[AIMS] Arizona Imaging and Microanalysis Society

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

Program, Abstracts & Biographical Information

March 12th, 2010

Ventana Ballroom
Memorial Union
Arizona State University

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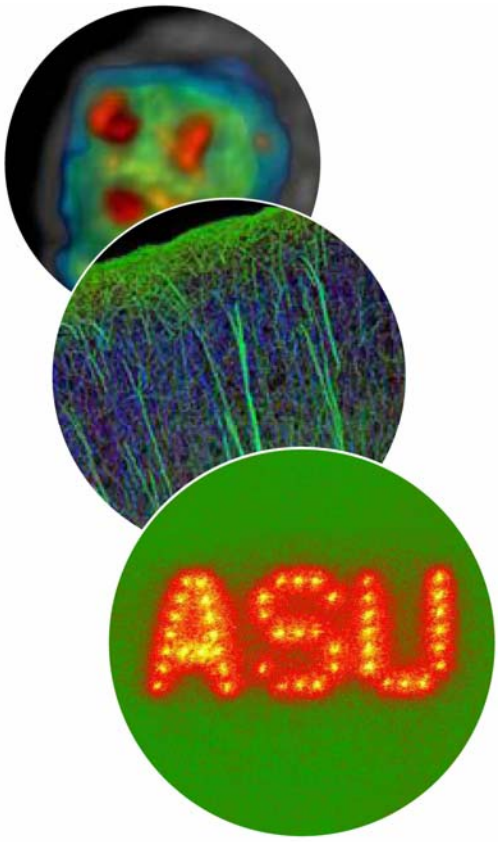


AIMS - Program - 2010

Check-In
| 7:30 - 8:15 |

Morning Session (subject to change)
Opening remarks
Page Baluch - AIMS President
| 8:15 - 8:30 |

Observations of the Synthesis and Evolution of Active Nanostructures
Peter Crozier , Associate Professor, Ira A. Fulton Schools of Engineering, ASU
| 8:30 - 9:15 |

Using Hubble Space Telescope Object Finding and Classification Software to Find Potential Markers for Diabetes Type 2 in an Early Stage
Rogier Windhorst, Regents' and FoundationProfessor, School of Earth & Space Exploration, ASU
| 9:15 - 10:00 |

Morning Break – Vendor Demonstrations
Coffee, Tea and Cheese Platter
| 10:00 - 10:45 |

Plasticity in Transient Dynamics of Early Olfactory Processing
Brian Smith, Professor, School of Life Sciences/Neuroscience, ASU
| 10:45 - 11:30 |

Buffet Lunch – MU241C
| 11:30 - 1:00 |

Afternoon Session (subject to change)

Single-cell Optical Tomography to Reveal Cell Structure-Function Relationships
Laimonas Kelbauskas, Assistant Research Professor, Biodesign Center for Ecogenomics, ASU
| 1:00 - 1:45 |

Array Tomography: Imaging the Molecular Architecture and Ultrastructure of Neural Circuits
Stephen Smith, Professor, Department of Molecular and Cellular Physiology, Stanford University School of Medicine
| 2:00 - 3:00 |

Student Poster Oral Presentations
| 3:00 - 3:30 |

Afternoon Break/Vendor Exhibits/Poster Session
| 3:30 - 4:15 |

Student Awards and Closing remarks
| 4:15 - 5:00 |

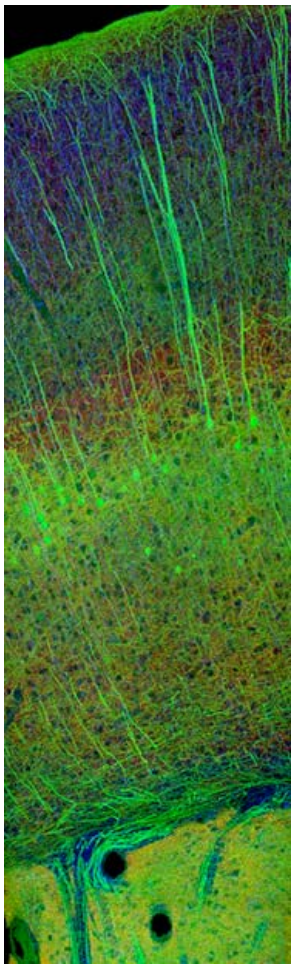
Business Meeting
Annual Society general meeting – open to the public
| 5:00 - 5:45 |

No Host Dinner - Meet at Casa Reynoso (3138 S Mill Ave)
| 6:30 - |

Speaker Abstracts & Biographies

Array Tomography: Imaging the Molecular Architecture and Ultrastructure of Neural Circuits

