Arizona Imaging and Microanalysis Society

AIMS **Poster Session**

March 22, 2024



Arizona Imaging and Microanalysis Society azmicroscopy.org

SCHEDULE

8:00-8:45 AM	Check-In
8:45-9:00 AM	Opening Remarks Page Baluch – AIMS President
9:00-10:00 AM	Tera Lavoie, UC Advanced EM Core Assistant Director, University of Chicago, Chicago, IL
10:00-11:00 PM	Moring Break – Vendor Demonstrations/Student Poster Session
11:00-12:00 PM	Elizabeth Wright, CryoEM Core Director, University of Wisconsin- Madison, Wisconsin, IL
12:00-1:00 PM	Buffet Lunch – sponsored by ThermoFisher
1:00-2:00 PM	Bill Graves, CXFEL Project Scientific Director, Arizona State University, Tempe, Az
2:00-2:45 PM	Beth Cimini, <i>Cell Profiler Senior Group Leader, Broad Institute of MIT and Harvard, Cambridge, MA</i>
2:45-3:45 PM	Matt Tyska, <i>Cornelius Vanderbilt Professor, Dept of Cell and Dev Bio, Scientific Director, Cell Imaging Shared Resource, Vanderbilt University School of Medicine, Nashville, TN</i>
3:45-4:20 PM	Lightening Talks from Diamond/Platinum Sponsors
4:20-4:30 PM	Afternoon Break
4:30-5:30	Claudia Lopez, OHSU Multiscale Microscopy Core and the Pacific NW CryoEM National Center Microscopy Core Director, Oregon Health and Sciences University, Portland, OR
5:30-5:45 PM	Awards and Closing Remarks
5:45-6:15 PM	AIMS Business Meeting – open to the public

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SPONSOR LIGHTENING TALKS

3:45-3:50 PM	 Dan Giardina, Benchtop Microscopy Product Specialist, Andor, an Oxford Instruments Company Democratizing Confocal Microscopy
3:50-3:55 PM	Steven Hernandez, Product Applications and Sales Specialist, Carl Zeiss Microscopy, LLC Seeing in the dark: how microCT can dramatically improve your serial block-face workflows
3:55-4:00 PM	Barnaby Levin , Senior Research Scientist, Direct Electron, LP Apollo: A Fast Electron Counting Detector for High-Throughput Cryo- EM
4:00-4:05 PM	Brian Miller, Senior Research Scientist, Bruker Nano Analytics
4:05-4:10 PM	Gavin Ryan, Research Imaging Specialist, Evident Scientific/Olympus A brief intro of new imaging technologies from Olympus – now Evident!
4:10-4:15 PM	<u>Chad Tabbatt</u> , Senior Product Specialist, Materials & Structural Analysis, Nanoscience Instruments, Inc.
4:15-4:20 PM	Dylan Wood, Senior Research Scientist, Protochips Machine-Vision Based TEM Workflow Solutions, Data Management and AI Expansion Platform

Cryo-EM structure of the tetra-phosphorylated R-domain in Ycf1 reveals key interactions for transport regulation

Rodolpho S. A. de Carvalho, Md Shamiul I. Rasel, Thomas M. Tomasiak

Many ATP-binding cassette (ABC) transporters are regulated by phosphorylation on long and disordered loops which makes their interactions a challenge to visualize. We have trapped an activated state of the regulatory domain (R-domain) of Yeast Cadmium Factor 1 (Ycf1) by enzymatically enriching the phosphorylated state. A 3.2 Å cryo-EM structure reveals an R-domain structure with four phosphorylated residues and a position for the entire R-domain. The structure reveals key R-domain interactions including a bridging interaction between NBD1 and NBD2 as well as an interaction the R-insertion, another regulatory region. We systematically probe these interactions with a linker substitution strategy along the R-domain and find a close match with these interactions and survival under Ycf1-dependent growth conditions. We propose a model where four overlapping phosphorylation sites bridge several regions of Ycf1 to engage in a transport-competent state.

Electrospun Scaffolds Impregnated with Verteporfin to Treat Keloid Scars

Joel R. Clegg, Aaron J. Tabor, Ph.D., CWCA, Adonna M. Rometo, Ph.D., and Robert S.

