Michigan Microscopy & Microanalysis Society

2019 Annual Meeting
Thursday, October 31, 2019
Kellogg Hotel and Conference Center, Michigan State University
219 S Harrison Rd, East Lansing, MI 48824

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Michigan Microscopy & Microanalysis Society Conference. October 31, 2019

8:30 – 8:55 Registration (South Lobby) and Refreshments (Lincoln Room)

Morning Sessions Auditorium

8:55 – 9:00 Welcome – **Vickie Kimler**, President MMMS.

Invited

9:00 – 9:30 **Courtney Akitake,** Zeiss Research Microscopy Solutions:
Gentle Imaging of Fragile Samples with Light Sheet Fluorescence Microscopy

Keynote

9:30 – 10:20 **Michael Velbel,** Michigan State University:
A Comet's Tale: A Story of the Solar System, Told From the Miniscule Minerals Sampled by NASA's Stardust Mission

10:20 – 10:40 Morning Break (Lincoln Room) – **Sponsored by** ThermoFisher SCIENTIFIC

Volunteered

10:40 – 10:55 **Y Hoang,** Michigan State University:
Cellular shape change and nucleoid dynamics during Myxococcus xanthus development

10:55 – 11:10 **Mark Kelsey,** Bruker Nano:
Micro XRF: The Perfect Complement to SEM/EDS

11:10 – 11:25 **Farid Badar,** Oakland University
Quantitative measurement of chondrocytes and collagen in articular cartilage by polarized light microscopy

Mid-Day Break

11:30 – 1:00 Luncheon (Red Cedar Rooms A&B)
12:30 – 2:00 Vendors and Poster Sessions (Lincoln Room)

Afternoon Sessions Auditorium

Keynote

2:00 – 2:50 **Sara Miller,** Duke University School of Medicine:
It's a Small, Small World

Invited

2:50 – 3:20 **Dalen Agnew,** Michigan State University:
Hyrax under glass: Microscopy tools to solve some exotic cases

3:20 – 3:40 Afternoon Break (Lincoln Room) – **Sponsored by** HITACHI Inspire the Next Electron Microscopy Sciences

Volunteered

3:40 – 3:55 **Amber Ide,** Central Michigan University:
Copine A has a role in contractile vacuole function and postlysosome maturation

3:55 – 4:10 **Erving Laryea,** Oakland University
The aggregation propensities and morphological studies of the six isoforms of TAU

4:10 – 4:25 **Robert Monteverde,** Direct Electron
Direct Detection Camera Technology - Enabling the Resolution Revolution

4:30 – 4:40 **Vickie Kimler**: Awards and Closing

4:40 – 4:45 Closing followed by Tours of Veterinary Diagnostic Labs or Center for Advanced Microscopy

4:45 – 5:30 MMMS Business Meeting

Posters in Lincoln Room

#	Name	Affiliation	Department	Title
B1	Nusrat Jahan and Philip L. Hertzler	Central Michigan University	Biology	Wnt pathway regulation of gastrulation and segmentation in marine shrimp.
B2	Hailee Butler and Dalen Agnew	Michigan State University	Pathobiology & Diagnostic Investigations	Hepatic Iron Overload Disorder in Captive Rhinoceros
B3	Syeda Batool and Yang Xia	Oakland University	Physics	Quantitative μMRI and PLM Study of Rabbit Cartilage at Microscopic Resolutions
B4	Amber Anger and Cynthia Damer	Central Michigan University	Biology	cpnC Knockout Cells have Defects in Development and Cytokinesis
B5	Devon Leroux and Joanne Dannenhoffer	Central Michigan University	Biology	Maize Endosperm Conducting Tissue: The Basal Intermediate Zone and Conducting Zone
B6	Mouhamad Hammami, Syeda Batool and Yang Xia	Oakland University	Physics	Site-specific Quantitative Polarized Light Microscopic (PLM) study of Young Rabbit Femur Cartilage
B7	Mohanad Ahmad, Colin G. Wu and Dr. Sanele Martic - Milne	Oakland University	Chemistry	Electron Transmission Microscopy Analysis of Gelsolin peptide aggregation
PS1	JoAnn Ballor	Michigan State University	CHEMS	Influence of Composition and Structure on Measured H Concentration in beta-Ti Alloys via Atom Probe Tomography
PS2	Shatadru Chakravarty, Jeremy M. L. Hix and Erik M. Shapiro*	Michigan State University	Radiology and Institute of Quantitative Health Sciences and Engineering	Investigating Tantalum Nanoparticles for X-ray CT and Therapeutic Use
PS3	Lei Zhang	Oakland University	Mechanical Engineering	Characterization of Multiple-layered Electrical Contacts Enabled by Surface Ion Polishing
PS4	Benard Kavey and Gabriel Caruntu	Central Michigan University	Chemistry and Biochemistry	Morphology-Controlled Synthesis of Colloidal Superparticles from Ti-Containing Perovskite Nanocubes for Thin Film Technology and Energy Storage Application
PS5	Nilave Chakraborty and Gabriel Caruntu	Central Michigan University	Chemistry and Biochemistry	Solvothermal Synthesis and Characterization of Pristine Barium Titanate (BaTio3) and Chromium-Doped Barium Titanate (BaTi1-xCrxO3) Colloidal Nanocrystals
PS6	Geeta Kumari	Michigan State University	Chemical Engineering & Materials Science	Microstructural evolution with varying solutionizing temperature in Allvac 718plus