Dr. Stephen Smith [Keynote Speaker]
Stanford University School of Medicine, Department of Molecular and Cellular Physiology

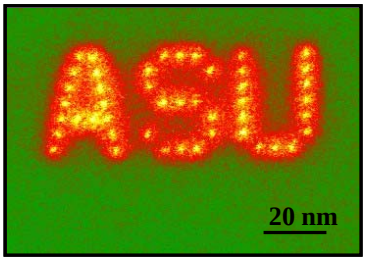


The Smith laboratory invents new light and electron microscopy methods to probe brain circuit structure, development, and function. Using these tools, the laboratory has discovered several previously unknown brain signaling pathways, including the NMDA-Ca signal (now widely recognized as fundamental to most synaptic plasticity), and the astrocytic calcium signal (now recognized as linking synaptic activity to vascular response and the NMR BOLD signal). The lab also was first to describe the filopodial dynamics stage of synaptogenesis. Most recently, the lab has invented a new ultra-high-resolution microscopy method called "array tomography". This method is proving a breakthrough in quantitative power for the study of brain synapse populations in health and in neurodevelopmental and neurodegenerative disorders. Array tomography is also giving us our first glimpse of the really stunning **beauty of the brain's vastly intricate cellular and molecular** architectures.

Stephen Smith earned his PhD at the University of Washington in 1977, and did his postdoctoral training at the University of California, Berkeley. The Smith Lab focuses on structural neuroscience research at Stanford University, developing and applying new methods to explore brain circuit structure. Dr. Smith has published over 100 peer reviewed articles in the area of neuroscience dealing with synaptic vesicle trafficking, neuronal development and growth dynamics as well as pre and post synaptic signaling pathways. Dr. Smith teaches neuroscience and microscopy at Stanford as well as courses focused on imaging neural structure and function at Woods Hole and Cold Spring Harbor and numerous other international teaching venues. He has been at Stanford since 1989 and is currently a professor in the Department of Molecular and Cellular Physiology at the Stanford University School of Medicine.

Observations of the Synthesis and Evolution of Active Nanostructures

Dr. Peter Crozier
Arizona State University, Ira A. Fulton Schools of Engineering



Gas-solid reactions play a major role in the evolution of materials structure and composition. In many applications, materials function in the presence of reactive gases at elevated temperature and systems which are relatively inert at room temperature can become active and undergo significant phase changes under ambient conditions. Moreover, fundamental processes taking place during nanomaterials synthesis and processing may involve gas-solid reactions (e.g. oxidations, reductions, CVD etc...). These phase changes can be particularly pronounced in porous nanometerials such as heterogeneous catalysts where the high surface to volume ratio gives rise to large gas contact areas. Transmission electron microscopy (TEM) is a powerful technique for elucidating the structure and chemistry of materials at atomic resolution. However, conventional TEMs operate under high vacuum conditions preventing observation of the structures and dynamic processes that take place in the presence of a reactive gas atmosphere. To address this so-called “pressure gap”, *in situ* environmental transmission electron microscopes (ETEM) have been developed which permit reasonably high gas pressures around the sample area while maintaining high vacuum conditions throughout the rest of the TEM column.

This presentation will review current approaches to performing electron microscopy under gaseous environments. Examples will be presented illustrating the phase changes that take place under different gas environments on metal and oxide nanocatalysts relevant to sustainable energy applications related to hydrogen production, solid oxide fuel cells and photocatalysis. There is wide recognition of the importance of understanding the detailed role of the nanoscale decomposition, diffusion, nucleation and growth processes taking place during catalyst synthesis and operation. Nanoscale identification of the phase with the highest catalytic active is a major goal of ETEM. We are also using the combination of a subnanometer focused electron beam in combination with reactive gases to explore novel approaches to synthesis and processing of nanostructures. Electron beam induced deposition (EBID), electron beam induced transformations (EBIT) and gas enhanced etching can be used to synthesize nanostructures of arbitrary shape at well-defined positions as shown below.

Peter A. Crozier obtained a PhD in Physics from the University of Glasgow in 1985 in the area of high spatial resolution analytical electron microscopy. He has extensive experience in developing and applying advanced transmission electron microscopy techniques to nanomaterials including, semiconductors, catalytic materials and atmospheric aerosols. He is a member of the Microscopy Society of America, the Materials Research Society and the American Institute of Chemical Engineer. He is a recognized expert in quantitative imaging and analysis of materials by electron microscopy and has published over 100 archival journal and book articles on these topics. His honors and awards include the Cosslett Award from the Microbeam Analysis Society (1998), Best Poster Award - EMAG (2001) and organizer of numerous workshops, schools and symposia on electron microscopy. He is currently an Associate Professor in the School of Mechanical, Aerospace, Chemical and Materials Engineering at Arizona State University.