Kellar

Keloids are a form of aberrant scarring which is caused by an injury to the dermis and epidermis of the skin. Keloid scarring occurs much more frequently in darker skinned individuals of African, Asian, and Hispanic descent compared to Caucasians. The incidence of keloid scarring in these populations ranges from 4.5% to 16%, with the higher incidence rates occurring in darker skin tones, representing an ethnic disparity in health care and the treatment of specific patient populations. Current treatments for keloids do not address the root mechanism that drives keloid formation but rather involve replacing the existing scar with more aesthetically appearing tissue. The overall goal of this project is the development of a biomimetic wound healing device that accelerates wound healing and encourages the functional regeneration of tissue following a keloid scar resection. The technique of electrospinning can be "tuned" to generate biomimetic wound healing devices that mimic the porosity, pore size, surface area, permeability, and structural integrity of the native extracellular matrix. Previous studies have demonstrated that a drug called verteporfin (VISUDYNE) is a promising candidate for the treatment of keloid scars due to its ability to impede the body's scarring response and promote regeneration of tissue. Upon the incorporation of the drug therapeutic, verteporfin, these electrospun wound healing devices function as drug delivery systems while simultaneously promoting cellular migration, adhesion, and proliferation. To examine the impact of impregnating electrospun scaffolds with verteporfin, SEM images were taken of scaffolds made with varying concentrations of drug. Morphometric analysis of fiber diameter, fiber alignment, and porosity of scaffolds were conducted utilizing MATLAB. There was no statistical significance observed when comparing the relative percent porosity of each scaffold concentration (control, 1% verteporfin, 2.5% verteporfin, 5% verteporfin). A statistically significant difference in average fiber diameter of the 5% concentration of verteporfin and the control scaffold was observed. Furthermore, SEM was used to visually confirm the presence of dermal fibroblasts adhering to the scaffold and therefore confirmed the cellular compatibility of the material.

Visible Light Photocatalysts: Studying Dopant Distributions in Rhodium Doped Strontium Titanate

Blake A. Dorame, Piyush Haluai, and Peter A. Crozier

Energy production is one of the largest carbon polluting sectors of human development, and in response to climate change, reliable renewable energy production is being sought out as a solution around the world in order to reduce carbon emissions [1]. Of these developing renewable energy technologies, photocatalytic water splitting using nanoparticles is an attractive one since it can convert solar energy into H2 and O2 by splitting

water [2]. These gases can then be stored and later used as solar fuels via electrochemically (hydrogen fuel cell) or combustion for energy production [3]. Semiconductors are used as the photon absorbing element in the photocatalyst to create electron-hole pairs to be used in the water splitting reaction. For real world applications, the bandgap of the light harvesting semiconductor should be tuned to absorb visible light, the largest portion of the light energy spectrum that reaches the earth's surface (~42%). A successful example of this is doping strontium titanate (STO) with Rh to reduce the bandgap from 3.2 eV to 2.3-2.7 eV [4] in order to generate charge carriers when exposed to visible light (1.65-3.1 eV). A major drawback in this technology is photogenerated charge carrier recombination [6] which suppresses water splitting. Often a co-catalyst is loaded onto the light-harvesting semiconductor to facilitate the separation of the photo-generated charge carriers and prevent recombination [5]. The efficiency of the charge separation process is enhanced when the co-catalyst is in close proximity to the charge generating site. Because the visible light generated charge carriers originate from regions with Rh doping, any heterogeneity of the dopants may give rise to regions of weak absorption of visible light. To explore this issue, we are investigating the heterogeneity of Rh doping in STO.

Exploring mitochondrial transcellular transfer in D. melanogaster as a response to neuronal injury

Aditi Ghosh, Gamaliel Luna, Susan Sharpe, Kiara Bachtle, and Martha R.C. Bhattacharya

