an anatase crystal and occupied the edge of the crystal. The rutile phase then gradually grew towards the other side of the cube. The phase concentration calculated from Raman spectra revealed that the ART transition following the first-order reaction mechanism. In the other growth pathway, multiple rutile nucleation sites formed simultaneously on the edges and corners of the microcrystal. The rutile phase then spread over the whole crystal from these nucleation sites. Our study on the ART of micro-sized crystals bridges the material gap between bulk crystals and nano-sized TiO_2 particles. The anatase/rutile co-existed particle will provide a feasible platform to study the synergistic effect between the anatase phase and the rutile phase in their catalytic performances.

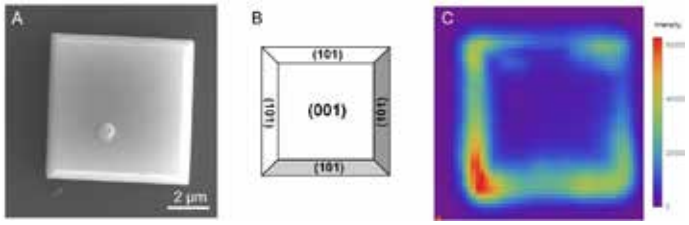

Figure 1: SEM image and Raman map of an anatase TiO_2 micro-crystal. A) Top view SEM image; B) Schematic diagram of anatase micro-crystal; C) Raman map of anatase E_g mode at 144 cm_{-1}.

COMPARISON OF CONVENTIONAL AND RESONANT ACOUSTIC MIXING OF AP/HTPB PROPELLANTS. FELIX A. RODRIGUEZ, JAMES C. THOMAS, ALVIN C. HONG, ERIC L. PETERSEN
Texas A&M University, J. Mike Walker '66 Department of Mechanical Engineering, College Station, TX

Composite solid propellants can be mixed by a variety of techniques. Recent R&D efforts have considered implementation of resonant acoustic mixing (RAM) strategies for improved homogeneity, reduced complexity, and faster mixing capabilities. In the current study, a standard high-performance propellant formulation (85% trimodal AP, 1.3% Fe_2O_3, 1.0% Al_2O_3) was mixed by standard laboratory techniques (i.e., hand mixed) and RAM to allow for direct comparison of these methods. Propellant samples were burned in a constant-volume, optically accessible strand burner at pressures between 3.45–20.7 MPa (500–3,000 psia). Propellant microstructures were evaluated with scanning electron microscopy (SEM). Implementation of RAM yielded a significant increase in the propellant burning rate (~25%) in comparison to the standard hand-mixing strategy. The observed performance improvement in the RAM formulation was attributed to improved catalyst/oxidizer contact, yielding improved catalysis during propellant combustion. Representative SEM images of the conventionally mixed and RAM propellants are displayed in Figure 1. The bright white spots, representing Fe_2O_3 catalyst particles, in the SEM image for the RAM propellant are smaller (Figure 1b, bottom panel) than in hand mixed samples (Figure 1b, upper panel). It can be reasoned that dispersion of the catalyst and other components is better with RAM than conventional mixing.

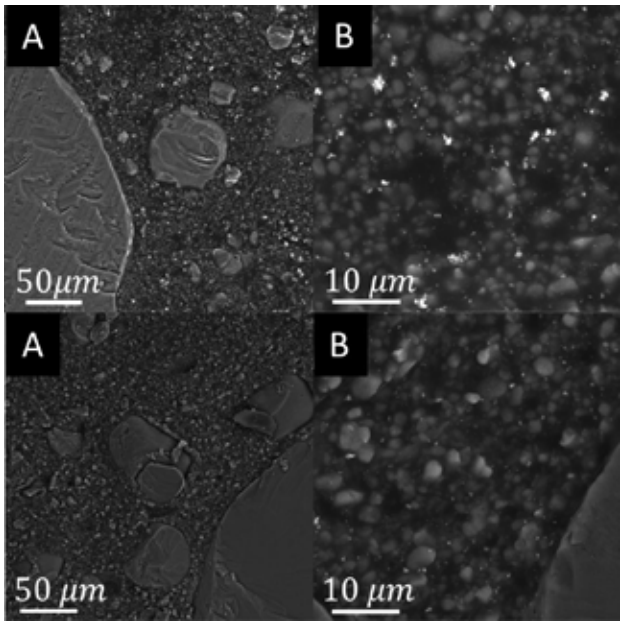

Figure 1. Representative backscattered electron SEM images of conventional mixed (top) and RAM (bottom) propellant formulations. A) and B) notation represent images taken with a primary magnification of 1000x and 6000x, respectively. Bright white spots in the B) images correspond to the Fe_2O_3 catalyst particles.