Using Hubble Space Telescope Object Finding and Classification Software to Find Potential Markers for Diabetes Type 2 in an Early Stage

Dr. Rogier Windhorst
Arizona State University, School of Earth & Space Exploration

First, I will summarize how the Hubble Space Telescope (HST) project has revolutionized astronomy since the 1990's, and how it has measured the process of galaxy assembly over cosmic time. Next, I will show the sequel to Hubble --- the James Webb Space Telescope --- will continue to revolutionize astronomy after its launch in 2014 through its search for Cosmic Dawn and the epoch of First Light and Reionization. A fundamental issue in these NASA projects is that faint and distant galaxies are so densely packed on the sky, that finding and quantifying individual galaxies has mathematically become a very difficult problem --- one literally can no longer see the forest through the trees!

Astronomers have therefore by necessity become experts in finding faint objects in very crowded dense fields, and so I briefly review the Hubble galaxy detection, deblending, and classification methods.

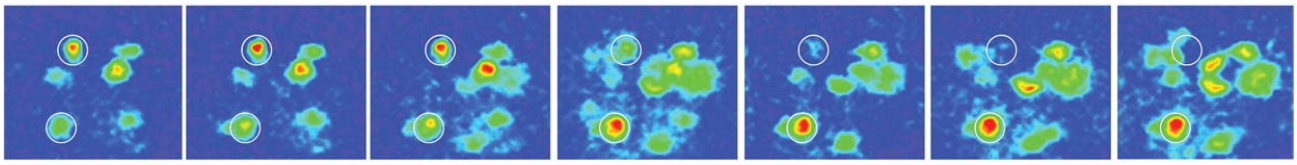
Next, I will illustrate how NASA Hubble's galaxy classification software can be used to help find potential markers for Diabetes Type 2 in an early stage.
Laser Scanning Confocal Microscopy was done of punch skin-biopsies from normal and obese patients. The Hubble object detection and classification software was used for semi-automated automated detection and quantification of cutaneous Small Sensory Nerve Fibers (SSNFs or C-fibers) in these biopsy's dermal and epidermal layers, and in the intervening basement membrane. Comparison between the semi-automated method and visual classification techniques on identical images demonstrates that the semi-automated method provides a quick and reliable method to determine the density of SSNFs, help eliminate systematic and personal biases, while reducing the time, effort and cost of manual counting of medical features of interest.

I will also show possible application of the Hubble object finding and classification software to study the spreading of cancer cells in confocal images of cancer tissues.

Rogier Windhorst earned his PhD in Astronomy and Physics at the University of Leiden in the Netherlands in 1984. Dr. Windhorst has worked with NASA since 1986 in the development of the Hubble Space Telescope project. He collaborates with over 150 astronomers from 10 different countries, has published numerous papers associated more than 70 different projects funded by NASA and the NSF. Recently he began a collaborative study to apply object finding and classification algorithms to create an automated detection system that identifies small sensor nerve fibers in tissue biopsies as a diagnostic tool for diabetes. Dr. Windhorst teaches astronomy at ASU and is currently a Regents' and Foundation Professor in the School of Earth & Space Exploration at Arizona State University.

Plasticity in the Transient Dynamics of Early Olfactory Processing

Dr. Brian Smith
Arizona State University, School of Life Sciences

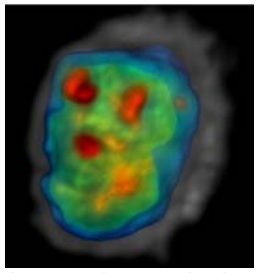


Early olfactory processing is fundamentally similar in animals as different as mammals and insects. Because of this similarity there have been very significant advances in understanding of sensory encoding by primary sensory cells the olfactory epitheliums of these animals. Yet a similar understanding of how sensory information is transformed at the first synapses in the brain remains elusive. The primary objectives of this research are to understand how early processing in the brain transforms sensory input, and how this transformation is modified by associative and nonassociative plasticity. The honey bee Antennal Lobe (AL) is the analog of the mammalian Olfactory Bulb (OB). The networks in the AL and OB both transform sensory inputs into spatiotemporal patterns that encode odors by a sequence of activity states, or transients, and information about the identity of the odor is in the specific sequence of states in the transient. Moreover, these networks are sensitive to modulation by feedback from other areas in the brain that represent reinforcement (e.g. food). When odors are associated, or explicitly not associated, with food these modulatory systems change the way that odors are represented by the AL and OB networks. We have shown in the honey bee, using bioimaging and electrophysiological data, that the paths that the transients take are pushed farther apart by association of odors with food reward in Pavlovian conditioning paradigms. Furthermore, nonassociative plasticity induced via repetitive unreinforced exposure to odors modifies competitive interactions between neural representations. We have also shown that disruption of this modulation by RNA interference disrupts behavioral conditioning of odors. We are integrating this information into computational models of the antennal lobes. Furthermore, this information about modulation can have far reaching impacts for understanding disease states that affect early sensory processing.

