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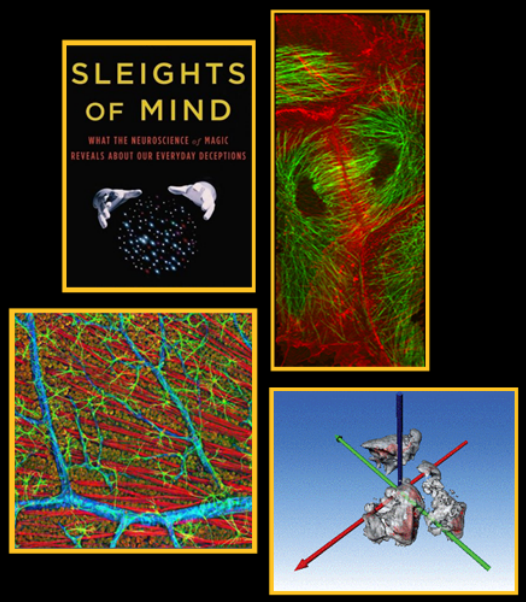

2012 AIMS Conference Program

Program, Abstracts & Biographical Information

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March 2, 2012

Arizona Ballroom, Memorial Union

MSA Microscopy Society of America

ASU School of Life Sciences Arizona State University

Arizona Imaging and Microanalysis Society

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

AIMS 2012 Program
MU Arizona Ballroom MU221

Check-In
| 8:00 - 8:45a.m. |

Opening remarks
Page Baluch - AIMS President
| 8:30 - 8:45a.m. |

High Resolution 3-D Characterization of Nanomaterials using Tilt Tomography in the Scanning Transmission Electron Microscopes
Ilke Arslan, University of California-Davis
| 8:45 - 9:45a.m. |

Student Presentations
| 9:45 - 10:45a.m. |

Morning Break – Vendor Demonstrations/Poster Session
| 10:45 - 11:25a.m. |

Abnormal capillary vasodynamics contribute to neural degeneration in Kv1.1 epileptic mutant mice
Steve Macknik, Barrows Neurological Institute, Phoenix, AZ
| 11:30 - 12:30p.m. |

Buffet Lunch – MU 202 Alumni Room
| 12:30 - 1:30p.m. |

Poster Session
| 1:30 - 1:55p.m. |

High-Throughput Robotic 3-D Electron Microscopy: The future is here
Tom Deerinck, NCMIR, Univ. California San Diego
| 2:00 - 3:00p.m. |

Sleights of Mind: What the Neuroscience of Magic Reveals About Our Brains
Steve Macknik & Susana Martinez-Conde, Barrows Neurological Institute, Phoenix, Az
| 3:00 - 4:00p.m. |

Afternoon Break/Vendor Exhibits/Student Awards
| 4:00 - 4:30p.m. |

Imaging protein dynamics in live mitotic cells
Patricia Wadsworth, Director MCB Dept, Univ Mass Amherst
| 4:30 - 5:30p.m. |

Business Meeting
Annual Society general meeting – open to the public
| 5:45 - 6:00p.m. |

Speaker Abstracts & Biographies

High-Throughput Robotic 3-D Electron Microscopy: The future is here

Dr. Tom Deerinck [Keynote Speaker]
Senior Research Scientist, National Center for Microscopy and Imaging Research (NCMIR), University of California, San Diego

Since its inception, biological electron microscopy has been a highly technical, labor-intensive and time-consuming endeavor. However, this situation is poised to change with the advent of automated serial blockface scanning EM (SBSEM), which enables the rapid acquisition of vast amounts of 3-D EM data with minimum operator involvement. SBSEM uses an ultramicrotome fitted inside a FEG-SEM to successively remove a thin layer of a specimen between imaging using a low voltage electron beam, with the entire process under computer controlled automation. Recent advances in heavy metal staining of biological specimens and improvements to instrumentation allow for both high-resolution blockface imaging and the rapid acquisition of very large field-of-view images by this technique. Additionally, new genetic methods to selectively introduce EM contrast into specimens promises to revolutionize the localization of proteins, macromolecular complexes, organelles and cells in tissues by providing high-resolution imaging in 3-D with excellent preservation of cellular architecture. Future enhancements to both SBSEM instrumentation and specimen preparation techniques should usher in a new era of automated 3-D nanohistology that is readily applicable to a wide variety of biological challenges.