TECHNICAL ABSTRACTS
Spring 2022

μCT CHARACTERIZATION FOR GEOLOGICAL AND DRILL CORE SAMPLES. ANGELA CRISWELL AND AYA TAKASE.
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X-ray techniques are powerful tools for characterizing geological and drill core samples. In particular, X-ray computed microtomography (μCT) is a fast and non-destructive tool that allows both qualitative and quantitative examination of internal sediment structure, fractures, porosity and other features. When used in combination with X-ray diffraction (XRD) and X-ray fluorescence (XRF), one can identify discrete materials within core samples and identify chemical composition. In this study, we use μCT to examine several core samples, including carbonates and sandstones. Each core sample was analyzed using Rigaku's CT Lab HX130 μCT systems. μCT tomograms were used to analyze distribution of minerals throughout the 3D samples. These results show that μCT provides valuable information when characterizing rock and mineral samples.

UNIQUE ANALYTICAL COMBOs… THE ULTIMATE ANALYTICAL APPROACH. JOHN MASTOVICH
Bruker AXS Inc., 5465 East Cheryl Parkway, Madison, WI 53711

The Scanning Electron Microscope (SEM) using electron beam excitation and Energy-Dispersive (EDS) detector has becomes the industry standard, as throughput and general performance has steadily improved. Today, MicroXRF technologies are available, providing very high X-ray flux with Trace Analysis and rapid data acquisition. The unique Dual Excitation approach provides many analytical advantages beyond trace detection, when dealing with beam sensitive low voltage applications and yields the ultimate Trace Analysis throughput and workflow.

Numerous applications ranging from soft materials [polymers & biologicals] to devices [microelectronics & semiconductor] and materials [metals & composites] will be discussed to illustrate the analytical benefits of the Dual Excitation approach. Significant advantage is the enabling of low dose/low voltage applications, while also using X-ray excitation to observe the extended range of the spectrometer as depicted in Figures 1A and 1B. Clear data are generated from samples that typically produce few to no X-rays via e-beam, from depths of many microns from within the sample, as shown in Fig. 1C. When the ultimate spectral and spatial resolution is required, the data in Fig. 1D conveys Wavelength Dispersive Spectroscopy (WDS). Simultaneous EDS - Electron Backscatter Diffraction (EBSD) is possible from thinned samples, gathering the utmost spatial resolution during in-situ work as illustrated in Figures 1E & 1F.

Overall, due to the FlatQUAD detector to the sample design, the In-situ work provides superior collection efficiency regardless of whether the SEM or Scanning Transmission Electron Microscopy (STEM) applications are implemented.