Keynotes
Auditorium

Michael A. Velbel
A Comet's Tale: A Story of the Solar System, Told From the Miniscule Minerals Sampled by NASA's Stardust Mission
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The Stardust spacecraft collected thousands of particles from comet 81P/Wild 2 and returned them to Earth for laboratory study. The preliminary examination of these samples shows that the nonvolatile portion of the comet is an unequilibrated assortment of materials that have both pre-solar and solar system origin. The bulk of the comet 81P/Wild 2 (hereafter Wild 2) samples returned to Earth by the Stardust spacecraft appear to be weakly constructed mixtures of nanometer-scale grains, with occasional much larger (over 1 micrometer) ferromagnesian silicates, Fe-Ni sulfides, Fe-Ni metal, and accessory phases.

During hypervelocity (6.1 km/s relative velocity between spacecraft and comet coma) capture in aerogel, half of the recovered comet-dust grains were disaggregated and extensively or completely melted and mixed into immediately adjacent melted silica aerogel capture-medium, and rapidly cooling to a vesicular glass with inclusions of solidified metal-sulfide droplets. These strongly thermally modified samples may have originated from a fine-grained primitive material consisting of loosely bound Wild 2 dust aggregates, which were heated and melted more efficiently than the relatively coarse-grained material of the crystalline particles found elsewhere in many of the same Stardust aerogel tracks. The least-affected thermally altered grains are nearest the track's entry apertures, an observation recently highlighted as an important criterion for selecting the specific least-altered indigenous grain remnants of Stardust aerogel capture to seek pre-solar grains hosted therein. Stardust quenched metal-sulfide droplets have size distributions slightly smaller than but otherwise identical to those of intact sulfide minerals in interplanetary dust particles with carbonaceous-chondrite affinities and in the anomalous carbonaceous chondrite Acfer 094. Input of grains from cometary sources, with the sizes (tens of nm) inferred from Stardust metal-sulfide beads, is required to maintain steady-state populations of such grains against grain loss by sublimation and collisional attrition in hot dust debris disks around early main-sequence stars.

The comet contains an abundance of coarse crystalline silicate grains that are much larger than predicted from interstellar grain models and were not thermally modified during aerogel capture. Many of these are high-temperature minerals that appear to have formed in the inner regions of the solar nebula. The very wide range of olivine and low-Ca pyroxene compositions in comet Wild 2 requires a wide range of formation conditions, probably reflecting very different formation locations in the protoplanetary disk. The restricted compositional ranges of Fe-Ni sulfides, the wide range for silicates, and the absence of hydrous phases indicate that comet Wild 2 experienced little or no aqueous alteration. Less abundant Wild 2 materials include refractory particles, whose presence appears to require radial transport in the early protoplanetary disk. The presence of anhydrous high-temperature minerals in a comet proves that the formation of the solar system included radial mixing on the grandest scales.

Sara E. Miller
It's a Small, Small World
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As electron microscopists, we are accustomed to working with tiny specimens; in fact, it's required. Sometimes, however, the material supplied to us to process is minuscule, even by our standards, and somehow, we're supposed to keep up with it during multiple steps and find it in the electron microscope.

Tiny-tipped tubes are available for concentrating small amounts of non-adherent cells such as cytology specimens into a pellet, which can be encased in agar for holding it together and enlarging the mass so that it does not get lost during processing. Also, pointed-tipped BEEM capsules are available where small numbers of cells can be entirely processed without removing them from the capsule. Very small numbers of white blood cells or platelets can be processed in a capillary tube to produce a buffy coat or platelet-rich plasma. Another technique is to amplify an "invisible pellet" with 0.5 µl of red blood cells. This increases the pellet bulk and permits keeping up with the specimen; while remaining in the sample, these red cells can be simply overlooked during the ultrastructural examination.

When the points of interest in tissue are small and focal, embedding multiple blocks of tissue is one way of hoping to locate them; however, this can be time-consuming and frustrating. Alternative methods, include use of a confocal microscope to select pertinent areas of pathology that can be cut out of wet tissue and embedded, or if the specimen has already been processed into paraffin and sections cut onto slides, the 6-µm section can be embedded in situ and glued onto a blank epoxy block for ultrathin sectioning. The stained paraffin section serves as the "thick" section for selection of pathological change for examination by EM.

Minuscule specimens such as particulate cell organelles and viruses can be pelleted in a Beckman Airfuge with an EM-90 rotor directly onto filmed grids and negatively stained. Other methods of concentration include immunoaggregation, whereby antibodies are added to a virus suspension, which is centrifuged at low speed, and the pellet is placed onto a grid and negatively stained. In agar diffusion, the virus suspension is placed onto a 1% agar surface, and a grid is placed onto the virus drop; the fluid diffuses into the agar, concentrating the particles. Then the grid is removed and stained. In a variant of this procedure, called pseudoreplica technique, Formvar is spread over the agar after the fluid has diffused while leaving the viruses on the agar surface; after drying, the film is floated off, like making Formvar-coated grids. Particles are stuck in the film, and grids are placed on the film in the water and picked up for negative staining.