Thomas Deerinck is a research scientist at the National Center for Microscopy and Imaging Research (NCMIR) at the University of California, San Diego. He attended the program in electron microscopy at San Joaquin Delta College in 1978 and since then has been working to develop new techniques to facilitate all types of microscopic imaging including confocal, multiphoton and electron microscopy as well as electron tomography. His work has garnered numerous awards including first place in the 2002 Nikon Small World, 2006 Olympus BioScapes Competition and 2008 Sony World Photography Awards and the same year was named the H. H. Crowley Award winner for outstanding contributions to the field of electron microscopy by the Microscopy Society of America. He has co-authored over 100 scientific papers, abstracts and book chapters and his work has appeared in various periodicals such as National Geographic, Scientific American, Discover and Time magazine. He is currently working with teams headed by Mark Ellisman, director of NCMIR and Roger Tsien, winner of the 2008 Nobel Prize in Chemistry, in advancing methods for correlated light and electron microscopic imaging and robotic electron microscopy.

High Resolution 3-D Characterization of Nanomaterials using Tilt Tomography in the Scanning Transmission Electron Microscopes

Dr. Ilke Arslan
Senior Research Scientist, Pacific Northwest National Laboratory, Richland, WA

Nanotechnology has become a key component in the field of materials science. Rather than analysing and determining the properties of bulk single or poly-crystals where the third dimension is assumed to be uniform, we must now analyse materials that have a finite size and shape in three dimensions, and not necessarily uniform in any of the directions. This new demand on materials characterization has led to the development of electron tomography for inorganic materials using Z-contrast imaging in the scanning transmission electron microscope (STEM). This technique involves taking a series of images of the sample at different tilt angles, normally ranging between -70° to +70° every 1 to 2 degrees, and using these two dimensional images to reconstruct a three dimensional volume of the sample. This tilt range may increase depending on the sample geometry and the holder used, but we are constantly battling against an artefact in the reconstruction called “the missing wedge.” This effect may be reduced greatly by performing dual axis tomography, or overcome completely using new holder technologies, but each technique has its pros and cons. These benefits and limitations will be discussed through examples of different inorganic materials.

Ilke Arslan obtained a MS in Physics from the University of Illinois Chicago and a PhD in Physics from the University of California Davis. She is an adjunct professor at the University of California Davis and Senior Research Scientist at the Pacific Northwest National Laboratory in Richland, WA. Ilke is an MSA Touring Speaker and in 2010 was among 85 researchers chosen by President Barack Obama to receive the Presidential Early Career Award for Scientists and Engineers which is the highest honor bestowed by the U.S. government to outstanding scientists and engineers at the beginning of their careers. She studies the structure-property relationships and physics of materials using advanced techniques in the scanning transmission electron microscope (STEM). Three dimensional morphology of nanostructures using high resolution STEM tomography; atomic and electronic structure of defects and nanoscale systems using aberration corrected/monochromated STEMs; theoretical simulations to complement experimental analyses.

Abnormal capillary vasodynamics contribute to neural degeneration in Kv1.1 epileptic mutant mice

Dr. Steve Macknik
Laboratory Director, Barrow Neurological Institute, Phoenix, AZ

The damaging effects of epilepsy are insidious: seizure-driven neural degeneration accumulates over time, especially in the hippocampus, leading to sclerosis, cognitive

decline, or even death. Excitotoxicity driven by calcium overload is the prevalent model to explain the degeneration, though the same cellular molecular pro-apoptotic cascades are also activated during ischemia-driven hypoxia. An untested possibility is that microscopic capillary vasospasms –driven by seizures -- lead to hypoxic micro-ischemia events and neural degeneration. Here we show, in the hippocampal capillary beds of awake and spontaneously epileptic Kv1.1 knockout mice, that pericyte vasoconstrictions occur in abnormally high number and magnitude within 80 seconds following the onset of seizures, and that degenerating neurons are tightly-coupled spatially to the microvasculature. This cannot be explained by excitotoxicity.

Sleights of Mind: What the Neuroscience of Magic Reveals About Our Brains

Drs. Stephen L. Macknik and Susana Martinez-Conde
Laboratory Director, Barrow Neurological Institute, Phoenix, AZ

All our life, every object we see, every person we know and every incident we experience, are derived from brain processes, and not necessarily the result of an event in the real world. The same neural machinery that interprets the sensory inputs also creates our thoughts, imaginations and dreams; thus the world we experience and the world we imagine have the same physical bases in the brain. Just as physicists study the most minute subatomic particles and the largest galactic conglomerates to understand the universe, neuroscientists must examine the cerebral processes underlying perception to understand our experience of the universe. Visual illusions are one of our most important tools to understand how the brain builds our experience of reality. Likewise, the principles developed by magicians and illusionists throughout history can be very useful to manipulate attention and awareness in the laboratory. Here we will discuss how the visual and cognitive illusions developed by artists and magicians can be applied to the study of the neural bases of consciousness and perception.

