[AIMS] Arizona Imaging and Microanalysis Society

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

Program, Abstracts & Biographical Information

March 24, 2011

South Ballroom, Memorial Student Union
University of Arizona, Tucson, AZ

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[AIMS] Program

Morning Session (subject to change)

7:30 - 8:15	Registration
8:15 - 8:30	Welcome: **Doug Cromey** - President AIMS
8:30 - 9:15	**Glen MacDonald, University of Washington** *Toxicity testing and High Content Screening of Compounds in Zebrafish*
9:15 - 10:00	**Jeff Rodriguez, University of Arizona** *Customized Techniques for Automated Image Analysis*
10:00 - 10:45	Coffee Break - Vendor demonstrations
10:45 - 11:30	**Neal R. Armstrong, University of Arizona** *Nanomaterials for the formation of fuels from sunlight: Semiconductor nanocrystals in and on polymer hosts*
11:30 - 1:00	Buffet Lunch – Tucson room

Afternoon Session (subject to change)

1:00 - 2:00	**Warren Zipfel (Keynote), Cornell University** *Optimizing in Vivo Multi-photon Imaging*
2:15 - 3:00	**Russ Witte, University of Arizona** *Special Effects with Light, Sound and Electricity for Biomedical Imaging*
3:00 - 3:30	Student Presentations
3:30 - 4:15	Break with Student Poster Session
4:15 - 5:00	Student Awards and Closing Remarks
5:00 - 5:45	Business Meeting Annual Society general meeting – open to the public
6:30 -	No host dinner - *more information at the meeting*

[AIMS] Speakers

Glen MacDonald is a Research Scientist at the University of Washington. He manages the Digital Microscopy Center, an instrumentation facility shared by the Center on Human Development and Disability, and the Virginia Merrill Bloedel Hearing Research Center, as well as the Microscopy and Imaging Core for the VM Bloedel Hearing Research Center. Mr. MacDonald has been a faculty member at the Live Cell Microscopy course held at the University of British Columbia for 15 years. He has a great deal of experience with light microscopy.

Screening for Ototoxicity and Protection using the Zebrafish Neuromast

Glen H. MacDonald[1,3], Anne L. Corke[1,3], David Raible[2,3], Edwin W Rubel[1,3]

[1]Dept. of Otolaryngology-HNS, [2]Dept. of Biological Structure, [3]Virginia Merrill Bloedel Hearing Research Center, University of Washington, Seattle, WA 98195

Sensorineural hearing loss affects 33,000,000 people in the USA. Loss of inner ear auditory mechanosensory hair cells most commonly results from aging, chronic and acute noise damage, and therapeutic drugs. New drugs are not tested for ototoxicity even though iatrogenic deafness is a known side-effect of several pharmaceuticals. Our center has developed the lateral line hair cell of the zebrafish, *Danio rerio*, as a model for the mammalian auditory hair cell. Zebrafish are well-suited for large-scale drug screens due to the small size of the organism and the external location of the lateral line hair cells and their support cells in sensory organs called neuromasts.

Fluorescent vital labels that differentially label hair cells allowed us to employ simple, rapid imaging methods to screen drug and compound libraries for qualitative assessment of ototoxicity as well as for compounds that attenuate the effects of ototoxic agents. Compounds imparting toxicity or protection were verified by replication and dose-response curves in zebrafish. Several compounds have been further tested by auditory brainstem response (ABR) tests in rats.

Over 13,000 compounds have been screened among the investigators at our Center. Most recently, a library of 640 drugs and bioactive compounds approved for human and veterinary use was tested against 4 known ototoxins: aminoglycoside antibiotics neomycin, gentamicin, kanamycin, plus cisplatin, a potent anti-neoplastic agent. In this screen, 11 drugs were found to provide robust protection against one or more of the 4 ototoxins.