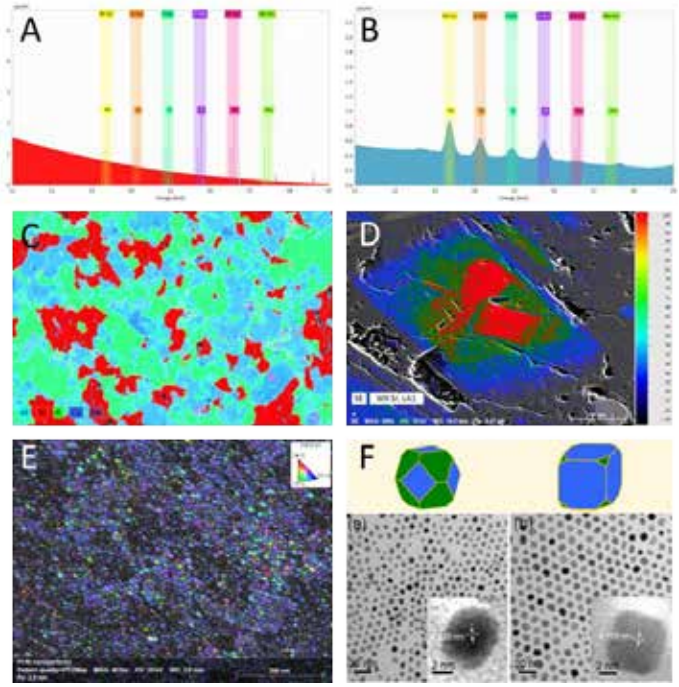


Figure 1. Analytical benefits of the Dual Excitation approach. A) Electron beam excitation; B) X-ray beam excitation; C) MicroXRF elemental mapping; D) Wavelength dispersive mapping; E) Platinum Nickel nanocrystals; F) Bright and dark field-like imaging.

RECENT ADVANCEMENTS IN INTEGRATED X-RAY SOURCES FOR SCANNING ELECTRON MICROSCOPY. ROBERT C. TISDALE
IXRF, Inc., AUSTIN, TX

Since the beginning of the 21st century, many advances for scanning electron microscopy (SEM) have been commercialized. One of the most significant of these has been the introduction of X-ray sources into the SEM to extend and enhance elemental analysis capabilities. Demonstration of the efficacy of employing small X-ray tubes, that have been modified for mounting on SEMs, date as far back as 1986 (Wherry and Cross, 1986). These X-ray sources fall into two categories: pin-hole collimated low-power transmission target miniature tubes that afford small spots, and micro-focus higher-power tubes with integrated polycapillary optics that produce microXRF scale (10μm) beam spots at the

sample. Both of these approaches are analytically viable options for an SEM enhancement, allowing samples to be excited by either an electron beam (SEM/EDS) or X-ray photons (SEM-XRF). It is also possible to analyze samples by employing both excitation strategies simultaneously or sequentially (Combo, see Fig. 1). Quantitative analysis using this combined approach uses the advantage of e-beam excitation for lighter elements below 2.0 keV, and the more efficient photon excitation for X-ray lines above 2.0 keV (see Fig. 2, NIST 610 example) (Cross and Witherspoon, 2009). MicroXRF with XY-stage scanning may be used to collect X-ray elemental maps similar to those collected with e-beams, except that the stage is scanned instead of rastering the e-beam (Witherspoon et al., 2009). Benefits of microXRF within the SEM are illustrated, including the analysis of multi-layer thin film samples, a larger elemental range, true bulk measurements, microXRF mapping, enhanced sensitivity down to ppm levels, scattering fundamental parameters for trace elements in low-Z matrices and non-destructive measurements (no e-beam damage and no coatings are required for microXRF analyses).