Brian Smith received his PhD in Biology at the University of Kansas in 1985. He went on to do postdoctoral studies in many labs such as the Institute for Neurobiology at the Free University of Berlin, the University California Berkeley and the University of Arizona specializing in the study of the olfactory system in honey bees. The Smith lab studies learning and memory under controlled laboratory conditions in order to investigate how neural and molecular mechanisms encode information in the peripheral and central nervous system. Dr. Smith is currently a professor in the School of Life Sciences at Arizona State University and serves as director of the interdisciplinary graduate program in neuroscience between the School of Life Sciences and Barrows Neurological Institute and faculty leader of the Organismal, Integrative, & Systems Biology research interest group.

Single-Cell Optical Tomography to Reveal Cell Structure-Function Relationships

Dr. Laimonas Kelbauskas
Arizona State University, Biodesign Center for Ecogenomics



At the Center of Ecogenomics we have implemented a novel optical tomography method for single cells (cell CT). It is based on absorption imaging of fixed cells stained with hematoxylin, Oil Red O or other absorption dyes. The method features isotropic spatial resolution of ~375 nm in all three dimensions, reliable data collection and reconstruction algorithms and moderate throughput. It permits unique insights into three-dimensional cellular and nuclear morphology and enables quantitative studies of cell architecture-function relationships. Utilizing hematoxylin staining, which specifically stains chromatin and the nuclear envelope, we have conducted a comparative study on nuclear morphology using two different cell lines representing normal and dysplastic stages of Barrett's esophagus, a disease which may culminate in esophageal cancer. We developed algorithms for extraction of 3D nuclear morphometric features for direct quantitative comparisons between the two cell lines. We find that, among the studied morphometric features, the normal esophageal cell line EPC-2 shows lower nuclear-to-cytoplasmic volume ratios, fewer nucleoli, and smoother nuclear surface texture as compared to the dysplastic CP-D cell line.

To complement and extend the microstructural absorption imaging mode to functional imaging, we are implementing fluorescence CT for fixed and live cells. To this end we have developed a custom, biocompatible gel that can support live cells for prolonged periods of time. We have modified the cell CT instrument to accommodate specific excitation and emission detection optical paths for transmission and epi fluorescence illumination modes. Cell CT imaging of live cells will facilitate studies of gene transcription and protein expression related to the nuclear structure in the context of disease progression. Fluorescence functional cell CT will also permit assessment of protein expression levels localized to the subcellular microdomains in which the proteins function.

Laimis Kelbauskas received his Ph.D. in experimental physics/ biophysics from the Friedrich-Schiller-University in Jena, Germany in 2003. He did his post doctoral training in Germany and at the Center for BioOptical Nanotechnology, the Biodesign Insitute at Arizona State University. His studies focus on intracellular protein-protein and protein-DNA interactions in bacterial photosynthetic reaction centers and tumor cell lines using advanced imaging methods such as multi-dimensional fluorescence lifetime measurements, time-resolved emission detection, and optical computed tomography. His interest is in cell phenotype development and architecture-function relationships in a context of cell differentiation and neoplastic progression. He is currently an Assistant Research Professor in the Center for Ecogenomics, in the Biodesign Institute at Arizona State University.

Student Abstracts

Elucidation of Metallic Conductivity in ZnO/Cu/ZnO multilayers with the Use of X-ray Photoelectron Spectroscopy and Transmission Electron Microscopy Analyses

K. Sivaramakrishnan, N.D. Theodore, and T. L. Alford
School of Materials and Flexible Display Center at ASU, Arizona State University, Tempe, Arizona 85287

Transparent conducting ZnO/Cu/ZnO films on flexible PEN substrates have been obtained with the highest conductivity of dielectric-metal-dielectric films reported in literature. The carrier concentration of the films was 1.2×10^{22} cm^{-3} and resistivity was 6.9×10^{-5} Ω-cm at the optimum copper layer thickness. The peak transmittance, photopic averaged transmittance, and Haacke figure of merit are 88%, 75%, and 8.7×10^{-3} Ω^{-1}, respectively. This study uses transmission electron microscopy (TEM) and X-ray Photoelectron Spectroscopy in concert to elucidate the conduction mechanism in these stacks. TEM results reveal the formation of discontinuous island films for thin thicknesses and these layers turned continuous with increasing metal thickness. X-ray photoelectron spectroscopy data confirms that the Cu is in an unoxidised state. These results corroborate a conduction mechanism which involves carrier injection from the metal to the oxide at low copper layer thickness and conduction through the contiguous metal pathway at higher thicknesses. Reflection and absorption from the continuous metal layers and scattering losses from the discontinuous islands are used to explain the optical transmission behavior of the films.