Stephen L. Macknik and Susana Martinez-Conde are Laboratory Directors at the Barrow Neurological Institute in Phoenix, AZ, and they are the founders of the exciting new discipline of neuromagic. In their new book Sleights of Mind: What the Neuroscience of Magic Reveals About Our Everyday Deceptions (published in 15 languages and > 100 countries) they have convinced some of the world's greatest magicians to reveal their techniques for tricking the brain. The Evening Standard (London) recently listed it as one of the 36 Best Books of the Year. Stephen and Susana are columnists for Scientific American Mind, the world's premier lay magazine of mind and brain. Their fascinating work has taken them on a multi-year, worldwide exploration of illusions as well as magic and its ancient principles, and how they can be explained using the latest findings of cognitive neuroscience. The secrets behind illusions and magic tricks reveal how your brain works not just when watching experiencing entertainment, but also in everyday situations.

Stephen and Susana are among the premier science communicators in the United States and have made television appearances on NOVA:scienceNow, CBS Sunday Morning and the Discovery Channel. They've also appeared on dozens of radio shows including NPR's Science Friday, PRI's The World, and in dozens of other TV and radio shows around the world. Susana and Stephen's research and scientific outreach activities have been featured in print in the New York Times, The Wall Street Journal, The Times (London), The Chicago Tribune, The Boston Globe, The Los Angeles Chronicle, Der Spiegel, among hundreds of media stories all around the world. They've given over 100 public presentations about their work and they have each published over 100 publications in academic journals including Nature, Nature Neuroscience, Neuron, Nature Reviews Neuroscience, and the Proceedings of the National Academy of Science.

Imaging protein dynamics in live mitotic cells

Dr. Patricia Wadsworth
Terrance R. Murray Commonwealth Honors College Professor, University of Massachusetts, Amherst

Advances in imaging technology and probes have revolutionized our ability to examine dynamic processes in live cells. Work in my lab has focused on using the light microscope to examine live mammalian cells during cell migration, cytokinesis and mitosis. Using cells permanently expressing proteins tagged with GFP, or photoactivatible variants of GFP, we can follow subcellular structures in individual cells as they progress through division. Using both FRAP and photoactivation we can examine the kinetics of protein turnover. Recent work has focused on spindle assembly, including the behavior of microtubules, motors and microtubule associated proteins. We are using TIRF microscopy to observe and record the behavior individual (or small clusters of) GFP-tagged motor proteins. Automated particle tracking is used to quantify the dwell time and velocity of motors throughout mitosis. Our goal is to understand the spatial and temporal dynamics of components of the mitotic machinery in mammalian cells.

Patricia Wadsworth received her Ph.D. in Molecular and Cellular Biology from Dartmouth College and did her post-doctoral training at the University of North Carolina with Ed Salmon. She is currently a Terrance R. Murray Commonwealth Honors College Professor at the University of Massachusetts, Amherst. The Wadsworth Laboratory focuses primarily on studying and imaging intracellular microtubules to elucidate their role in cell division, intracellular transport and other vital processes. Pat is an accomplished photomicrographer and authority in fluorescence imaging, and has won awards in Olympus BioScapes and is now a judge of the competition.

Student Abstracts

Effects of hematite nanoparticles on human intestinal cells

Chakravadhanula, M., Faust, J., Zhang, W., Chen, Y and Capco, DG.
Arizona State University, School of Life Science, Tempe, AZ

The human body is constantly exposed to various nanoparticles used in commercial consumer products. One such nanoparticle is hematite which is used in commercial products such as pigments, catalysts, medical devices, sensors, and recording media. This may accumulate in the environment which can have a potential impact on the human intestinal system. In our study, we hypothesize that hematite nanoparticles at the sizes and concentrations tested will negatively impact Caco-2 cells the human intestinal epithelial cells, with increasing time. We have used various techniques such as trans-epithelial electrical resistance (TEER) measurements, immunocytochemistry and microscopic analysis (confocal and electron microscopy) to support our hypothesis. Three sizes of nanohematite were used for the study namely, diameters of 17nm, 53nm, 100nm. Each of these nanoparticles was used at three concentrations namely, 1ppm, 10ppm, and 100ppm and the study was conducted at increasing time intervals. The results from our study show that the smallest and the largest nanohematite sizes affect the Caco-2 cells more than the intermediate sizes with increasing time. Also, our results show that nanohematite particles affect the cells at both physiological and non-physiological concentrations. Our results thus support our hypothesis that nanohematite at certain sizes and concentrations is detrimental to the human intestinal Caco-2 cells with increasing time.