Mitochondria are crucial for energy regulation and cell survival, especially for neurons which have an energyintensive nature, making them particularly susceptible to stress and injury. Maintaining proper mitochondrial function is thus essential for neuroprotection. Additionally, mitochondria have been shown to transfer between glial cells and neurons after nerve injury in a process called mitochondrial transcellular transfer (mitoTCT). This exchange of mitochondria presents a novel method of cell-cell communication and may be involved in whether neurons undergo protective or destructive consequences following damage and glial cell intervention. While neuron-glia mitoTCT has been observed in cultured conditions and mouse stroke models, the major pathways regulating mitoTCT remain unclear. Our project employs confocal microscopy and imaging flow cytometry to visualize and quantify neuron-glia mitoTCT post-injury in D. melanogaster. We hypothesize that neuronal injury will trigger an increase in mitoTCT frequency between neurons and glia as a rescue mechanism. Here, we present a detailed methodology of the two imaging techniques used, as well as our preliminary findings. Understanding the triggers and regulation of mitoTCT will provide insight into intercellular communication, especially in cases of neurodegeneration, where mitochondrial dysfunction is considered a major contributor.

Sourcing Scaffolding from Aloe vera for Tissue Regeneration

Christopher S. Groberg, Aaron J. Tabor, Ph.D., CWCA, and Robert S. Kellar

At the intersection of botany and tissue engineering, this study presents a novel approach to wound healing by harnessing the microarchitecture of decellularized Aloe vera leaves. Inspired by the plant's historical medicinal use and structural similarity to human skin, Aloe vera is identified as a sustainable and advantageous scaffold for dermal wound healing. This research leverages advanced imaging techniques, including SEM and light microscopy, to investigate the preserved intricate cellular structure of Aloe vera. The presence of vascular bundles to stomata that are sized similarly to human skin pores demonstrates the potential for supporting human dermal fibroblasts and facilitating the integration of human vasculature. By analyzing the size of the plant's stoma, epidermis, and gel cell structures and the size of available vasculature, using light and electron microscopy, a comparison can be made to the available commercial scaffolds and the human dermis.

The current approach addresses traditional skin grafts' ethical and cultural limitations, offering a cost-effective, universally acceptable, and environmentally friendly alternative. The utilization of Aloe vera for dermal regeneration showcases the practicality and benefits of plant-derived scaffolds in medical applications. This current research sets the stage for future exploration into the compatibility of human cells with plant-based scaffolds. Preliminary findings suggest that Aloe vera has a compatible cellular architecture for wound healing. This research emphasizes the role of biomimicry in medical device development and the importance of creating accessible healthcare solutions.

Comparing Adenovirus morphology using transmission electron microscopy to determine genome reduction due to repeated culturing

Paige Hawkinson, Ron Allen, and Crystal Hepp

Adenoviridae is a family of viruses capable of causing illnesses in various vertebrates, including humans. There are seven different species of human adenoviruses in the Mastadenovirus genus known to cause illnesses such as conjunctivitis and gastroenteritis. Human Adenovirus-41 (HAdV-41) causes acute gastroenteritis in children and was recently linked to a global outbreak of acute hepatitis in pediatric patients. Transmission electron microscopy done on a live control of HAdV-41, purchased from ATCC, reveals that the live adenoviruses were anywhere from 7.5 to 9 nm in diameter, remarkably different from previously reported 70 to 100 nm diameters. Comparative imaging of another live human adenovirus control from ATCC (HAdV-10) to HAdV-41 revealed similar results with diameters of virions being between 7 to 9 nm. We hypothesize this size discrepancy to be a consequence of genome reduction of the HAdV-41 and HAdV-10 control following repeated culturing. Further analysis with scanning electron microscopy (SEM) found the diameters of both adenoviruses to be within 40 to 85 nm. While inconclusive, these results demonstrate irregularly small virions and support the possibility of genome reduction. Furthermore, these results emphasize the importance of repeated measurements for data analysis.

Investigating the Molecular Basis of Secondary Substrate Recognition by the Human Lcystine/L-glutamate Antiporter, xCT