In these ways, specimens too small for handling by routine methods can be kept up with and processed for EM examination.

Invited Presentations
Auditorium

Gentle Imaging of Fragile Samples with Light Sheet Fluorescence Microscopy
Courtney Akitake
Zeiss Research Microscopy Solutions

Light Sheet Fluorescence Microscopy, an extremely powerful alternative to established fluorescence imaging techniques, especially for 3-D imaging within whole live organisms and large tissue explants. By selectively illuminating the observed optical section with a thin sheet of light, photo bleaching is reduced to a minimum, making light sheet microscopy ideal for nondestructive imaging of fragile samples over extended periods of time.

Hyrax under glass: Microscopy tools to solve some exotic cases
Dalen Agnew
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When dealing with exotic species, be they pet, zoo, or free-ranging wildlife, accurate disease diagnosis depends on a range of skills and techniques. While it is important to extrapolate from domestic animal and human medicine, there are many unique anatomic and physiologic differences as well as a many unique pathogens and conditions that a diagnostician must keep in mind. Special stains, electron microscopy, immunohistochemistry, and in situ hybridization are among the techniques we may employ to arrive at a diagnosis; however, the most important tool we have is our colleagues with whom we can consult. Access to a variety of experiences and perspectives can be critical in identifying unique diseases in exotic beasts.

Volunteer Talks
Auditorium

Cellular Shape Change and Nucleoid Dynamics During *Myxococcus xanthus* Development

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The bacterium *Myxoccocus xanthus* provides an attractive experimental system to study multicellular development. When starved, cells send signals to each other, and alter their movements to construct multicellular mounds. Within mounds, rod-shaped cells differentiate into spores. Other cells undergo lysis or remain outside of mounds as peripheral rods. Using confocal laser scanning microscopy in combination with a fluorescent membrane stain, we determined when and where cells change shape within mounds. At 24 h post-starvation (PS), cells were rod-shaped throughout mounds. By 30 h PS, cells near the bottom of mounds were mostly rods, but progressively toward the top, more cells were transitioning. By 72 h PS, cells near the bottom were transitioning, and cells farther up were spherical spores. The results suggest that cells changed shape as they move upward in mounds. Association of a key signal-dependent transcription factor, FruA, with nucleoids was also examined. The nucleoid in growing cells localized primarily to the central portion of the cell, while the nucleoid of starving cells was localized along one side. In transitioning cells at 48 h, two condensed and segregated nucleoids were observed, and FruA was nucleoid-associated. At 72 and 96 h, round spores formed progressively, FruA exhibited less nucleoid association, and nucleoids decondensed. Efforts are underway to create a reporter of FruA activity in order to understand how signal-dependent activation of FruA impacts cell fate determination during multicellular development. The results are expected to provide new insight into how microbial communities use signaling to control complex behaviors.

Micro XRF: The Perfect Complement to SEM/EDS

Mark Kelsey
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Micro XRF uses X-rays to generate the same spectral information that is generated by the electron beam of an electron microscope (SEM). Like an SEM spectra from selected point(s) and area(s), line profiles and maps can be collected. However, micro XRF
offers the following advantages:

1. No sample preparation.
2. The ability to do liquids and samples with volatiles.
3. Larger samples.
4. Better limits of detection for elements above silicon.
5. No overvoltage required so the higher energy end of the spectrum is revealed.
6. No charging issues since electrons are not being used.
7. Screening to see if electron microscopy is necessary.

The information is easily understood by users of SEM/EDS because the spectra and types of information are the same.

Quantitative measurement of chondrocytes and collagen in articular cartilage by polarized light microscopy

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Introduction: Articular cartilage with its heterogeneous zones degrades in debilitating diseases such as osteoarthritis (OA). Polarized light microscopy (PLM) is capable of quantifying the depth-dependent orientations of the collagen fibers and the chondrocytes in healthy and OA cartilage (1).
Methods: Cartilage-bone specimens were harvested from 11 knee joints (5 healthy, 3 contralateral, 3 OA). 6-µm slices were obtained and imaged using PLM at 5x for collagen orientation (°) and retardation (nm) and at 20x/40x magnification to quantify the morphology of cells. ImageJ was used to collect data on the elliptical-shaped chondrocytes: the area, the major and minor axes, and the angle of orientation.
Results: Retardation and azimuth maps of cartilage showed the near- 90° collagen orientation changes from the articular surface to the subchondral bone. The measurements of cells showed their changes in orientation, as well as cell aspect ratio (long/short axis) and area. Using a fitting equation, the relative zone thickness was calculated for all tissue slices using the collagen and cell orientations. The cell area and aspect ratio were averaged across each zone showed the cell changes per zone, disease, and strain. Under external loading, the orientational changes of collagen and cell were also studied across different strains and disease stages, showing a limited linear relationship between strain and relative zone thickness. Conclusion: Quantitative measurement of collagen and chondrocyte at 2.0 and 0.5/0.25 µm pixel resolution respectively showed changes in cartilage from healthy to OA, and under unconfined external loading. Ref: (1) Xia et al, Osteoarthritis Cartilage 10, 370 (2002)