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In-situ Environmental TEM studies in understanding the catalytic activity of supported Ni nanoparticles for Partial Oxidation of Methane

Santhosh Chenna,* and PeterA. Crozier*
* School of Mechanical, Aerospace, Chemical and Materials Engineering, Arizona State University, Tempe, AZ, USA 85287-6106

Heterogeneous catalysis is a dynamic process of gas-solid interactions that takes place on the surface of the catalyst. The challenge in catalysis lies in recognizing the active sites of interest which may only exist in a unique environment inside the reactor. A fresh catalysts may undergo significant changes during a reaction (such as transformation, shape changes, etc.) which may affect their catalytic properties. *In-situ* environmental electron microscopy (ETEM) can allow us to probe the dynamic changes taking place in the catalyst when the system is exposed to reactive gases at relevant temperatures providing information on morphological and chemical changes which may not be available by ex-situ studies. In the present work, we employ Ni nanoparticles supported on well-defined amorphous silica spheres for partial oxidation of methane to syngas ($CO+H_2$). We performed *in-situ* ETEM studies to correlate the nanostructure changes in

different gaseous environments with the catalytic activation process obtained in parallel from the reactor experiments. Many ETEM experiments under different gas conditions have been performed to simulate the various gas-solid interactions that take place in
the *ex-situ* reactor. We will present the effect of nano-scale changes on the performance of Ni catalyst for partial oxidation methane reaction.

Direct Observation of Nucleation and Growth of III-Nitride Nanowires

Rosa E. Diaz*, R. Sharma*†, and S. Mahajan*
* School of Mechanical, Aerospace, Chemical and Materials Engineering, Arizona State University, Tempe, AZ85287
† National Institute of Standards and Technology, Gaithersburg, MD20899

III-nitride semiconductor nanowires have great potential as building blocks for nanoscale electronic and optoelectronic devices. Important features of these semiconductor nanowires are their wide band gaps and stability at high temperature. Also, due to their low dimensionality, nanowires often exhibit unique thermal, electrical, and optical properties. The synthesis of III-nitride nanowires has been reported using different growth methods such as chemical vapor deposition and metalorganic chemical vapor deposition. These methods usually use a metal catalyst to grow the nanowires, following the vapor-liquid-solid (VLS) mechanism as proposed by Wagner in 19701. Although this mechanism is widely accepted, there is a lack of understanding of the processes involved during nucleation and early stages of growth of III-nitride nanowires. Some questions, still to be answered concern the phase of the catalyst, the nature of the nucleation sites, and the evolution of the catalyst-nanowire interface. This work reports direct observations of the nucleation and early stages of growth of GaN nanowires. The nanowires were grown by the formation of Au + Ga droplets on Si films, and their subsequent nitridation with ammonia (NH3) at high temperature.

The Physics of Solid State Lighting and the Characterization of Semiconductors through Cathodoluminescence

Reid K. Juday* and Fernando A. Ponce*
***Department of Physics at Arizona State University, Tempe, AZ

Large-scale solid state lighting will be a key ingredient in reducing worldwide energy demands. Light emitting diodes (LEDs) are becoming increasingly prevalent in today's society due to their high efficiencies, long lifetimes, and ever-decreasing cost. My research is centered on GaN-based LEDs. We hope to continue to improve the efficiency and light output of these LEDs through the full spectrum of colors. This work is based on identifying the acceptor and defect levels in Mg-doped GaN and InGaN. Through scanning electron microscopy and cathodoluminescence we can gain valuable insight into the characteristics and behavior of these important materials

Sub-dendritic targeting of GABAergic and cholinergic synapses to a Drosophila motorneuron

Claudia Kuehn and Carsten Duch
School of Life Sciences, Arizona State University, Tempe AZ, 85287