Clare F. Hotze, Tik Hang Soong, Tarjani M. Thaker, Thomas M. Tomasiak

Solute carrier (SLC) transporters are a superfamily of secondary carriers, which play important roles in transport of molecules across the cell membrane. One of the members of the SLC family, the system xc- transporter (xCT), is a dimer of L-cystine/L-glutamate antiporter SLC7A11 and the chaperone SLC3A2 (or CD98). xCT supports redox homeostasis in cells by neutralizing reactive oxygen species (ROS) through the intake of cystine, which is used in the production of glutathione (GSH). However, in cancer cells, xCT is overexpressed in order to combat the oxidative stress of upregulated metabolic activity. Kynurenine, a metabolite used as a secondary method to combat ROS in the cell, has been shown to be a substrate of xCT, but the mechanism of its transport is still unknown. My research focuses on determining the mechanism of kynurenine binding to xCT in order to develop an inhibitor of xCT to be used in chemotherapy. Having performed transport assays with kynurenine, we hypothesize that kynurenine interacts with amino acids arginine-135, arginine-396, and tyrosine-244 in the cystine binding pocket and have used site-directed mutagenesis to create mutants in this region. Our preliminary results indicate that kynurenine binds the wild-type protein and I plan to perform the transport and thermostability assays on the mutant proteins in order to determine the amino acid residues implicated in substrate binding.

Lung Capillary Endothelium to Arterial Endothelium Transition in Pulmonary Arterial Hypertension

<u>Bin Liu</u>, Dan Yi, Xiaomei Xia, Karina Ramirez, Ryan Dong, Hongxu Ding, Vladimir Kalinichenko, Michael B. Fallon, Zhiyu Dai

"Introduction: Pulmonary arterial hypertension (PAH) is characterized by a progressive increase of pulmonary vascular resistance and obliterative pulmonary vascular remodeling that result in right heart hypertrophy, failure, and premature death. The underlying mechanisms of loss of distal capillary endothelial cells (ECs) and obliterative vascular lesion formation remain unclear.

Method and Results: Our recent single-cell RNA sequencing, spatial transcriptomics analysis, RNASCOPE, and immunostaining analysis showed that arterial ECs accumulation and loss of capillary ECs were evident in human PAH patients and pulmonary hypertension (PH) rodents. Pseudotime trajectory analysis of the single-cell RNA sequencing data suggest that lung capillary ECs transit to arterial ECs during the development of PH. Our study also identified CXCL12 as the marker for distal arterial ECs in PH. General capillary EC lineage tracing

approach using Plvap-DreERT2;Tdtomato mice demonstrated that general capillary ECs gave rise to arterial ECs during PH development. Genetic deletion of HIF-2a or Notch4 neutralized antibodies normalized the arterial programming in PH.

Conclusion: In conclusion, our study demonstrates that general capillary endothelium transits to arterial endothelium through the HIF-2a-Notch4 pathway during the development of PAH. Thus, targeting arterial EC transition might be a novel approach for treating PAH patients.

Acknowledgement: This work was supported in part by NIH grant R00HL138278, R01HL158596, R01HL62794, R01HL169509, R01HL170096, AHA Career Development Award 20CDA35310084, The Cardiovascular Research and Education Foundation, Arizona Biomedical Research Centre funding (RFGA2022-01-06), and University of Arizona institution funding to Z.D."

Understanding Ferritin biomineralization using cryogenic electron microscopy (Cryo EM)

Sagnik Sen, Brent Nannenga

Biomineralization refers to the processes via which organisms synthesize inorganic materials. Proteins have been found to be key mediating agents for the process of biomineralization. There is significant interest among researchers into the molecular mechanisms of biomineralization as the knowledge gained could be used to make highly tunable nanomaterials under eco-friendly and sustainable conditions. Currently there is a scarcity of high-resolution structures showing the organic-inorganic interface during biomineralization. In our previous work we have established single particle cryo EM as an effective tool to study biomineralization.

In this study we use single particle cryo EM to study interaction of Ferritin with iron oxide nanoparticles. Ferritin is a ubiquitous, globular protein which acts as an iron homeostasis agent in organisms. We have obtained a high-resolution map displaying the Human Light Chain Ferritin (HuLF) - Iron Oxide nanoparticle interface. Fitting this map into an atomic model of HuLF, the amino acid residues involved in the nucleation and crystal growth have been identified and some functional tests have been done.