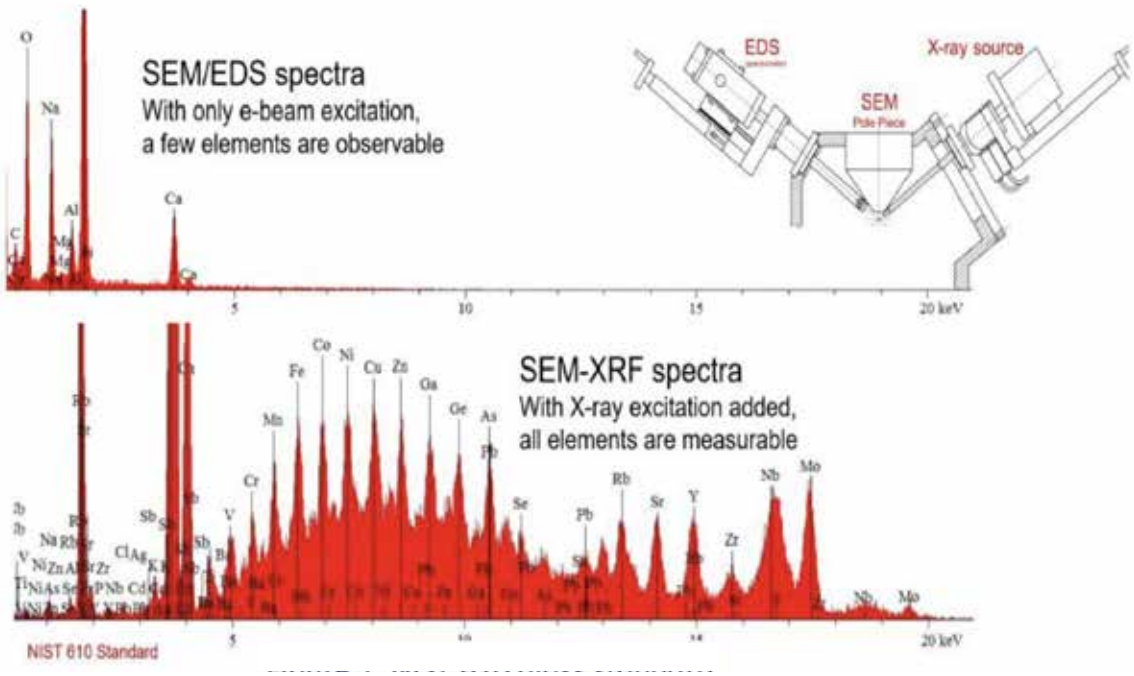


Figure 1. NIST 610 glass standard.

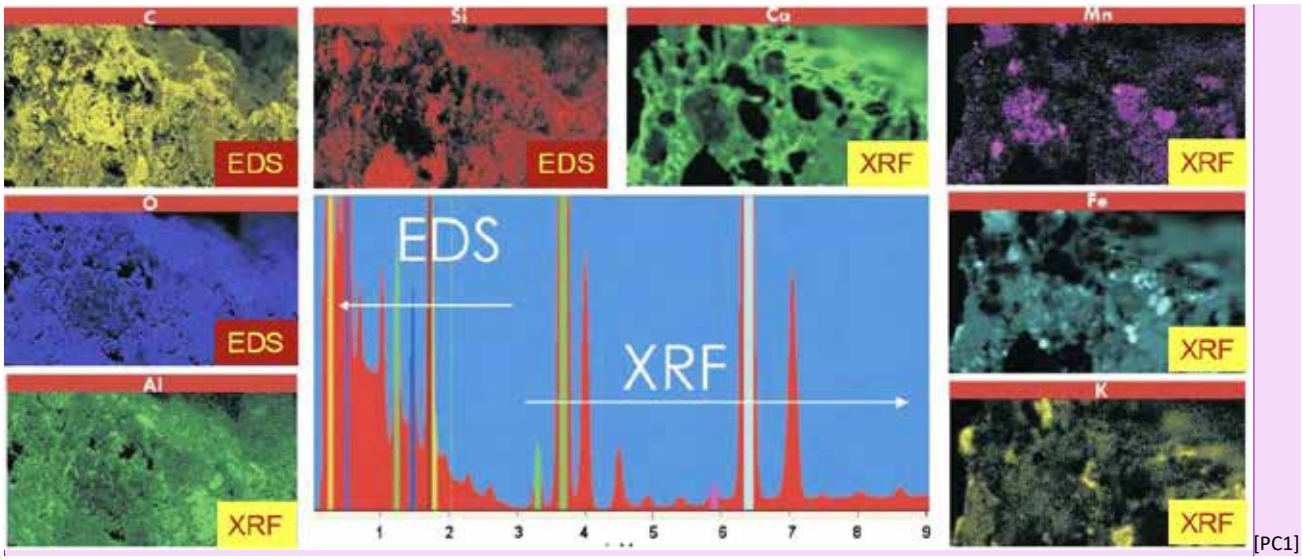


Figure 2. Example of Combo analysis using X-ray and e-beam excitation.

References

D.C. Wherry and B.J. Cross, Kevex Analyst, (Aug. 1986) 8.

Cross, B., & Witherspoon, K. Integrated XRF in the SEM. Microscopy and Microanalysis, 10(S02), (2004) 104-105. K.C. Witherspoon, B.J. Cross, R.D. Lamb and P.-O. Sjoman. Integrated XRF in the SEM. Microscopy and Microanalysis, 15(S01), (2009) 79-128.