For efficient synaptic transmission, postsynaptic neuronal receptors need to be localized in precise apposition to presynaptic terminals that release the neurotransmitter. Modelling and electrophysiological studies have shown that the location of input synapses in complex dendritic trees can affect the computational properties of the postsynaptic neuron. Here we investigate whether sub-dendritic targeting and clustering occurs in a transmitter specific manner in complex dendritic trees of the identified flight motor neuron MN5, located in a non-layered neuropil in *Drosophila melanogaster*. Nicotinic acetylcholine receptors containing α7 subunits mediate fast excitatory signaling at most synapses in the Drosophila escape circuit, including synapses to MN5. The most abundant fast inhibitory receptors in the insect CNS are ionotropic GABA receptors. We use immunocytochemistry and targeted expression of tagged receptors to map the expression of the RDL subunit of the GABAAR and the α7 subunit of the nicotinic AChR onto 3 dimensional geometric reconstructions of the MN5. We find a distinct spatial pattern for the distribution of GABAARs. Interestingly, the majority are clustered on identified dendritic sub-trees in the anterior distal region of the dendritic tree, near the spike initiating zone. Therefore, specific sub-dendritic recognition mechanisms must exist for the targeting and maintenance of GABAergic synapses. In contrast, α7 subunit containing AChRs are evenly distributed over the whole dendritic tree. Previous studies have predicted that during flight MN5 integrates tonic excitatory cholinergic drive into steady firing frequencies, and that specific sequences of motor neuron firing are ensured by sharp inhibitory feedback within the central pattern generating network. We now test in multi-compartment models whether experimentally derived sub-dendritic distribution rules for cholinergic and GABAergic synapses aid behaviorally relevant input-output computations of this motor neuron.
Support by the German Science Foundation to C. Duch (DFG DU 331/4-1) is gratefully acknowledged.

Photoreduction of CO_2 with H_2 on well-dispersed Copper loaded TiO_2 photocatalysts.

Cesar Leyva* and Peter A. Crozier*
* School of Mechanical, Aerospace, Chemical and Materials Engineering, Arizona State University, Tempe, AZ, USA 85287-6106

In this work, the photoreduction of CO_2 in the presence of hydrogen (H_2) on the surface of copper loaded (Cu) titanium dioxide (TiO_2) photo catalysts was studied under UV illumination. By combining transmission electron microscopy with photoreactor data, the relationship between catalyst activity and nanostructure was determined. To enhance the photoactivity of the semiconductor, Cu nano-particles were finely dispersed on the surface of TiO_2. The results from the synthesis of the catalysts showed that the dispersion of the copper particles is closely related to i) the surface tension and (ii) volume of the solvent used for the metal impregnation and iii) the solvent vapor pressure. From the photoreaction experiments, the

calculated yields of methane were in the order of 0.1 micro moles with quantum yields of about 0.005%. The Cu loading and oxidation state seems to have a large effect on the activity of the photocatalyst for methane production.

Spreading of liquid AuSi on vapor-liquid-solid grown Si nanowires

Prashanth Madras[1], Eric Dailey[1] and Jeff Drucker[1,2,]
1. School of Mechanical, Aerospace, Chemical and Materials Engineering, Arizona State University, Tempe, AZ, 85287-6106
2. Department of Physics, Arizona State University, Tempe, AZ, 85287-1504

The vapor-liquid-solid (VLS) growth technique is a convenient and flexible method for producing nanowires (NW) and nanowire heterostructures in a variety of elemental and compound semiconductors. VLS growth of high quality Si NWs relies on the stability of the liquid metal seed. Aside from issues of growth control and perfection, understanding and controlling Au decoration of the NW sidewalls is essential for some applications. Au is known to degrade the performance of Si devices, so eliminating Au on the NW sidewalls is required for nanoelectronic applications. On the other hand, some plasmonic or sensing applications; or the growth of branched NWs may benefit from Au-decorated NW sidewalls. High-angle annular dark field imaging conclusively helps identifying condition that favor Au free sidewalls in ultra high vacuum(UHV) grown Si NWs. In situ transmission electron microscopy shows that a liquid AuSi seed spreads along the sidewalls of Si nanowires for some growth conditions. This liquid thin film phase separates to form solid Au clusters as the nanowire is quenched below the solidus temperature. Further, energy dispersive x-ray spectroscopy of UHV grown NWs reveals dependence of side-wall Au coverage liquid thin AuSi film during growth) on growth pressure, NW diameter and the distance from the tip. The length that the liquid film spreads from the seed, and its thickness, can be explained by considering the spreading thermodynamics of droplets on cylinders.