A new screen has been developed to quantify uptake of gentamicin conjugated to Texas Red (GTTR) to test whether protectants prevent gentamicin from entering hair cells. Larvae were treated for 5 to 60 minutes with a protectant, then exposed to GTTR in the presence of the protectant, rinsed and anesthetized. 3-5 larvae in a drop of embryo medium were mounted between coverslips, then imaged through a 20X/.75 NA objective on an inverted microscope. A z-series was collected with 3-5 neuromasts within the field, always including the 3 neuromasts dorsal to the eye, SO-1, SO-2 and SO-3, with dark frame and flat field corrections. A minimum of 15 neuromasts were segmented and measured for each datapoint, with their mean intensities normalized to background. The results allowed us to compare the efficacy of protectants at disrupting gentamicin uptake as well as to compare variants of the protective compounds that will be created.

Future directions include using this assay to evaluate newly identified protectants, chemical variants of identified protectants or mutant zebrafish strains that display altered ototoxicity. Other fluorescently-conjugated ototoxins will be tested as they become available. Another goal is to increase the level of automation for our imaging systems to increase throughput as we prepare to screen additional compounds and screen zebrafish mutants.

Dr. Jeff Rodriguez is an Associate Professor and Director of Graduate Studies for the Department of Electrical & Computer Engineering at the University of Arizona. In addition Dr. Rodriguez is the Director of the Signal and Image Laboratory (http://www.ece.arizona.edu/~sail), which is involved in research into signal/image/video processing and analysis, combined with pattern recognition techniques, to develop novel solutions to interdisciplinary problems in biology, medicine, homeland security, and consumer electronics.

Dr. Rodriguez' presentation is entitled **Customized Techniques for Automated Image Analysis.**

Dr. Neal Armstrong is a Professor of Chemistry and Optical Sciences at the University of Arizona. Dr. Armstrong's research is involved with the interface science of materials which lead to new solar electric and solar fuel energy conversion technologies. These materials include thin-film molecular semiconductors (polymers and small molecules), semiconductor nanocrystals (quantum dots), and the contacts which allow them to harvest energy efficiently. In addition to his several other honors, he was recently named a 2011 member of the UA College of Sciences' Galileo Circle of Fellows.

Nanomaterials for the formation of fuels from sunlight: Semiconductor nanocrystals in and on polymer hosts.

Neil Armstrong

Dept. of Chemistry and Biochemistry, University of Arizona

The formation of chemical fuels is one way of efficiently converting solar energy into a form that can be stored and used when the sun is not shining. To do so efficiently, however, requires exquisite control of the energetics of the entities that absorb sunlight and carry out the critical chemical reactions.

This talk will focus on our recent work to:

1. create new semiconductor nanocrystals (like those made from CdSe) with nanometer scale control over their sizes;
2. cap them with unique ligands and tether them into or on top of conducting polymer hosts,
3. characterize the energetics of photoinduced electron transfer as a function of small variations in nanocrystal size and;
4. incorporate them into new energy conversion and light emitting platforms.

Microscopy, especially electron microscopy, plays a key role in each step of this research and will be strongly featured in this presentation.

Dr. Warren Zipfel (keynote speaker) is a Professor of Biomedical Engineering at Cornell University. He is the director of the Developmental Resource for Biophysical Imaging & Optoelectronics (DRBIO, http://www.drbio.cornell.edu/), a nationally funded resource for the "creation and optimization of quantitative optical instrumentation for biophysical and biomedical research, specifically multiphoton laser-scanning microscopy for biological research and biomedical imaging." Dr. Zipfel has been a long-time colleague of multiphoton pioneer, Dr. Watt Webb. The DRBIO laboratory is currently working on several projects, including intravital multiphoton, developing novel femtosecond lasers, endoscopic & clinical imaging, and applications of Fluorescence Correlation Spectroscopy.

Optimizing *in Vivo* Multiphoton Imaging.