K.C. Witherspoon, B.J. Cross, R.D. Lamb and P.-O. Sjoman. Integrated XRF in the SEM. Microscopy and Microanalysis, 15(S01), (2009) 79-128.

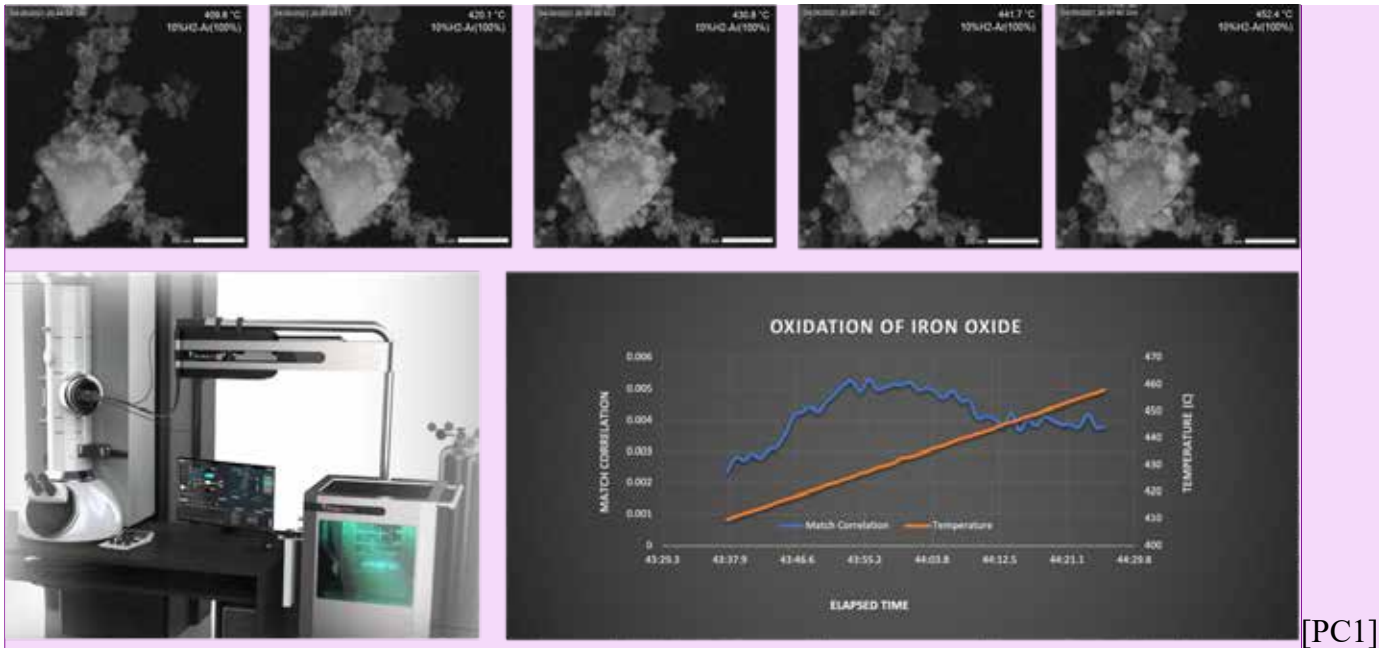
IN-SITU TEM: A NANOSCALE LABORATORY.

MADELINE J. DRESSEL-DUKES

Protochips, 3800 Gateway Centre Blvd #306, Morrisville, NC 27560

The transmission electron microscope (TEM) has long been the gold standard for high resolution imaging, providing atomic level detail about a sample's structure. In a conventional setup, the sample is deposited onto a 3 mm copper grid which contains a layer of amorphous material, such as carbon. The sample is then inserted into the high vacuum environment of the TEM for imaging and/or elemental analysis. One significant limitation of this setup is the expectation that the sample must be stable in a high vacuum environment. In-situ transmission electron microscopy (*in-situ* TEM) techniques were developed to overcome this limitation, enabling samples to be imaged with TEM in non-vacuum environments, and simultaneously introduce real-time stimuli, such as temperature or electrical currents, during a TEM imaging session. Thus, users can record dynamic processes in real-world conditions at resolutions not obtained using non-TEM techniques. In-situ studies require the use of designated sample holders which protect the sample from the high-vacuum TEM column, deliver stimuli, and accurately measure signal output. The concurrent development of advanced detectors, cameras, software and designated *in-situ* holders has enabled researchers across diverse fields to unravel previously impossible results which range from observing viral transcription in physiological relevant, liquid environments, to changing the gas environment of catalyst particles from oxidizing to reducing during a single experiment.