Copine A has a role in contractile vacuole function and postlysosome maturation

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Copines make up a family of cytosolic proteins that associate with membranes in a calcium-dependent manner and are found in many eukaryotic organisms. The presence of two C2 domains suggests copines may have a role in membrane trafficking. *Dictyostelium discoideum* has six copine genes (cpnA-F) and cells lacking cpnA have been shown to have defects in cytokinesis, chemotaxis, adhesion, and development. GFP-tagged CpnA was shown to associate with the plasma membrane, endosomes, lysosomes, phagosomes, and contractile vacuoles. Here, we use cpnA- cells to investigate the role of CpnA in contractile vacuole function and endocytosis. When placed in water, cpnA- cells made unusually large contractile vacuoles that took longer to expel. Visualization of contractile vacuoles with the marker protein GFP-dajumin indicated that the contractile vacuoles of cpnA- cells made fewer, larger, more persistent vacuoles that began refilling before complete emptying. In endocytosis assays, cpnA- cells took up small fluorescent beads by macropinocytosis at rates similar to parental cells. However, at the later timepoints, cpnA- cells had less fluorescence and the beads were found in smaller endolysosomal organelles. In a feed-chase experiment, cells were fed FITC- and TRITC-labeled dextran to distinguish neutral postlysosomes from acidic endosomes and lysosomes. Postlysosomes appeared sooner in cpnA- cells, did not become as large, and disappeared at a faster rate. p80 antibody staining of postlysosomes also indicated that cpnA- cells have smaller postlysosomes. These results suggest that CpnA is involved the regulation of contractile vacuole size and expulsion, and the maturation, size, and exocytosis of postlysosomes.

The aggregation propensities and morphological studies of the six isoforms of TAU

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Alzheimer's disease (AD) is a progressive irreversible neurodegenerative disorder that slowly destroys memory as well as thinking skills and eventually the ability to carry out the simplest tasks. The two proteins implicated in AD are amyloid beta (Aβ) and TAU protein. Recent AD studies are TAU related because AD and the primary motor disorders also characterized by dementia all show TAU deposition. When TAU protein is hyperphosphorylated, it detaches itself from the microtubules forming fibrils and filaments. These clusters are deposited into neuronal bodies causing synaptic failures and motor dysfunction. However, the mechanism via which TAU aggregates and deposits into neuronal membranes affecting cognitive function and motor activity is yet to be fully deciphered. However, a greater understanding of the aggregation properties and the structural changes that occurs in the TAU isoforms will lead to the development of effective therapies for AD treatment. In our study, human recombinant TAU 441, 412, 410, 383, 381, 352 isoforms were first expressed and purified from E. coli cells. The purity of the various TAU isoforms was assessed by SDS-PAGE and Western Blots. The aggregation properties of the six isoforms were monitored by fluorescence assay as a function of time. The morphological changes that occurred via clustering of TAU were studied by transmission electron microscopy. All six TAU isoforms showed increased aggregation in a time dependent manner when aggregation was induced with heparin, with

increased aggregation over time been prominent in TAU 412 (Fig 1a) and 410 (Fig 1b). Fibrils and filaments, the main characteristic seen in AD brains were observed in the all the TEM images of all six TAU isoforms. In conclusion, the aggregation of all the TAU isoforms may contribute to memory loss and motor function in AD pathways.

Direct Detection Camera Technology - Enabling the Resolution Revolution

Robert Monteverde
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Direct Detection Devices (DDD) are a class of advanced sensors used for transmission electron microscopes that directly detect incident electrons. By eliminating the scintillator and fiber-coupling layers, DDD sensors provide improved resolution, lower noise, and faster frame-rates. These benefits are a key enabler for the "resolution revolution" in TEM that led to the Nobel prize in Chemistry for Dubochet, Frank, and Henderson in 2017. This talk will review the motivations that led to the development of DDD sensors, describe their design, and explain their advantages. The key figures of merit for DDD sensors (MTF, DQE, ...) will be discussed. And the key applications and future directions for DDD sensors will be examined.

Posters

Lincoln Room

Biology 1

Wnt pathway regulation of gastrulation and segmentation in marine shrimp.

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In dendrobranchiate shrimp the 5th cleavage produces a 32-cell stage embryo having two mesendoblasts (MEs). Vegetal cytoplasmic determinants are hypothesized to specify ME fate, and signaling from ME cells may induce other gastrulation movements. Gastrulation movements start when ME cells ingress into the blastocoel. Subsequently, nine naupliar mesoderm (NM) cells, surrounding the ME cells, invaginate at the vegetal pole. NM cells surround the blastopore and divide along the axis of invagination. The molecular mechanism of ME specification or ME signaling is unknown. One candidate is the Wnt pathway, which controls axis formation, convergent-extension movements, and cell polarity in other systems. The Wnt pathway has three variants: the canonical Wnt pathway, the planar cell polarity (PCP) pathway and Wnt/Ca2+ pathway. In sea urchins, overstimulation of the canonical Wnt causes vegetablization while inhibition of the canonical Wnt causes animalization. Furthermore, the inhibition of the PCP pathway blocks polarized movement of cells. Here, we study the role of Wnt pathway in the dendrobranchiate shrimp, *Sicyonia ingentis* during early developmental stages through various drug treatments. Inhibition of PCP pathway has no effect on ME ingression and NM invagination. However, it blocks segmentation of the embryo. Interestingly, both inhibition and overstimulation of the canonical Wnt pathway perturbs proper NM invagination and segmentation. Our data suggest that the PCP pathway functions neither in ME ingression nor in NM invagination. Furthermore, an optimal level of canonical Wnt signaling appears to be necessary for segmentation.