Structural basis for oxidized glutathione recognition in the Yeast Cadmium Factor 1, Ycf1

Tik Hang Soong, Clare Hotze, Thomas Tomasiak

The ATP Binding Cassette (ABC) transporter superfamily originated as an ancient system that plays an essential role in regulating molecular flux across membranes to maintain cellular homeostasis. In particular, the ABCC subfamily is primarily responsible for detoxifying harmful electrophilic compounds like xenobiotics or heavy metals that are conjugated to glutathione. In yeast, the Yeast Cadmium Factor 1 (Ycf1) sequesters glutathione-conjugated heavy metals, including major environmental contaminants Cd2+, Hg2+, As2+, and Pb2+ within the vacuole. Ycf1 can also transport oxidized glutathione alone to recycle glutathione and scavenge reactive oxygen species. Here, we determined a new high-resolution structure of Ycf1 bound to oxidized glutathione using single-particle cryo-EM. Our structural insights, complemented with cellular assays, led us to establish a molecular framework for substrate recognition to such a wide variety of substrates. We propose a "flexpocket" mechanism in which the pliable and pleiotropic binding pocket in Ycf1 enables flexibility within the transporter for varying metal complex accommodations.

Effect of Rhamnolipids on Bacterial Biofilms and Fibroblast Migration

<u>Abby Stoecker</u>, Brenda Padilla, Marjorie Nguyen, Andrew T. Koppisch, Ph.D., Robert S. Kellar Chronic wounds are unable to heal naturally and are stalled in an inflammatory, unhealed state. Over 90% of chronic wounds are inhabited by bacteria or fungi that have formed extensive biofilm networks. The presence of biofilms within chronic wounds contributes to the wound's inability to heal properly. In particular, Staphylococcus aureus is a common microbe found in chronic wounds, typically present in 93.5% of studied chronic venous leg ulcers. A focus on eradicating biofilms present in a chronic wound could help facilitate healing. Rhamnolipids, a class of glycolipids, have been studied for their antimicrobial effects due to their biocompatibility and biosurfactant properties. In this study, the rhamnolipid RhaC10C10 was investigated as a

possible wound healing therapeutic for biofilm-colonized wounds using an in vitro fibroblast wound model and a biofilm-eradication assay. First, cell viability of human dermal neonatal fibroblasts (HDFn) treated with rhamnolipid was determined. Following the viability assay, the effects of rhamnolipids on cellular migration was studied using an in vitro wound assay with HDFns treated with rhamnolipid. Finally, a minimum biofilm eradication concentration assay (MBEC) was performed to determine the anti-biofilm properties of rhamnolipids against methicillin-sensitive Staphylococcus aureus (MSSA) biofilms.

Histologically Assessing Wound Healing Progression Using Adipocyte Count and Epithelial Thickness in Acute and Chronic Bacterial Contaminated Wounds Treated with CAGE Ionic Liquid

<u>Samuel S. Stoecker</u>, Kyla Thomas, Aaron J. Tabor, Ph.D., CWCA, Marjorie Nguyen, Brenda Padilla, Taylor Gilmore, Chris Groberg, Elki Cederquist, Abby Stoecker, Joel Clegg, Emily K. Cope, Andrew T. Koppisch, and Robert S. Kellar

Bacterial contaminated wounds present significant challenges to the wound healing process and are a large concern for many populations worldwide. The prevalence of these pathologies is due in part to the presence of bacterial biofilms which allow bacterial colonies to resist standard therapies and avoid immune responses. Therapeutics such as ionic liquids have been shown to possess antimicrobial properties for common skin wound pathogens. Novel treatment delivery devices, such as electrospun protein scaffolds, have promise to provide a matrix for fibroblast migration while simultaneously delivering treatment to the wound. In addition, the presence of intradermal adipocytes in skin wounds has been linked to fibroblast recruitment and dermal reconstruction. Previously, we incorporated the ionic liquid solvent choline geranate (CAGE) into electrospun scaffolds and demonstrated its compatibility with supporting fibroblast proliferation and the inactivation of bacterial pathogens. Further work utilized an in vivo murine study to assess the effect of CAGE on acute and chronic bacterial contaminated wounds. Tissue samples were collected for histological assessment after eight days and were sectioned and stained with hematoxylin and eosin. Outcome measures included adipocyte count, epithelial thickness, and gross wound closure as indicators of wound healing progression.

Herpes Simplex Virus (HSV): Neurons, Vectors, and Viral Dynamics

Wesley Tierney and Dr. Ian Hogue

Herpes Simplex Virus 1 infects approximately two-thirds of the world's population. Some infection mechanisms are not yet well understood and multiple tools are implemented to help observe said mechanisms.