State-of-the-art *in-situ* tools and software enable users to observe real-time reactions and behavior under a variety of conditions, such as liquid, gas and high temperatures and introduce a range of stimuli to the sample in those environments. These systems incorporate semiconductor MEMs technology into the sample support. MEMs technology enables vacuum-sensitive samples to be encapsulated between ultrathin electron transparent windows for imaging in liquid and gas, provides exceptionally low drift rates at high temperatures, and allows patterning of a variety of features such as electrical contacts. Here, we will review

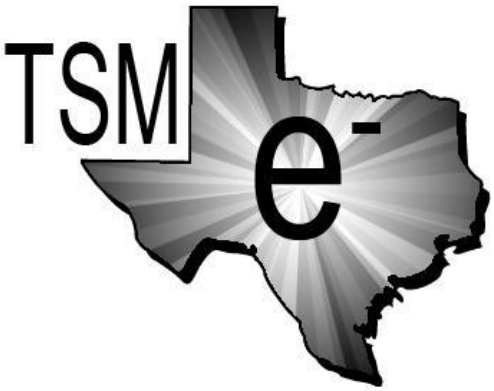


[PC1]

Figure 1: Reduction of Iron Oxide Nanoparticles Using a Commercial In-Situ Gas TEM System. Top Row: STEM images showing the morphological change in iron oxide nanoparticles during a heating ramp from 409°C to 452°C under a 10% hydrogen atmosphere. Bottom Left: Setup of the Protochips Atmosphere in-situ system on a TEM. Bottom Right: Metadata analysis using Protochips' AXON software.

the functionality and use in-situ systems developed by Protochips Inc. for dynamic *in-situ* studies including high-temperature and electrical studies, nucleation and material growth in liquid, electrochemistry, catalysis and corrosion.

Shown in Fig. 1 is an example of one such study. Here, a catalytic reaction, the reduction of iron oxide nanoparticles under 10% H_2 in argon, is observed over a temperature ramp. As the temperature is increased the reduction rate increases, resulting in the morphological changes observed in the nanoparticles. Combined with powerful, machine vision software, these systems enable the TEM to be converted to a real time laboratory merging high resolution images and movies with multiple data streams from both the sample environment and the microscope/detector.