Warren R. Zipfel

Dept. of Biomedical Engineering & Applied and Engineering Physics, Cornell University

Multiphoton microscopy has enabled fluorescence imaging with subcellular resolution at depths into living specimens not possible with other forms of fluorescence microscopy. Using this imaging modality one can collect optical sections as deep as a millimeter in biological tissues, follow cell trafficking, study cell vitality and apoptosis, measure local transport phenomena and physiologic parameters such as calcium signaling and pH gradients. Although nonlinear microscopy can provide a level of investigation previously unattainable using other forms of intravital microscopy, we are at times still limited by imaging depth, motion artifacts and signal-to-noise when imaging deep in tissue. This talk will provide an overview of the current state of multiphoton imaging and cover several technical developments that should produce future practical improvements in multiphoton intravital microscopy.

Dr. Russell Witte is an assistant professor of radiology, optical sciences, and biomedical engineering at the University of Arizona. His Experimental Ultrasound and Neural Imaging Laboratory develops new methods using a combination of light, ultrasound and radio frequencies that potentially affect a variety of medical disorders from epilepsy to cancer.

Dr. Witte's presentation is entitled: **Special Effects with Light, Sound and Electricity for Biomedical Imaging**.

[AIMS] Student abstracts

Neuroanatomical characterization of the marine harpacticoid copepod Tigriopus californicus, a minute crustacean with an elaborate brain.

David R. Andrew and Nicholas J. Strausfeld
Neuroscience, University of Arizona

Arthropods are the most species-rich group of Metazoa and accordingly exhibit an incredible diversity of morphological form, behavior, and ecological specialization. The evolutionary success of arthropods is due, in part, to their nervous systems, which enable the ability to sense salient environmental stimuli and tailor behavioral responses appropriately. Early comparative work on arthropod nervous systems revealed certain anatomical characteristics as ubiquitous and which differentiated arthropods from other metazoan Phyla, as well as other characters that only a subset of arthropods possessed. The field of cladistics posits that shared derived characters are the basis for proposing evolutionary relationships and the application of this concept to arthropod nervous systems has provided insights into the interrelationships of the major arthropod groups. Certain evolutionarily and ecologically important arthropod lineages, however, have been overlooked as candidates for neuroanatomical evaluation of their nervous systems for a variety of reasons. This study examines the neuroanatomical organization of one such taxon, the marine copepod *Tigriopus californicus*, a species that has recently become an important taxon for studies in population genetics, toxicological monitoring, genotype/phenotype interaction, and a candidate for whole-genome sequencing. We used serial 1mm epoxy sections to produce 3D reconstructions of the nervous system in *Tigriopus*. In addition, we used confocal microscopy to reveal structural details of cell bodies and their associated neuropil. Despite their diminutive size (~1 mm at maturity), we find anatomical elaborations in the brain of *Tigriopus* comparable to much larger malacostracan crustacean that suggest evolutionary affiliations. These elaborations include a central complex with distinct protocerebral bridge, the presence of deuterocerebral glomeruli, and ascending heterolateral projections from these glomeruli to a center in the lateral protocerebrum. These traits connect *Tigriopus*, and by extension other copepods, to malacostracan crustaceans in terms of shared anatomical complexity and suggests an ancient origin of these traits.

Neuronal and glial localization of corticosterone-sensitive organic cation transporter 3 (OCT3) in hierarchical systems regulating the hypothalamic-pituitary-adrenal (HPA) axis in rats.

Jeremiah Molinaro[1], Luke Mumaw[1], Sean Barton[1], Miles Orchinink[1], Christopher Lowry[2], Kenneth Renner[3]
[1] Arizona State University, [2]University of Colorado, [3]University of South Dakota