Biology 2

Hepatic Iron Overload Disorder in Captive Rhinoceros

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All species of rhinoceros are critically endangered, and some even extinct in the wild, making captive breeding programs especially important to maintaining these species. Captive populations of rhinoceros also act as ambassadors for their wild counterparts, both educating and inspiring the public about important conservation issues. Iron overload disorder, a commonly reported condition in captive browsing rhinoceros, has the potential for causing severe morbidity and even mortality. The potential for iron overload disorder to cause disease on its own is currently under debate, though it is likely that it predisposes or exacerbates other conditions in rhinos. Currently the most common antemortem diagnostic test that is performed to diagnose iron overload is to look at ferritin levels in serum, but recent studies have shown that this may not be a reliable diagnostic tool. This deficit is a challenge to caretakers because it is often difficult to determine whether an animal should be treated or not. The interaction of other dietary minerals such as copper and nickel are also an unexplored area of interest. In the hopes of developing a better understanding of the pathogenesis of iron overload disorder, we are performing post-mortem analyses on the liver tissue of multiple species of rhinoceros; including histopathology, special stains for iron, nickel, and copper, and correlating those findings with species, age, sex, mineral analysis, and antemortem serum ferritin levels. With this knowledge we can help improve long term health in captively managed populations and the wild populations of rhinoceroses.

Biology 3

Quantitative μMRI and PLM Study of Rabbit Cartilage at Microscopic Resolutions

Syeda Batool and Yang Xia
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Osteoarthritis is a degenerative joint disease characterized by the gradual degradation of articular cartilage, a thin protective layer at the ends of long bones. Pain, stiffness and impaired movement are common symptoms. This study aimed on the quantification of T2, T1ρ and T1 relaxation times in humeral and femoral cartilage of rabbit using μMRI, and complementing the results with Polarized Light Microscopy (PLM) at highest possible resolution. We plan in the future to study cartilage from the rabbit model of osteoarthritis. Multiple (1.8×2×2.5 mm³) cartilage-bone specimens were harvested from humeral and femoral heads and imaged in μMRI at 9.75 μm resolution. After μMRI, 6.0 μm thick sections were obtained from each sample to generate quantitative PLM images at 1 μm resolution.

Quantitative μMRI relaxation data and PLM fibril structural data showed distinct features in tissue properties at different depths of cartilage, different in individual histological zones. The thicknesses of the histological zones in μMRI and PLM were successfully obtained. This is the first correlated and quantitative MRI and PLM study of rabbit cartilage at sub-10 μm resolutions. The establishment of the characteristic features of rabbit cartilage based on multidisciplinary imaging techniques would provide a solid foundation for future utilization of the rabbit model in the OA investigation.

Biology 4

cpnC Knockout Cells have Defects in Development and Cytokinesis

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Copines are conserved membrane-binding proteins thought to be involved in calcium-dependent signaling and membrane trafficking. Copines are found in most eukaryotic organisms. The Damer lab uses *Dictyostelium discoideum* as a model organism to explore copine activity, which has six copine homologues (cpnA-cpnF). If copine homologues have similar functions, then knockouts for each cpn gene should produce similar defects. A cpnA knockout cell line (cpnA-) was previously shown to have aberrant development, slight cytokinesis defects, and large contractile vacuoles with inefficient exocytosis. Here we describe the generation of a cpnC- cell line and subsequent analysis of their development, cytokinesis, and contractile vacuole function. To explore development, we examined cpnC- cells using a dissecting microscope for 24-hours. Under starvation conditions, wild-type *Dictyostelium* cells signal each other to induce a multicellular development cycle in which individual cells aggregate and form fruiting bodies. The cpnC- cells displayed abnormal morphology throughout development and delayed developmental timing. These abnormal phenotypes differed from those found in the cpnA- cells. DAPI staining and fluorescence microscopy of dividing cells were used to investigate cytokinesis; cpnC- cells were found to have an increased number of nuclei suggesting a slight cytokinesis defect similar to cpnA- cells. Differential interference contrast (DIC) microscopy was utilized to examine contractile vacuole formation and expulsion; no key differences were found between cpnC- and parental cells. The highly conserved nature of copine proteins suggest they play an indispensable role in eukaryotes, but the differences in defects revealed here suggest Copine C and Copine A have unique functions.