SCIENTIFIC PROGRAM

56th Meeting of the Texas Society for Microscopy (TSM) | Friday, March 25, 2022

Baylor Research and Innovation Collaborative (BRIC) - Atrium

8:00 a.m. - 10:00 a.m.	**BRIC Atrium:** Registration | Breakfast
8:00 a.m. - 10:00 a.m.	**BRIC Atrium:** Vendor Setup | Poster Setup
8:30 a.m. - 8:45 a.m.	**Room BRIC 2160:** Opening Remarks Amy Jo Hammett, TSM President and Nabarun Ghosh, Program Chairman
	1st Session Room BRIC 2160 | Session Chair: Nabarun Ghosh Current perspectives in characterization methods of advanced materials along product life cycles
8:45 a.m. - 9:35 a.m. *Invited Speaker*	Christie M. Sayes, Baylor University, Waco, TX Comparison of conventional and resonant acoustic mixing of AP/HTPB propellants
9:35 a.m. - 9:50 a.m. *Student Competition (MS)*	Felix A. Rodriguez, Texas A&M University, College Station, TX In-situ TEM: a nanoscale laboratory
9:50 a.m. - 10:10 a.m. *Corporate Speaker*	Madeline J. Dressel-Dukes, Protochips Inc. Phase Transition of anatase TiO2 single crystals with large percentage of (001) Facets: a raman mapping and SEM study
10:10 a.m. - 10:25 a.m. *Student Competition (MS)*	Hao Zhu, Baylor University, Waco, TX Recent advancements in integrated X-ray sources for scanning electron microscopy
10:25 a.m. - 10:45 a.m. *Corporate Speaker*	Robert C. Tisdale, IXRF Inc. Polarization-dependent raman spectroscopy of anatase and rutile TiO_2 micro-crystals
10:45 a.m. - 11:00 a.m. *Student Competition (MS)*	Nolan Craft, Baylor University, Waco, TX Spatially-resolved photoluminescence of monolayer MOS_2 under controlled environment
11:00 a.m. - 11:20 a.m.	Blake Birmingham, Baylor University, Waco, TX
11:20 a.m. - 12:00 p.m.	**BRIC Atrium:** Poster Session and Vendor Exhibit
12:00 p.m.- 1:00 p.m.	**BRIC Atrium & Gallery:** Lunch
12:30 p.m.-1:30 p.m.	**Room BRIC 2160:** TSM Business Meeting
1:30 p.m. - 3:00 p.m.	**BRIC Atrium:** Poster Session | Vendor Exhibit
	2nd Session Room BRIC 2160 | Session Chair: Josefina Arellano Sample preparation of biological material for TEM and SEM investigations
3:00 p.m. - 3:50 p.m. *Invited Speaker*	Bernd Zechmann, Baylor University, Waco, TX Implementation of light microscopic techniques to aid identification of aeroallergen for diagnosis of allergy cases
3:50 p.m. - 4:05 p.m. *Student Competition (LS)*	Aubrey Howard, West Texas A&M University Chemical and cryo-collection of muscle samples for transmission electron microscopy using methacarn and dimethyl sulfoxide
4:05 p.m. - 4:20 p.m. *Student Competition (LS)*	Dylan Wilburn, Baylor University, Waco, TX Unique analytical combos… the ultimate analytical approch
4:20 p.m. - 4:40 p.m. *Corporate Speaker*	John Mastovich, Bruker AXS Inc. X-ray computed tomography (CT) of composite and its application for validation of ultrasonic inspection
4:40 p.m. - 5:00 p.m.	Pradip Acharya, Baylor University, Waco, TX Characterization of sub-surface wrinkles within a laminated composite using a phased array ultrasonic sectoral volumetric scan technique
5:00 p.m. - 5:15 p.m. *Student Competition (MS)*	Irrtisum Khan, Baylor University, Waco, TX From aluminum foil to two-dimensional nanocrystals using ultrasonic exfoliation
5:10 p.m. - 5:30 p.m.	Weigang Lu, Baylor University, Waco, TX μCT characterization for geological and drill core samples
5:30 p.m. - 5:50 p.m. *Corporate Speaker*	Angela Criswell, Rigaku Americas Corp.
5:50 p.m. - 6:00 p.m.	**Room BRIC 2160:** Student Awards | Final and Closing Remarks
Evening Off-Site	Dinner on Your Own

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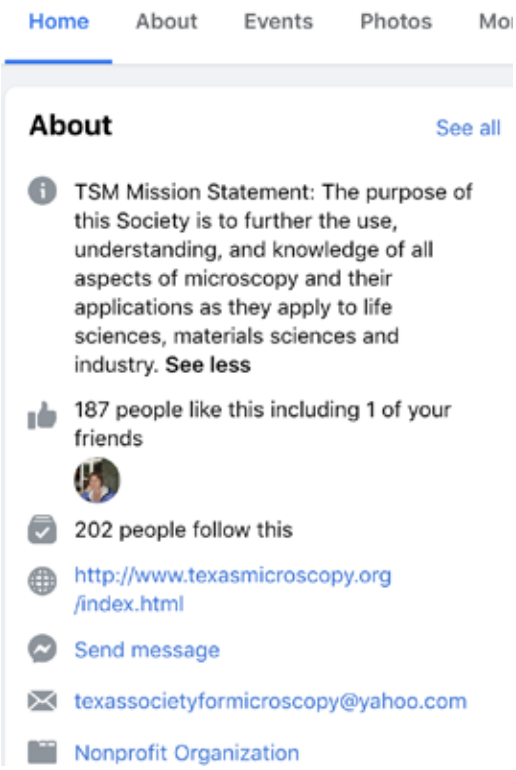