Estrone, a primary component of Premarin hormone therapy, impairs memory in the rat: relations with the cholinergic system

Engler-Chiurazzi, E.[1,2], Talboom, J.[1,2], Braden, B.[1,2], Tsang, C.[1,2], Andrews, M.[1,2], Mennenga, S.[1,2], and Bimonte-Nelson, H.A.[1,2]
[1] *Department of Psychology, Arizona State University, Tempe, AZ*
[2] *Arizona Alzheimer's Consortium*

Conjugated equine estrogen (CEE; tradename Premarin) is the most widely used hormone therapy (HT) in the United States. Clinical studies assessing the cognitive effects of CEE have been inconclusive, with some studies showing positive effects, and some studies showing negative effects. Our laboratory has been studying factors that influence the cognitive outcome of HTs such as CEE. We recently showed that CEE benefitted working memory and increased number of basal forebrain choline acetyltransferase immunoreactive (ChAT-IR) positive neurons (Acosta et al., 2009, Engler-Chiurazzi et al., 2010). CEE is a complex estrogen formulation composed of over 50% estrone sulfate, which gets converted to estrone (E1), and then to 17β-estradiol. E1 increases cortical nerve growth (Brinton, 1997) and protects against β-amyloid induced toxicity (Zhao and Brinton, 2006). However, for most measures in

which other components of CEE, such as equilin and delta8,9-dihydroestrone, were neuroprotective against insult, E1 was ineffective. The current study evaluated whether three doses of E1 treatment impacted cognition in middle-aged, ovariectomized rats. We also quantified number of ChAT-IR positive neurons in the medial septum (MS), and vertical (vDB) and horizontal diagonal bands (hDB), of the basal forebrain. These regions are susceptible to age-related changes (Luine and Hearns, 1990) and are influenced by 17β-estradiol (Gibbs and Aggarwal, 1998 for review) and CEE (Acosta et al., 2009). On the spatial working and recent memory delayed-match-to-sample maze, the highest E1 dose impaired performance during regular testing and after a 6-hour delay. E1 treatment did not impact number of ChAT-IR neurons in the MS, whereas in a comparison study using the same procedures, 17β-estradiol increased number of ChAT-IR neurons in the MS. In the vDB and hDB (regions combined), the lowest dose of E1 significantly decreased the number of ChAT-IR neurons. These findings indicate that E1 negatively impacts spatial working and recent memory as well as memory retention in the middle-aged surgically menopausal rat, in accordance with recent findings of others showing that E1 negatively impacts hippocampal-dependent fear conditioning (Barha, Dalton and Galea, 2010). Further, E1 does not appear to impact ChAT-IR basal forebrain neurons as does 17β-estradiol. Moreover, that we have previously shown that CEE can enhance cognition and increase number of ChAT-IR neurons, and now find that E1 does not have these effects, indicates that E1 is likely not the primary mechanism of these CEE effects.

Deposition of CuInS2 Absorber Layer for a Prototype Solar Cell

Fang, Y.
Department of Chemical & Environmental Engineering, University of Arizona, Tucson, Az

The goal of my research project is to manufacture a prototype solar cell with low cost materials. The complete solar cell contains the CuInS2 absorber layer, the CdS buffer layer, the ZnO window layer and metal contacts. My work focuses on the CuInS2 (CIS) absorber layer.

CIS is chosen as the absorber layer of a solar cell because of its low cost and its low environmental impact. So it is a competitive alternate to conventional Si solar cells.

The object of my project is to deposit a uniform CIS absorber layer with a thickness of 2-3. The residual contaminants, such as CuS, should be minimized to reduce defects and increase the quality. The deposition method should be easily scaled to manufacturing.

Both spin-coating method and painting method are compared. The category of the substrate, the oxidation temperature and method, the sulfurization temperature and method, the ratio of Cu/In, the ratio of S/acac, the concentration of S dissolved in the ink, are all varied and characterized.

Scanning Electron Microscope (SEM) is used to determine the quality of the film, including the thickness, the uniformity, and the grain size. X-ray Photoelectron Spectroscopy (XPS) is used to verify the surface composition of the coated film. X-ray Diffraction (XRD) is used to detect the crystallography of the film. UV-vis is used to

check the light transmittance of the CIS film, and to get the data for the calculations of the band gap and the thickness of the coated CIS film.
In the future, more work needs to be focused on varying the sulfurization method and temperature and monitoring the band gap and morphology of the CIS film, and to be focused on replacing a portion of the In in the CIS film with Ga and detect its effect on changing the band gap.

High levels of morphological and cellular differentiation in the filamentous euendolithic cyanobacterium *Mastigocoleus testarum* strain BC008

Guida, B.S. and Garcia-Pichel, F.
Arizona State University, School of Life Sciences, Tempe, AZ

Mastigocoleus testarum strain BC008 is a filamentous, true-branching, heterocystous cyanobacterium which plays a major role in environmental biogenic erosion of limestones and biogenic carbonates. The exact mechanism of mineral dissolution has only recently begun to be studied. The process occurs through active calcium ion pumping mediated by at least one P-type calcium ATPase pump. To gain a greater understanding of the mechanism behind calcium carbonate boring we resorted to various microscopy techniques that have uncovered a surprising level of intrafilamentous cellular heterogeneity, which we think may be functional. Multicellular filament regions seem to be specialized in concentrating calcium and contain very little photosynthetic pigments. Conversely, regions that contain typical pigment autofluorescent profiles, have no detectable calcium signal. We have also observed further cellular diversification regarding phycobiliprotein contents and composition. Several regions putatively containing concentrated intracellular accumulations of autofluorescent pigments and the development of long thin trichome extensions containing no light harvesting pigmentation have also been observed. We have also shown differential localization of P-Type calcium ATPases. This extreme cellular diversity can be seen within a very small sample of boring as well as in non boring biomass. This level of differentiation within a single species of filamentous cyanobacteria is unprecedented.