Biology 5

Maize Endosperm Conducting Tissue: the Basal Intermediate Zone and Conducting Zone

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Maize endosperm transfer and conducting tissues include the basal endosperm transfer layer (BETL) and the poorly studied basal intermediate zone (BIZ) and conducting zone (CZ). To substantiate these poorly studied tissues as unique or transitionary and elucidate their differences relative to each other and the BETL, we have analyzed them through a developmental time course. BIZ cells are slightly elongate and develop wall-in-growths (WIGs), whereas CZ cells are very elongate and lack WIGs. Both BIZ and CZ cell walls though, contain plasmodesmata connecting adjacent cells. Early in development, BIZ cytoplasm houses many organelles including mitochondria, membrane-containing plastids, lipids, ribosomes, and largely empty vacuoles with minimal granular contents that are easily identifiable. CZ cells possess many of the same organelles as BIZ but in far less abundance, with most of the cytoplasm appearing empty as an electron-light granular matrix, making CZ vacuoles and the cytoplasm difficult to distinguish. Unique to CZ cells are starch-containing plastids, vacuoles containing electron dense protein deposits of variable sizes, and large prominent nuclei, the latter of which may indicate endoreduplication. BETL, BIZ and CZ nuclei were found to endoreduplicate. CZ nuclei eventually reached a copy level peak (96°C) two rounds greater than that of BIZ or BETL (24°C). Our results indicate that the BIZ and CZ are different from each other, with the BIZ appearing more similar to the BETL. CZ cells however, are very distinct from BETL cells and have characteristics that align with a conducting function.

Biology 6

Site-specific Quantitative Polarized Light Microscopic (PLM) study of Young Rabbit Femur Cartilage

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Aim: The present study was designed to reveal site specific and depth dependent structural and organizational changes in the collagen network in young rabbit cartilage using Polarized light microscopy (PLM). Introduction: Articular cartilage (AC) is a thin complex load-bearing tissue mainly contains water, proteoglycans (PGs), and surrounding collagen network. The architecture of its collagen fibril network has a unique depth-dependent organization, which in mature cartilage has an arcade-like structure but it is absent in juvenile or inborn cartilage. Material and methods: 3 sample sites, anterior, central and posterior were chosen on femoral medial condyle of 12-14 weeks old White New Zealand male rabbits. Multiple 6.0 Âµm histological sections were obtained from these sample sites and imaged at 1 Âµm/pixel. In addition, thin histological sections that spanned the entire central femoral medial condyles were imaged to study the heterogeneity of the collagen fibril organization across the tissue. Results: We found significant topographical variations in both cartilage thickness and its collagen organization across the joint surface at different anatomical sites. For example, the central load bearing and posterior sites have developed the arcade like structure but the anterior sites are not the collagen fibers there run predominantly parallel to articular surface with increased cellular density and have the lowest retardation values. Conclusion: Since the collagen network organization plays a significant role in managing the mechanical properties of tissue, the findings in the site-specific differences in collagen network organization at early age can be useful to understand site specific mechanical properties and mechanobiology of tissue.

Biology 7

Electron Transmission Microscopy Analysis of Gelsolin peptide aggregation

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Electron Transmission Microscopy (TEM) analysis of amyloid peptides is a powerful tool used to study the morphology of fibrils formed as a result of peptide aggregation. Negatively stained peptide samples can be visualized at high resolutions in a dried state, allowing us to study and explore fibrillar networks unique to different amyloidogenic diseases. Formation of higher-order β-sheets structures by protein or peptide is a cornerstone of amyloidogenic diseases, such as Alzheimer's disease, and Parkinson's diseases, among others. Amyloidogenesis of the heart is a disease associated with misfolding and aggregation of proteins and peptides of the heart. Recently, hundreds of proteins have been identified in cardiac aggregates, and among these are transthyretin (TTR), gelsolin, and lactadherin. Gelsolin protein is localized intracellularly and extracellularly throughout the body. A 70-kb gene located on chromosome 9 codes for GSN, an 81-83 kDa protein with six homologous subunits. The six domains of GSN contain calcium-binding sites, which plays a regulatory role in cytosolic actin filament organization. In this study, an JEOL 100CXII transmission electron microscope was used to study the fibrillar morphologies of five Gelsolin peptides (GRRVV (1), RLFQVKG (2), NNGDCFILDL (3), CFILDL (4), and DCFILDL (5)) and their mutants, negatively stained with 5% uranyl acetate, following their aggregation in solution. Gel 3-5 peptides exhibited aggregation in solution at 10 mM concertation, with an average width of 20.2 nm ± 2.8 nm,13.2 nm ± 2.6 nm, and 34.4 nm ± 5.8 nm, respectively. Gel 3-5 peptides formed fibrils of varying characteristics, where Gel 3 showed a network of interconnected fibrils, whereas Gel 4 and 5 formed isolated and thinner filaments. Gel 1 and 2 peptides didn't inhibit aggregation and therefore no fibrils were apparent on their respective TEM images. TEM analysis was also utilized to investigate the effect of small molecule inhibitors (MB and EGCG) on the aggregation propensity of Gel 3-5 peptides. 50 µM of both inhibitors resulted in a significant reduction in the number of fibrils was noticed, demonstrating the ability of MB and EGCG in disaggregating CFILDL-containing Gelsolin peptides. Our study elucidated the benefits of employing TEM analysis in investigating fibrillization propensity of Gelsolin peptides, which can be used to enhance our understanding of the pathogenesis of Gelsolin amyloidosis diseases.