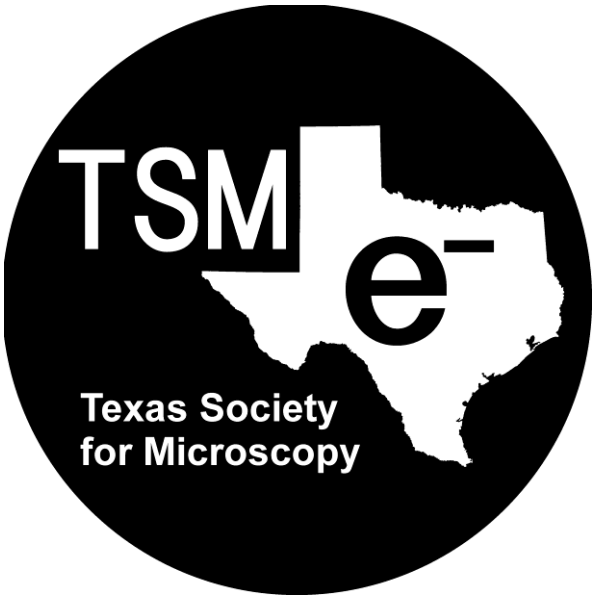

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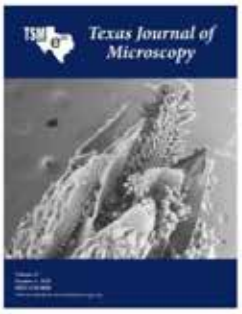

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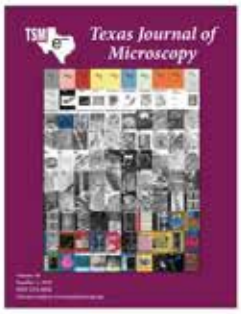

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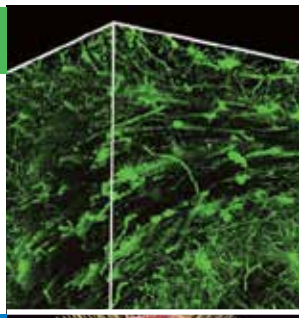
NEUROSCIENCE



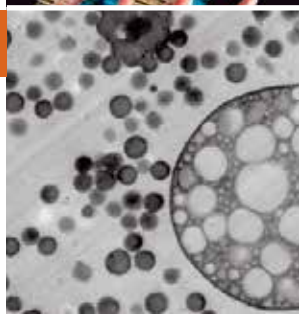
Micro-Optical Sectioning Tomography to Obtain a High-Resolution Atlas of the Mouse Brain Anan Li, Hui Gong, Bin Zhang, Qingdi Wang, Cheng Yan, Jingpeng Wu, Qian Liu, Shaoqun Zeng, Qingming Luo

Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics–Huazhong University of Science and Technology, Wuhan 430074, P. R. China.

CRYO

A single slice of a tomogram of an aldehyde fixed and sucrose infiltrated cryosection with a 3D reconstruction. Erik Bos and Peter J. Peters, Netherlands Cancer Institute, Amsterdam. (see: J. Lefman, P. Zhang, T. Hirai, RM. Weis, J. Juliani, D. Bliss, M. Kessel, E. Bos, P.J. Peters, S. Subramaniam: Three-dimensional electron microscopic imaging of membrane invaginations in Echerichia coli overproducing the chemotaxis receptor Tsr. J. Bacteriol. 2004 Aug; 186(15): 5052-61.)

MATERIALS



ABS, stained with OsO_4, sectioned at room temperature with the ultra sonic knife, section thickness 50nm. Note the almost perfect spherical shape of the large rubber particles and the preservation of the inclusions inside. Also the smaller dense rubber particles are well preserved. B.Vastenhout, Dow Benelux N.V. Terneuzen, The Netherlands.

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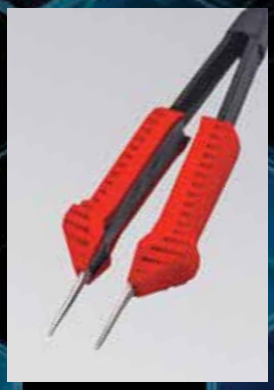

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