Chemical Bath Deposition (CBD) of Cadmium Sulfide (CdS) as a Buffer Layer for a Prototype Low-Cost Solar Cell

Joshipura, I.D., Jiang, F. and Muscat, A.J.
Department of Chemical & Environmental Engineering, University of Arizona, Tucson, Az

Cadmium sulfide (CdS) thin films were deposited by chemical bath deposition (CBD), and the film properties were studied by UV-visible spectroscopy and scanning electron microscopy (SEM). The CdS will be used as a buffer layer between a $CuInS_2$ (CIS) absorber layer and a zinc oxide (ZnO) window layer in a prototype low-cost solar cell. The CdS films were deposited by placing a substrate in the bottom of a flask containing thiourea [0.53 M], cadmium chloride [0.45M], and ammonium hydroxide. Ammonium

chloride [1.89 M] was added as a complexing agent for Cd^{2+}. A condenser was connected to the top of the flask to create a closed system, and the assembly was immersed in a temperature controlled water bath. On glass substrates, the deposition time was used to control CdS film thickness and at 70^{o}C, approximate thicknesses of 80 nm (10 min), 129 nm (15 min), and 218 nm (30 min) were obtained based on applying the Beer-Lambert Law to UV-vis spectra. A plot of $(Ah:)^2$ versus h:, where A is absorbance and h: is incident light energy, yielded a band gap of 2.4 eV, which compares closely to literature values. There was an induction time for film growth because no film was deposited before 5 min. Films appeared to have a light-yellow, uniform deposition with moderate transparency. SEM images verified uniform CdS deposition on bare glass slides and indium tin oxide (ITO)-coated glass. In addition, substrates were masked using both Kapton and ChemTape to selectively deposit CdS on only one side.

Effects of CDPPB on neuronal plasticity

Lacrosse, A.L. and Olive, M.F.
Arizona State University, School of Life Sciences, Tempe, AZ

Schizophrenia is a neuropsychiatric disorder that affects 1% of the population, and is partially characterized by cognitive deficits, ranging from mild to severe. Currently there are limited effective treatments available, with atypical antipsychotics being the most commonly used, but exhibiting very limited efficacy. It has been suggested that the cognitive deficits associated with schizophrenia occur because of overall hypoglutamatergic function in the central nervous system. Schizophrenic patients have also been shown to have retracted dendrites and spines in the hippocampus. The mGluR5 positive allosteric modulator CDPPB indirectly increases NMDA receptor function, and as a result can increase the overall glutamatergic tone within the central nervous system, including the hippocampus. The rationale of this study is to investigate the use of CDPPB as a potential treatment for the cognitive deficits seen with schizophrenia. Procedures to be used include confocal microscopy in order to assess how CDDPB affects dendritic spine plasticity. Specific microscopy techniques include DiOlistic labeling which is a procedure used for assessing the morphology of dendritic spines. The process of DiOlisitic labeling involves the use of DiO-coated tungsten particles which labels individual neurons. The DiO-coated particles are delivered via a helium “gene gun” into brain tissue sections that are fixed at 1.5% paraformaldehyde and then allowed to diffuse and fill entire neurons. Confocal laser scanning microscopy using Z-sectioning is used to image individual neurons, and individual dendrites are reconstructed in 3-D using Bitplane Imaris 7.4 software. The purpose of our study is to assess dendritic length as well as the density and morphology of dendritic spines in the hippocampus of rats treated repeatedly with either vehicle or CDPPB 30 mg/kg once daily for 10 days. The results will elucidate changes in structural morphology of dendrites and their spines following augmentation of glutamatergic tone via mGluR5 positive allosteric modulation.