Physical Sciences 1

Influence of Composition and Structure on Measured H Concentration in beta-Ti Alloys via Atom Probe Tomography

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Ingress of hydrogen (H) in titanium (Ti) causes hydride formation and embrittlement but is difficult to measure using electron microscopy. Atom probe tomography (APT) can measure H in Ti with sub-nanometer spatial resolution, but extraneous H can be introduced into Ti samples during focused ion beam (FIB) based APT sample preparation.

H was investigated in the beta-Ti alloys Ti-11Cr(at%) (TC) and Ti-11Cr-0.85Fe-5.3Al(at%) (TCFA) in beta-homogenized and heat-treated (400Â°C 12hr) conditions. Transmission electron microscopy determined that the heat-treated TC alloy contained the beta, alpha, and omega phases, and the heat-treated TCFA alloy contained the beta and alpha phases.

The effect of accelerating voltage (5kV or 30kV) during the deposition of a protective Pt cap before FIB-based APT sample preparation was investigated. H concentrations in TCFA samples prepared using an accelerating voltage of 5kV were significantly lower than TCFA samples prepared using the 30kV accelerating voltage. H-enriched regions were observed in the heat-treated TCFA samples prepared using 30kV. These regions are hypothesized to be metastable hydride phases based on Ti, H, Cr, and Al concentrations. Changing the accelerating voltage during Pt deposition had no effect on the H concentrations in the TC alloy samples. The heat-treated TC alloy samples formed the stable hydride phase TiH at both accelerating voltages. In both alloys, the samples without hydrides had higher H concentrations in the beta phase than the alpha or omega phases.

Physical Sciences 2

Investigating Tantalum Nanoparticles for X-ray CT and Therapeutic Use

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Advancements in nanoparticle (NP) design can transform Computed Tomography (CT) into a robust molecular imaging platform. The drawback of CT is the low sensitivity of CT contrast agents; currently 10's

mM is required for detection. As such, pre-amplified radiopaque contrast agents are necessary to realize the molecular imaging potential of CT. Nanocrystals (NCs) are such a pre-amplified system, with 1000's of radiopaque atoms per NC. Our idea was to develop flexible and biocompatible, pre-amplified NCs and NPs for CT. Two different NP platforms were fabricated using Tantalum Oxide (TaOx; k-edge 67.4 keV) NCs as the X-ray dense component. TaOx NCs with high Ta content (78%) were synthesized by a base-catalyzed sol-gel method. Encapsulation of bare NCs within mesoporous silica and PLGA was carried out to afford TaOx@MSNPs and TaOx@PLGA NPs, respectively. Both NP types were characterized for size, structure and composition using DLS, TEM, SEM, EDS and ICP-OES, and biocompatibility in cell culture. The TaOx NCs/NPs were also evaluated in vivo. Following IV injection in BALB/c mice, TaOx NCs circulated in blood for ~3 hours, accumulating eventually in RES organs. Bolus IM injections of TaOx@PLGA NPs and TaOx@MSNPs produced significant contrast enhancement in BALB/c mice. We also report an intrinsic mesoporous structure for TaOx NCs, similar to the intricate pore structure in MSNPs. Both TaOx NCs and TaOx@MSNPs displayed higher Doxorubicin Hydrochloride (DOX) loading as compared to empty MSNPs with a pH dependent DOX release over 72 h. This work shows considerable promise for TaOx NCs/NPs in theranostics.

Physical Sciences 3

Characterization of Multiple-layered Electrical Contacts Enabled by Surface Ion Polishing

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Multiple layer structured electrical connectors have drawn great attention for advanced electrical and electronic systems, especially in recent years, electric vehicles. Comparing to the single layer options, the advantages of multiple metal layers in electrical connectors include improved mechanical and electrical contact properties, and potential enhanced reliability of the contacts in long-term use. Normally a connector pin consists of a copper substrate on which multiple thin metal layers with different functions are electrically plated. Each plated metal layer only measures fraction to a few micrometers in thickness. It's challenging to characterize individual layer of the metal after it stacks with other layers, especially when soft layers such as tin are involved. Focused ion beam (FIB) can produce desired samples with localized details. Yet due to the high energy level, localized temperature elevation may pose a risk for preservation of metallurgical details. Gatan's Ilion has been proved as a versatile tool in preparation of such metal composite for microscopic analyses. It features flexible beam alignment and tunable beam energy level ranging from a few hundreds of eV to 8 KeV. With the particularly designed mask blades and alignment mechanism, both planar and cross-sectional surfaces can be well polished to enable grain-level visualization and diffraction patterns. This study presents our preliminary results on tin involved multi-layer metal contacts enabled by the Ilion II by Gatan.