Developmental plasticity in the tracheal system of *Zophobas morio* beetles as visualized by synchrotron x-ray phase contrast imaging

Elyse Muñoz*, Michael Weed*, Erica Heinrich, James Waters, Jon Harrison, and John VandenBrooks
School of Life Sciences, Arizona State University
* Presenting authors: Elyse Muñoz (elyse.munoz@asu.edu) and Michael Weed (mdweed@asu.edu)

Recent geological models indicate that oxygen has varied greatly over the last 500 million years, rising as high as 31% and dropping to as low as 12%. These findings are often correlated with observations of Paleozoic gigantism. It is widely hypothesized that elevations in oxygen partial pressure (hyperoxia) allowed increases in body size of vertebrates and insects. However, these models suffer from a high degree of error and there is currently no biological proxy for atmospheric oxygen levels in the past. Without being

able to establish the actual oxygen levels, the effect of oxygen on evolution can only be established as a correlation. Therefore, this study is aimed at developing the first-ever proxy for paleo-oxygen levels: namely the ratio of leg tracheal diameter to leg length in fossil insects. There are two components to developing this proxy, establishing a baseline by measuring tracheae in modern insects and imaging tracheae in fossil insects preserved in amber. In order to best analyze these trachea, synchrotron x-ray phase contrast imaging was performed on modern cockroach and beetle species. The major result was that tracheal volume is negatively correlated with rearing oxygen. Combining this result with the ability to image fossil tracheae data we will be able to generate a regression model that predicts atmospheric oxygen level from leg tracheal dimensions. By measuring the leg tracheae to body/leg size ratio we will be able to estimate the atmospheric oxygen partial pressure level in which the insect lived. The development of a biological proxy for atmospheric oxygen is a huge advance in biogeochemical modeling and evolutionary biology.

Single Cell Optical Tomography (cell CT) for Early Cancer Detection

Vivek Nandakumar[1], Laimonas Kelbaukas[2], Roger Johnson[2], Deirdre Meldrum[2]
[1] Electrical Engineering, Arizona State University, Tempe, Arizona
[2] Center for Ecogenomics, Biodesign Institute, Arizona State University, Tempe, Arizona

Nuclear morphology is a proven biomarker for early detection of deadly diseases such as cancer. High resolution 3D cell imaging may facilitate sensitive and specific early detection. We perform optical tomographic imaging on individual, hematoxylin-stained cells to quantify variations in nuclear morphology in several cell lines spanning the neoplastic progression spectrum in esophageal cancer. Our 3D cell images are obtained by applying principles of optical projection tomography to obtain isotropic resolution of ~350 nm. Each cell image is generated with mathematical reconstruction algorithms from 500 projection images acquired over 360°. Using this technique we observe qualitative and quantitative differences in nuclear morphology between esophageal cell lines representing normal and dysplastic cells. Our results validate the superiority of 3D over 2D quantitative cytometry.

Electron microscopy analysis of methicillin-resistant *Staphylococcus aureus* ultrastructure following exposure to antibacterial leachates

Caitlin C. Otto [1,2] and Shelley Haydel[1,2]
[1] School of Life Sciences, Arizona State University, Tempe, AZ 85287
[2] The Biodesign Institute Center of Infectious Diseases and Vaccinology, Arizona State University, Tempe, AZ 85287

As bacterial pathogens continue to develop antibiotic resistance, the pursuit of novel therapeutic agents is becoming increasingly urgent. To this end, we have identified two mineral mixtures, designated CB07 and BY07, that exhibit antibacterial activity against a broad spectrum of pathogens. We prepared

mineral leachates that maintain similar chemical components as the minerals, but are absent of physical particles. These leachates maintain antibacterial activity, demonstrating that the mechanism of action is dependent on chemical, not physical, interactions. To begin investigating the mechanism of action, we employed scanning and transmission electron microscopy to assess cellular ultrastructure and morphology of methicillin-resistant *Staphylococcus aureus* (MRSA) following antibacterial leachate exposure. Scanning electron microscopy (SEM) of MRSA following 24h leachate exposure shows intact cells, membrane blebs, and increased extracellular debris. Transmission electron microscopy (TEM) imaging of MRSA exposed to the antibacterial leachates for 24h shows misshapen cells, irregular membranes, condensation of the cytoplasmic contents, and distorted septa of dividing cells. Since both leachates exhibit a low pH, we also exposed MRSA to a pH 3.6 phosphate buffer for 24h to resolve the effects of pH alone. SEM images show surface crests both in the leachate-treated cells and in the buffer controls. In contrast to leachate-exposed cells, TEM images of buffer-exposed MRSA reveal an even distribution of cytoplasmic contents, demonstrating that the toxic effects induced by a low pH environment differ from that of the leachates. These microscopy studies have guided us in our efforts to evaluate the leachate antibacterial activity by demonstrating that the cells are not lysing, that the cell membrane remains intact, and lastly, that leachate-mediated toxicity differs from a strict low pH environment. While these minerals and their leachate derivatives show great potential for use as topical antibacterial agents, further research is needed to fully elucidate their specific mechanism of action.

Structural diversity among fungal hyphae: insights into cell growth and phylogeny

Jeff Propster, Bonnie Saucedo, Brant Unger, Dan MacLean, Matt Funderberg, Terrence A. Oneil, David Lowry, and Robert W. Roberson
School of Life Sciences, Arizona State University, Tempe, AZ 85287-1601. U.S.A.