Organic cation transporter 3 (OCT3) is a corticosteroid (CORT)-sensitive, polyspecific transporter whose substrates include norepinephrine, serotonin and dopamine and is located in many organs, including the brain. OCT3-mediated transport is unique as it is the only known monoamine transporter inhibited by physiological levels of CORT, such as those that occur during an acute stress response. We hypothesize that OCT3 functions in hierarchical systems regulating the hypothalamic-pituitary-adrenal (HPA) axis, such as the dorsomedial hypothalamus (DMH), to increase extracellular monoamine concentrations during an acute stress response and thereby regulate HPA axis activity. OCT3 may complement other steroid-

insensitive polyspecific transporters such as the plasma membrane monoamine transporter (PMAT) and OCT1 and OCT2 in regions of the brain that are innervated by monoaminergic terminals, especially in regions with few specific presynaptic re-uptake transporters. Although previous work has described the regional distribution of OCT3 mRNA and protein in the brain, research characterizing the specific cell types that express OCT3 has been limited. We used dual immunofluorescence of OCT3 and markers of either glia (GFAP) or neurons (NeuN) and confocal microscopy to identify cell types that express OCT3 in brain regions regulating the HPA axis in adult male Sprague-Dawley rats. We found that OCT3 is localized on ependymal cells as well as presumed non-monoaminergic neuronal soma in the DMH. The functional role of this CORT-sensitive non-monoaminergic neuronal clearance of monoamines is currently unclear. Additionally, we have developed a novel technique to observe and quantify, in real-time, OCT3-mediated transport of the fluorescent substrate 4-di-2 ASP (ASP+) in acute slice preparations with confocal microscopy.

Immunolocalization of Ghrelin and the Growth Hormone Secretagogue Receptor 1a (GHS-R1a) in the Reproductive Tract of Dairy Cattle.

S.E. Deaver, P.B. Hoyer, S.M. Dial, R.J. Collier and M.L. Rhoads
University of Arizona, Tucson, AZ

It is well known that energy status influences reproduction, yet the mechanisms by which metabolism impacts reproductive success are not well understood. The orexigenic hormone ghrelin has been identified as a potent regulator of energy homeostasis. Given that dairy cattle typically experience a period of negative energy balance during early lactation (when fertility is low), we postulate that ghrelin is involved in the metabolic regulation of reproductive function. Indeed, studies in rodents have demonstrated potent inhibition of early embryonic development in the presence of ghrelin. Ghrelin and its active receptor GHS-R1a have been identified in the reproductive tract of several species including humans, mice, sheep, and dairy cattle. However, despite this initial characterization, ghrelin and GHS-R1a proteins have not been localized within bovine reproductive tissues. The aim of this experiment was to localize ghrelin and GHS-R1a proteins within the reproductive tract of dairy cattle. Reproductive tissues (ampulla, isthmus, uterine body, corpus luteum, and follicle) were harvested from 3 Holstein heifers immediately following exsanguination. Duodenum and hypothalamus were collected simultaneously as positive controls for ghrelin and GHS-R1a, respectively. Tissues were fixed in 10% formalin for 24 hrs then embedded in paraffin for microscopy. Immunofluorescent and immunohistochemical techniques were used to identify ghrelin and GHS-R1a proteins within the reproductive tissues. Positive staining for ghrelin and GHS-R1a was evident in all tissues of the reproductive tract and the control tissues. Ghrelin was detected in the cytoplasm of the cell while GHS-R1a was localized to the plasma membrane. The current study provided evidence for the presence of ghrelin and GHS-R1a proteins within the reproductive tract of dairy cattle. Such widespread distribution indicates that ghrelin could affect fertility via direct or indirect actions on the oocyte or early embryo; particularly during the period of negative energy balance that dairy cattle experience during early lactation.

Improved Quantification of Acetylcholine Receptor (AChR) Clustering in C2C12 Myotubes Using an Objective Computational Algorithm

D.H. Campbell[1], M. Hicks[1], W. Grow[2], P.R. Standley[1]
[1]The University of Arizona College of Medicine – Phoenix
[2]Midwestern University – Arizona College of Osteopathic Medicine

Introduction/Hypothesis: We have previously reported the affects of caffeine treatment on agrin-induced AChR clustering in myotubes (Kordosky-Herrera and Grow et. al, 2009); caffeine decreased AChR cluster number as determined by human count ($p<0.01$). In an effort to improve upon and advance the objectivity of our protocol, we developed a quantification algorithm using CellProfiler, a free open-source computer program. We hypothesize that automated analyses of fluorescent pixel intensity yield faster, more sensitive, and more accurate data acquisition than visual count by a human observer.