A High-throughput Method for Metabolic Phenotype Characterization at the Single-Cell Level

Myers, J.[1,2], Ray, T.[1,3], Zhu, H.[1], Yaron, J.[1], Kelbauskas, L.[1], and Meldrum, D.[1,3]
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[2] *The School of Biological and Health Systems Engineering, Arizona State University*
[3] *The School of Electrical, Computer, and Energy Engineering, Arizona State University*

Intercellular heterogeneity is an intrinsic feature of tissues and cell populations that plays an important role in normal cell functioning and pathogenesis. Single cell assays for profiling metabolic phenotypes can provide insight into disease mechanisms and biological pathways important for early disease diagnosis and discovery of new therapeutic targets. A large number of disease states, including cancer, are associated with alterations in cellular metabolism. However, technological challenges have limited investigations of metabolic phenotypes with single–cell resolution thereby limiting studies to population-based measurements, where essential cellular heterogeneity information is averaged out and lost. The ability to characterize single cell metabolic phenotypes utilizing high-throughput methodologies may provide detailed insights into transdifferentiation processes taking place in the progression of tissue from a normal to a cancerous state. Herein we present an experimental platform consisting of a hermetically isolated array for non-invasive, real-time determination of oxygen consumption rates for a population of 225 single cells. An array of microwells containing fluorescent, immobilized extracellular oxygen sensors is precisely positioned over an identically oriented single cell array and creates a hermetically sealed microenvironment wherein local oxygen concentration is reduced by cells via oxidative phosphorylation and dynamically measured as changes in the sensor fluorescence intensity. Cell viability in the isolated array was verified pre- and post-measurement by confocal fluorescence imaging with Ethidium homodimer-1 and Calcein AM. We demonstrate the ability to efficiently micromanipulate single cells, create an array of hermetically sealed microchambers, and quantify the oxygen consumption rates of single cells with fluorescence microscopy.

A Correlative Electron Microscopic Study and Immunofluorescent Localization of Proton-pyrophosphatase in *Physcomitrella Patens*

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Arizona State University, School of Life Science, Tempe, AZ

Type I Proton Pyrophosphatase (H^+-PPase) is an evolutionarily conserved hydrophobic transmembrane protein that hydrolyzes the pyrophosphate (PPi) molecule to pump H^+ across membranes. H^+-PPase is found in archaea, protists, eubacteria, and plants, but not in animals or fungi. This protein has been implicated in auxin transport [1], and

phloem development in *Arabidopsis thaliana* [in prep.], and has been localized in tonoplast [3], plasma- [4], and thylakoid membranes of *A. thaliana* [in prep]. Here, immunofluorescent localization of H^+-PPase ortholog in moss *Physcomitrella patens* was done, suggesting its localization in the chloroplasts of the stem. Transmission Electron Microscopy (TEM) revealed the ultrastructure of the leaves, stems, and rhizoids of *P. patens* gametophyte. Scanning Electron Microscopy (SEM) was also done to visualize the conducting tissues of this model Bryophyte. This work will serve as the basis for the immunogold localization of H^+-PPase in *P. patens* in the near future.

In-situ Environmental TEM Study of Phase and Morphological changes of TiO_2 Nanotubes.

Santra, S., and Crozier, P.A.
School for Engineering of Matter, Transport and Energy, Arizona State University, Tempe, Az

TiO_2 is known to be a photo catalytically active material. The photocatalytic activity of TiO_2 may vary depending on the phase and composition, which is affected by heat treatment conditions. We are using TiO_2 nanotubes for solar fuel reactions and in order to get superior performance, we want to understand and improve the material from structural point of view. In the present work, we are focusing on understanding the phase and morphological changes in the nanotubes under various *in-situ* and *ex-situ* heat treatments and annealing environments. We employ environmental transmission microscopy (ETEM) to investigate morphological changes during *in-situ* annealing.
Self-organized TiO2 nanotubes were synthesized [1] by anodization of polished and cleaned Ti foils in fluoride mediated ethylene glycol solvent, using a Pt foil as cathode. The as synthesized tubes were cleaned in distilled water and annealed in ambient atmosphere at different temperatures to produce tubes composed of different percentages of anatase and/or rutile. The tubes were characterized by XRD, SEM and TEM techniques. *In-situ* TEM (ETEM) studies were performed on as-prepared tubes in the presence of 1 Torr of air under different annealing conditions.
Figure 1 (a-d) shows the SEM images of tubes annealed in air at different temperatures in *ex-situ* condition (760 torr) along with the XRD. The onset of tube disintegration is noticed at ~600^{o}C and at 800^{o}C the tubes are completely destroyed. As-prepared tubes are amorphous, and the tubes annealed at 280^{o}C are predominantly anatase. Both anatase and rutile phases exist at 600^{o}C and complete transformation to rutile takes place at 800°C. Figure 1e, shows a high resolution TEM image of TiO_2 nanotube annealed at 280^{o}C along with the Fast Fourier Transformations (FFT) from regions 1 and 2. Though XRD shows the formation of anatase at 280^{o}C, from figure 1e there is still a lot of amorphous phase that has not been transformed to anatase, as shown in the FFT from region 1.
Figure 3(a-c) shows the *in-situ* ETEM images of nanotubes in the presence of 1 Torr of air at different temperatures. No change in the tube morphology was observed at 450^{o}C. At 650^{o}C the tubes are destroyed leaving behind a skeleton of nanoparticles.