Physical Sciences 4

Morphology-Controlled Synthesis of Colloidal Superparticles from Ti-Containing Perovskite Nanocubes for Thin Film Technology and Energy Storage Application

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In this communication, we report on the controlled synthesis of pristine and rare-earth doped titanium-containing perovskite nanocrystals. The resulting aggregate-free nanocubes have been formulated into simple superlattice structures such as spheres and cubes. By using transmission electron microscope (TEM), the morphological features of the as-synthesized nanocrystals and their superlattice structures have been determined. Also, by adopting the Williamson-Hall model, the crystallite size and structural strain existing in these nanocrystals have been determined. It was determined that, the theoretical nanocube crystallite size calculated by using the Williamson-Hall model is similar to the nanocrystal size obtained from the TEM. In addition, by using advance microscopy technique such as scanning probe microscopy, the ferroelectric and piezoelectric properties of these structures have been explored.

Physical Sciences 5

Solvothermal Synthesis and Characterization of Pristine Barium Titanate (BaTio3) and Chromium-Doped Barium Titanate (BaTi1-xCrxO3) Colloidal Nanocrystals

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Barium titanate, BaTiO3, nanoparticles are widely known due to their excellent dielectric, ferroelectric, and piezoelectric properties. The demand for BaTiO3 nanocrystals is augmenting sharply due to their advanced applications in microelectronics industries. The doping of BaTiO3 (BTO) nanoparticles with Cr3+ exhibits a combination of magnetic and electric orderings, known as multiferroics, have significant potential for high- density memory storage and the design of complex multistate memory elements. A solvothermal method was developed to synthesize well-isolated, nanocrystalline BaTiO3 and Cr-doped BaTiO3 with a narrow size distribution. The synthesized nanoparticles were characterized by using powder X-ray diffraction (XRD), transmission electron microscopy (TEM) and Raman spectroscopy. In addition, the properties of the nanoparticles were determined and explained according to the concentration of the dopant. Furthermore, the synthesized nanocrystals were utilized to study the measurements of the electric polarization and the coupling between the electric dipole and magnetic moments.

Physical Sciences 6

Microstructural evolution with varying solutionizing temperature in Allvac 718plus

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Alloy Allvac 718Plus is relatively new superalloy developed to improve the properties of the widely-used superalloy Inconel 718. It shows improvement in service temperature up to 704ºC (55ºC more than IN718) because of its chemical composition, microstructure and major strengthening phase, γ'. The high temperature stability of the microstructure influences the life and mechanical properties of these alloys. Therefore, it is necessary to understand the phase evolution as a function of temperature. In the present work, the solutionizing temperature has been varied during heat treatment which led to varied grain size and phase volume fraction. The as-received samples were solution annealed at 1100ºC, 1050ºC, 1000ºC, and 954ºC followed by aging at 788ºC for 8 hours then furnace cooled to 704ºC for 8 hrs. (two-step ageing). Metallographic samples of the as received and heat-treated samples were prepared to identify different phases using optical microscopy and scanning electron microscopy (SEM). Electron Backscatter Diffraction and Energy dispersive spectroscopy was performed to investigate the composition of the various phases. All the data obtained were rationalized and compared with the existing data. This data will help in investigating the dependency of time and temperature on the microstructural changes.

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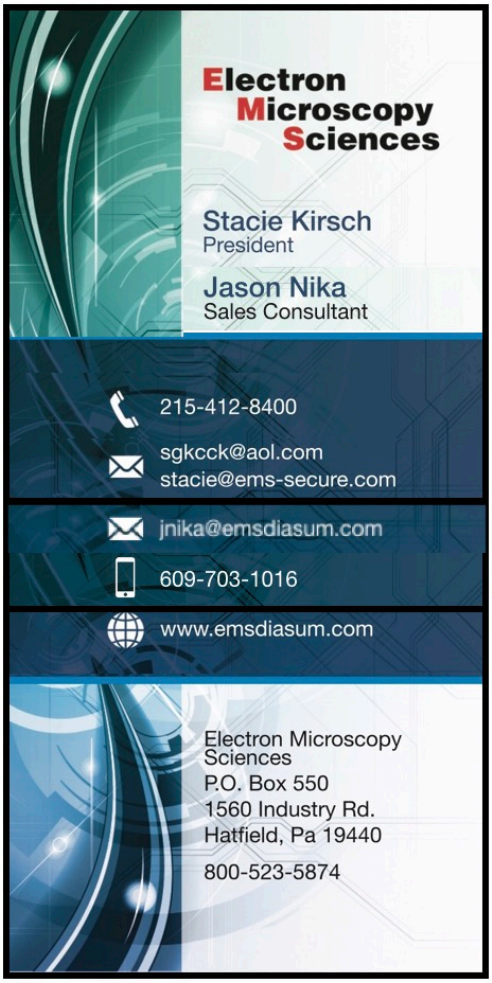

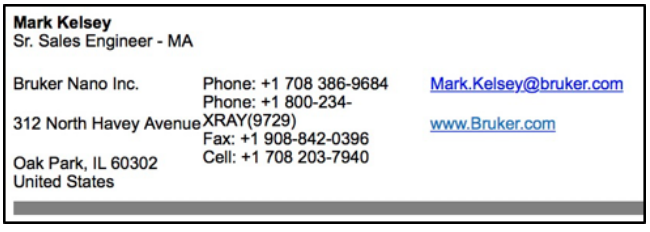

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