Method: CellProfiler was calibrated to assign each pixel in a monochromatic image a weighted intensity between 0 and 1. Using image overlays generated by numerous iterations of our algorithm, we determined that ≥4 adjacent pixels (1.73μM) with weighted intensities ≥0.7 provide an accurate minimum threshold for designating AChR clusters. Contiguous pixels meeting these criteria were quantified to obtain cluster number, area, and intensity. Using the developed algorithm, we reanalyzed the human-counted fluorescently-labeled images (n=50/treatment) pertaining to tenfold increases in caffeine doses (0.001-1000μM). The automated reanalysis of 400 images was completed at ≈100 images/hr.

Results: Caffeine significantly attenuated AChR clustering via human count and CellProfiler count (≥1μM, $p<0.01$). However, CellProfiler-derived cluster area and total cluster intensity yielded a significant decrease in AChR clustering in every caffeine treatment (≥0.001μM, $p<0.01$), revealing a more sensitive measurement. Linear regression analyses of total intensity exposed a more acute dose response to caffeine (m=-5.30%, $r2=0.936$) than human count (m =-4.90%, $r2=0.876$; $p<0.05$).

Conclusion: While laborious pre-existing microscopy methods determined the effects of caffeine on AChR clustering by quantifying number only, our new method not only confirms these data, but also reveals a more sensitive means for quantification by taking into account both area and fluorescent intensity. Measuring total cluster intensity provides access to richer data sets, significantly faster and consistent quantification, and more objective observations.

Screening for Defects in Axon-Glial Cell Interactions in the Drosophila Visual System

Jason P. Town, Lynne Oland
Neuroscience, University of Arizona

The general principles of biological organization and the cellular strategies associated with them are often evolutionarily conserved. We are using the *Drosophila* visual system as a model for examining the cellular strategies underlying the formation of complex biological systems. We are particularly interested in how the organization of the system depends on interactions between the photoreceptor axons and glial cells. Using a mosaic generating method developed by Dr. R. Stowers, we are currently conducting a screen on a set of flies donated to our lab by Dr. K. Zinsmaier. These flies have recessive lethal mutations that cause functional blindness without causing superficial defects in the structure of the retina. To examine the phenotype of these mutations, we use the EGUF-Hid mosaic generating system to restrict homozygous expression of a mutation to the photoreceptor cells in an otherwise heterozygous animal. The optic lobes of these offspring are then analyzed using confocal microscopy. We are using an array of antibodies to reveal the structures of the nervous system. 24B10 was used to analyze

the position, trajectory and morphology of photoreceptor axons. NC82 was used to examine the organization of the synaptic neuropil. Finally, 8D12 was used to determine the position and morphology of glial cells.

Initiation and Progression to Colon Cancer

Huy Nguyen
Cell Biology & Anatomy, University of Arizona

Colon cancer causes the second highest frequency of cancer mortality. Important steps in reducing colon cancer mortality would be identifying factors causing colon cancer initiation and progression. A high fat diet is associated with increased risk of colon cancer. A high fat meal causes bile acids to be released into the digestive tract to emulsify and digest fat. Recent evidence shows that the bile acid, deoxycholic acid, causes oxidative stress and DNA damage, which may contribute to colon cancer risk.

By performing immunohistochemistry on colon tissues obtained from colonoscopies, we found that colon cancers were surrounded by large areas (>11 cm on a side) that frequently had intestinal glands (crypts) which were reduced or absent in expression for two DNA repair proteins: ERCC1 (nucleotide excision repair) and Pms2 [DNA mismatch repair and also for apoptosis (cell suicide)]. A frequent defect for two DNA repair proteins would give an area of great mutability. Thus double defects in ERCC1 and Pms2 appear to be central to progression to colon cancer.