The defining feature of filamentous fungi is the hypha; a tube-shaped cell that grows at its tip. The mode of hyphal growth is a complex process that has allowed the fungi to successfully utilize a wide range of ecological habitats and develop multiple lifestyles. The hyphal cytoplasm contains many of the organelles and sub-cellular inclusions found in other heterotrophic eukaryotic organisms. However, because of their mode of growth and diverse interactions with the ecosystem, hyphae also maintain a cytoplasmic structure and contain elements that are unique to the eukaryotes. Some cytoplasmic features show significant structural plasticity within the fungi themselves or are present in only certain fungal groups. These elements (e.g., Spitzenkörper) are of particular importance in understanding aspects of hyphal growth and are being used as indicators of fungal evolution. In this presentation, light and electron microscopy are used to review cytoplasmic features of hyphae from diverse taxonomic groups. Characteristics of cytoplasmic order, behavior, and selected organelles that are relevant to understanding hyphal growth and fungal phylogeny will be presented.

Effect of TiO_2 Nanotube Structure on Photocatalytic Production of Methane

Sanjitarani Santra,* C. Leyva-Porras and Peter A. Crozier*
* School of Mechanical, Aerospace, Chemical and Materials Engineering, Arizona State University, Tempe, AZ, USA 85287-6106

Photocatalytic hydrogenation of CO_2 is a potential approach for creating useful solar fuels. TiO2 based materials are effective photocatalysts although they work only under UV irradiation. Tubes provide potential advantages over powders owing to their higher surface areas, since heterogeneous reactions occur on the surface of the catalyst. Even though a great number of works have been accomplished related to TiO2 nanotubes, few studies have been performed on their use for photocatalytic reduction of CO2. In the current work, we synthesized aligned TiO2 nanotubes by anodization of Ti foils in fluoride mediated ethylene glycol solvent, using a Pt foil as cathode. The obtained nanotubes were heat treated at different temperatures. Structural changes upon heat treatment were observed in SEM and TEM. Photocatalytic hydrogenation of CO_2 with H_2 to produce methane was performed in photoreactor with UV lamp (λ= 254 nm). We are comparing the structure and catalytic efficiency of the titania nanotubes for the same reaction based on different annealing temperatures and phase compositions.

Automated microscopy for single-cell stimulus response measurements: cytotoxicity of metalloporphyrins on normal and cancerous cells

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Metalloporphyrins are commonly used to monitor the metabolic activity of single cells as well as for photodynamic therapy in the excise margin of tumors so their cytotoxic effects are of particular interest. In order to quantify the cytotoxicity of these compounds, an automated fluorescence microscopy approach is presented. Automated microscopy is useful because a non-averaging, microplate based imaging approach to measuring cytotoxicity is broadly applicable, devoid of investigator bias, and is capable of achieving higher throughput with equivalent accuracy as compared to other cell counting cytotoxicity methods. Beyond this, the method is uniquely suited to observing the progression of toxicity in real-time and may use confluency and proximity as additional analysis. Further, correlations with ecll state may be made depending on the dye system that is used. We present a dye system composed of Calcein AM as a live state marker, Ethidium Homodimer-1 as a dead state marker, and Hoechst 33258 as a high-affinity nucleic acid stain for cell cycle determination. Our results are compared to other methods, including MTT, WST, and Trypan Blue viability assays. The correlation between cell cycle and cytotoxicity of metalloporphyrins is examined.

[AIMS]

Dendritic tree and dendritic territory organization of an adult Drosophila motoneuron

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Different types of neurons can be distinguished by the specific targeting locations and branching patterns of their dendrites, which form the blueprint for wiring the brain. Unraveling which signals control different aspects of dendritic architecture, such as branching and elongation, pruning and cessation of growth, territory formation, tiling, and self-avoidance requires a quantitative comparison in control and genetically manipulated neurons.

The highly conserved shapes of individually identified Drosophila neurons make them well suited for the analysis of dendritic architecture principles. However, to date it remains unclear how tightly dendritic architecture principles of identified central neurons are regulated. This study uses quantitative reconstructions of dendritic architecture of an identified Drosophila flight motoneuron (MN5) with a complex dendritic tree to assess the natural variability of metric and topological dendritic architecture measure.

Furthermore, we test for stereotyped principles of dendritic territory formation. MN5 contains a fixed number of 23 dendritic sub-trees, each of which innervates a separate volume of the homogenous motor neuropil. The innervation volumes of these sub-trees are non redundant, non-overlapping, non interdigitating, reminiscent of tiling between the dendrites of different neurons of the same neuron types during efficient but non-redundant receptive field innervation.