Figure 3d shows the in-situ EELS O K-edge from the tube at 800^{o}C, which shows the nanotube is anatase. This *in-situ* result showing anatase at 800^{o}C is different from the *ex-situ*; this may be due to the difference in gas pressure. We are currently focusing on understanding the effect of gas pressure and gas environment on the anatase to rutile phase transformation. *In-situ* ETEM experiments under different gas environments and pressures will be presented and the mechanisms of phase and morphological changes will be discussed in detail.

Apis mellifera octopamine receptor 1 (AmOA1) expression in inhibitory neurons in olfactory centers of the honeybee

Sinakevitch, I.[1], Zolotova, N.[2] and Smith, B.H.[1]
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[2]*University of California Los Angeles, Neuroscience Program, Los Angeles, CA*

Octopamine underlies reinforcement during appetitive conditioning in the honey bee and fruit fly, and it acts via different subtypes of receptors. Antibodies raised against a peptide sequence of an identified octopamine receptor (AmOA1) were used to study the distribution of the AmOA1 receptors in the honey bee brain (Sinakevitch et al., 2011). We found in the honey bee antennal lobes that that AmOA1 receptors are mostly in GABAergic processes within the antennal lobe glomeruli. Inhibitory GABAergic neurons – specifically, multiglomerular projection neurons (PNs) and local interneurons - express AmOA1 receptors in cell bodies, axons and their endings in the glomeruli. The present study describes these neurons in detail by dye injections (into the antennal lobe, lateral protocerebrum lobe) and subsequent staining with anti-GABA and anti-AmOA1 antibodies. We found that there are in total 402±30 GABAergic cells (n=3) in the antennal lobe. Only twenty five GABAergic mPNs leave the antennal lobe through the two identified output tracts (ml-ACT and l-ACT) and branch into the lateral protocerebrum of the central brain. About 375 GABAergic neurons are local interneurons that make the intraglomerular connections in the antennal lobe. The data suggest the action of octopamine in the olfactory lobe takes place by the modulation of inhibitory neurons through the AmOA1 receptors.

A Drosophila model of neuronal MeCP2 function

Vonhoff, F. and Duch, C.
Arizona State University, School of Life Science, Tempe, AZ

Classic Rett Syndrome (RTT) and Rett related conditions are caused mutations in the Methyl-CpG-binding protein 2 (**MeCP2**), a multi-functional regulator of gene expression involved in chromatin remodeling, RNA splicing and histone modifications. Consistent with the wide range of phenotypes in patients with RTT, MeCP2 likely regulates a large number of target genes, not necessarily linked to their methylation status, together with context-specific molecular partners. Thus, different mutations in MeCP2 may potentially affect multiple different cellular features of developing neurons, and the discovery of

successful treatment strategies mandates a deeper understanding of the genetic and cellular bases underlying MeCP2 function in animal models. Although MeCP2 mouse models recapitulate RTT phenotypes and have provided valuable mechanistic insight into MeCP2 function, the identification and functional investigation of MeCP2 target genes in this system is time intensive and complicated. We propose ***Drosophila* as a high throughput animal model** to precisely define neural defects caused by MeCP2 and to identify downstream neuronal target genes of MeCP2. Although the Drosophila genome does not contain an ortholog of human MeCP2, multiple MeCP2 interactors, and most components of the chromatin machinery have well conserved orthologs in flies. Thus, in MeCP2 transgenic flies, the protein associates with chromatin, modifies the expression of multiple genes, and is phosphorylated at serine 421, as in mammals. We demonstrate that MeCP2 expression in an identified Drosophila motoneuron, MN5, specifically results in defects in dendrite development and maintenance, but does not affect membrane properties. Human MeCP2 induced dendritic defects in fly motoneurons require an intact methyl binding domain and can be rescued by genetic manipulation of the chromatin remodeling protein osa. These data demonstrate the validity of the Drosophila model to identify specific functions and genetic interactors of MeCP2 in neurons.

High Throughput Single Cell Image Cytometry

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Studies of cellular processes traditionally rely on bulk average measurements of large populations. However, cancer, inflammation and disease originate at the single cell level. Single cell analysis is therefore a critically important field for expanding the understanding of biological mechanisms. Measurements of cellular viability and cell cycle are commonly used in both the clinical and research setting to determine responsiveness to drugs or to track the progress of treatment. Despite their importance, the traditional methods of making these measurements have a number of drawbacks; cellular viability is usually measured by biochemical microplate assay, where single cell heterogeneity is obscured by a population measurement, while cell cycle analysis measured by flow cytometry is an expensive and technically complex method that lacks the ability to address individual cell morphology. Herein, an inexpensive, high throughput analytical imaging pipeline based on the open source image analysis software CellProfiler is presented for multiparameter single cell analyses. The pipeline rapidly processes fluorescent micrographs and exports data in a readily accessible format for downstream statistical analysis. We demonstrate our approach by correlating single cell viability with DNA content using a combination of commonly available fluorophores, Hoechst 33342, Calcein AM and Ethidium Homodimer-1. Our method provides an easy-to-implement alternative to commercial systems for research groups wishing to perform high content image analyses.