We also examined tissue samples (biopsies) from patients in four groups at different levels of risk of colon cancer for expression of CcOI. The groups were Risk Group (RG) 1 of patients with never a colonic adenoma, RG2 of patients with small polyps, RG3 of patients with large or misshapen polyps, and RG4 of patients with a previous colon cancer. We found that biopsies from the 4 risk groups had similar ascending frequencies of crypts defective for CcOI. While deficiencies for CcOI were not greatly increased in areas surrounding colon cancers, a generally increased level of crypts deficient for CcOI appears to be an indicator of increased general colon mutagenesis, a step in initiating colon cancer. Deficiency in CcOI may be a biomarker of risk of colon cancer.

Multi-modality optical imaging of ovarian cancer in a mouse model

Jennifer M. Watson[1], Photini Faith Rice[1], David L. Bentley[1], Samuel L. Marion[2], Molly A. Brewer[3], Patricia B. Hoyer[2], Jennifer K. Barton[1]
[1]Department of Biomedical Engineering, [2]Department of Physiology, The University of Arizona, [3]Division of Gynecologic Oncology, Carol and Ray Neag Comprehensive Cancer Center, University of Connecticut, Farmington, CT

Our goal is to use optical imaging to detect cancer development on the sub cellular scale. By determining the microscopic changes that precede ovarian cancer we hope to develop a minimally invasive screening test for high risk patients. A mouse ovarian cancer model has been developed by treating mice with 4-Vinylcyclohexene Diepoxide to induce ovarian failure and 7, 12-Dimethylbenz[a]anthracene (DMBA) to induce ovarian cancer. Using optical coherence tomography (OCT) and multiphoton microscopy (MPM) we have obtained co-registered en face images of sixty-seven mouse ovaries ex vivo and forty-two ovaries in vivo. Preliminary analysis indicates that OCT and MPM can visualize ovarian microstructure. During the next year we will be analyzing ex vivo data and completing a long term survival study in which ovaries are imaged in vivo at time points before and after treatment.

Evaluating the effectiveness of Sulindac and/or DFMO as chemopreventitive and chemotherapeutic agents in colorectal cancer using spectral domain OCT.

Susan LeGendre-McGhee, Photini Faith Rice, Justin Klein, Amber Luttman, and Jennifer K. Barton
University of Arizona

Colorectal cancer is the third most common cancer in men and women both in incidence and death rates in the United States. The investigation of disease progression, the role of specific genes in carcinogenesis, and the potential of chemopreventative and therapeutic compounds is instrumented through the use of mouse models. However, the typical paradigm for investigating colon cancer with mouse models involves sacrifice of the animals and tissue harvesting for analysis of tumor count, immunohistochemistry and various assays. Our project aims to replace the current destructive paradigm with analysis of tissue morphological features through time-serial imaging of disease development and regression using optical coherence tomography (OCT). Using a 2 mm diameter spectral domain OCT endoscopy system centered at 890 nm with 3.5 μm axial resolution in air and 5 μm lateral resolution, we have obtained 30 mm lateral images at eight different rotations over several time-points on sixty-seven mice. During the next year, these images will be analyzed to determine if treatment with α-difluoromethylornithine (DFMO) and/or sulindac regress adenoma and prevent adenocarcionoma.

Molecular Targeting of Ovarian Cancer with a Confocal Microlaparoscope

Jordan Barton, Marty Pagel, Arthur Gmitro
University of Arizona

Ovarian cancer is a leading cause of death for women with low survival rates for advanced disease. Since ovarian cancer is usually not diagnosed until it reaches an advanced state, there is a need for a means to detect it earlier, especially in women at high risk for the disease. We have developed a confocal fluorescence micrlaparoscope (CFML) to image the surface of the ovary at high resolution during a laparoscopic procedure. A critical need is for a targeted contrast agent that helps to identify cancer with during CFML imaging. 5-FAM and FITC single isomer fluorescence agents targeted to asioglycoprotein, folate and EGFR receptors were tested on 5 different ovarian cancer cell lines. Encouraging results from this preliminary work are presented.