Sol-gel deposition of ZnO thin films

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Zinc oxide (ZnO) and zinc oxide doped with gallium (ZnO:Ga) are commonly used materials in the production of photovoltaic cells. Typically, ZnO has a band gap of about 3.3 eV and a transparent appearance, lending itself well to the window layer of a solar cell. Specifically, research is being conducted in the production of a low-cost solar cell, with $CuInS_2$ (CIS) as the absorber layer, cadmium sulfide (CdS) as a buffer layer, and ZnO as the window layer. Many methods are currently available for the deposition of ZnO and ZnO:Ga, with a range of quality of results. In order to seek a low-cost production method, sol-gel deposition of ZnO thin films on the order of 50 nm to 300 nm in thickness has been explored. In this process, a precursor solution is made from zinc acetate dihydrate, 2-methoxyethanol, and monoethylamine (MEA). The solution is allowed to react for two hours and then sit for at least two days before usage. The solution is then applied to the substrate via spincoating, the solvent evaporated on a hotplate, and the sample annealed at a high temperature. Various annealing conditions, solvent evaporation temperatures, and wait times before usage were explored to determine their effect on film quality and composition. UV/vis spectroscopy was used to confirm the identity of the film as well as calculate band gap and thickness. A band gap of about 3.5 eV was obtained through this method. X-ray spectroscopy (XPS) was used to determine the exact chemical composition of the films and SEM images were taken to observe the quality of the film. Based on XPS results, a high quality film can be obtained with a two-week wait time, solvent evaporation at 350 °C, and annealing at 400°C in ambient air for one hour.

In Situ Analysis of TiO_2 Based Photocatalysts under Light Exposure in the Environmental TEM

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Materials for photocatalytic reactions like water splitting and CO_2 reduction have been attracting considerable research interest for decades. It's very meaningful and helpful to have a better scientific understanding of the fundamental questions in the light harvesting processes, like charge transfer to the catalyst surface, absorption/desorption of reactant molecules and corresponding surface structure changes. Such analysis at the nanometer and atomic level should be performed under *in situ* conditions in the presence of both reacting gases and light in an environmental transmission electron microscope (ETEM). Titania is an important photocatalyst with good UV-light sensitive photocatalytic properties. To make a visible light sensitive photocatalyst, methods like coupling with narrower bandgap semiconductors, metal or nonmetal doping to TiO_2 have been extensively researched. For example, semiconductor coupling of CdS with

TiO_2 is often used to produce visible light photocatalysts. Here we employ high resolution imaging and EELS in a modified ETEM to detect structural changes in titania based photocatalysts under photoreaction conditions.

In situ analysis was performed on a FEI Tecnai F20 field emission differentially pumped environmental transmission electron microscope (ETEM) operating at 200kV. The microscope was modified to allow samples to be illuminated with light from a broadband laser driven light source (EQ-99, Energetiq Technology, Inc.). The *in situ* light intensity can be up to 4 times solar intensity but with a higher fraction of UV light. Apertures and filters can be attached to control the intensity and range of wavelengths of the light. Analysis is being performed on a variety of titania catalysts including P25(80% anatase & 20% rutile), pure anatase particles, rutile nanowires and CdS sensitized TiO_2 catalysts. Pure anatase particles with rod and diamond shapes were prepared by transforming P25 to sodium titanate nanosheets and then to pure anatase using hydrothermal methods. Rutile nanowires were grown on FTO glasses also using hydrothermal methods[1]. CdS quantum dots(QDs) were loaded onto rutile nanorods using a sequential chemical bath deposition method[2].

We have performed preliminary baseline studies of P25, rutile nanorods and pure anatase to establish the stability of the system under *in situ* conditions. Figure 1 shows a series of low dose *in situ* HREM images of the anatase particles in 1 Torr of water vapor at 150^{o}C taken at different times with/without light exposure. From the images, we can see the sample surfaces were initially clean and 15 hours of exposure to H_2O results in the formation ofa disordered layer one or two monolayers thick partially covering the surface. Subsequent exposure to 30 minutes of light thickens the layer up to a few monolayer (~ 0.7 nm) and give continuous coverage over the surface of the nanoparticle. The layer could be disordered titanium hydrooxide which is consistent with a photo induced hydrophilic effect first reported by Wang et al. that with the exposure of UV light TiO_2 nanomaterials tend to be more hydrophilic by absorbing dissociative water forming hydroxyl groups at the oxygen vacancies[3]. Continued exposure to H_2O, light and heat over a period of 68 hours showed very little additional changes to the surface structure. The observed long term stability after the initial change is expected for this system since titania is known to be very stable for photocatalytic reactions in water.

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