Project 1: HSV and Synapses

Currently, HSV is thought to spread from epithelial tissues to sensory neurons via synapses. Due to large numbers of virus particles entering and exiting neurons simultaneously, studying intracellular transport/trafficking is difficult. This problem is compounded further by the unpredictability of synapse formation in cultured neurons. We plan to use the existing SynView technology that uses protein complementation to fluoresce induced synapses between neurons to induce PNS neurons to make synapses This will be combined with our existing primary sensory neuron culture methods to study synaptogenesis and fluorescence microscopy methods to image trafficking and egress of HSV-1 particles. With this model, we aim to better examine viral egress specifically at synapses and have a much-needed working model of PNS synaptogenesis.

Project 2: HSV, HSV Amplicons, and Viral Vectors

Viral vectors and other trans gene delivery systems are commonly used to observe cellular mechanisms and are used to study viral dynamics such as viral exocytosis. Unfortunately, using viral vectors is not effective for dual fluorescence while studying HSV viral dynamics. Fortunately, HSV Amplicons are more effective at infecting and dual tagging cells to study viral vectors. HSV amplicons take advantage of HSV high packaging limit and its consistent recombination events at amplification to make infectious agents that contain not only the original

HSV viruses, but the plasmid DNA complete with GOI's that effectively delivery many transgenes to study viral dynamics more effectively than dual transductions with other viral vectors.

FIJI Mediated Quantitative Analysis of Cerebrovascular Structure, Protein Expression, and Cytoskeletal Morphology and Co-Localization *Wendt TS, *Chen TA, and Gonzales RJ

In the field of stroke research, the ability to quantitatively analyze microscopy images is paramount for elucidating the intricate mechanisms underlying cerebrovascular pathologies. Such analyses provide crucial insights into overall vascular morphology, cellular responses, and molecular interactions, contributing to the development of targeted therapeutic interventions to mitigate cerebrovascular diseases. Implementing tools such as FIJI to enhance the study of cerebrovascular disease via quantification rather than subjective observations following image capture can provide more uniform conclusions which can inform future directions. We have previously reported that FIJI mediated analysis is capable of capturing autophagic punctuations and vacuole formations within the cerebrovascular smooth muscle cells following in vitro ischemic-like injury; however, its applications can also be applied to whole vessel as well as vascular smooth muscle protein and cytoskeletal morphology and this analysis approach has not been previously considered. Thus, we hypothesized that automated FIJI workflows would be able to capture structural alterations at the whole vessel as well as at the cellular level in a reproducible manner allowing for implementation in future studies. Whole brain from sixmonth-old male Sprague Dawley rats were cryo-sectioned at -3.48 from the bregma and subsequent sections were labeled with tomato lectin and DAPI to identity the cerebrovasculature and nuclei, respectively. Human brain or aortic smooth muscle cells were treated with lipopolysaccharide or oxidized low-density lipoprotein and immunocytochemical analysis for TLR4 expression (inflammatory mediator) within the brain smooth muscle as well as F-actin and SM22 (cytoskeletal associated proteins) within the aortic smooth muscle was performed. Following image acquisition, we performed four automated FIJI pipeline analyses to quantitate branch length of intact vessels, TLR4 expression within the brain smooth muscle, in situ super-resolution enhancement and colocalization of F-actin and SM22, and cytoskeletal actin organization analysis within the aortic smooth muscle. We observed that the automated pipeline analyses generated consistently reproducible outcomes regarding branch length, super-resolution enhancement, co-localization, and actin arrangement. Concomitantly, we observed that lipopolysaccharide treatment increased TLR4 expression within the cerebrovascular smooth muscle cells and that oxidized low-density lipoprotein exposure decreased aortic smooth muscle actin stability and orientation. In conclusion, the automated quantitative analysis of microscopy images allows for further investigation into the complex mechanisms underlying cerebrovascular diseases, offering vital insights into outcomes such as vascular morphology, cellular responses, and molecular interactions. The implementation of tools like FIJI enhances research capabilities, enabling reproducible and comprehensive examinations at both the whole vessel and cellular levels. Our findings highlight the effectiveness of automated FIJI pipelines in elucidating significant alterations induced by treatments such as lipopolysaccharide and oxidized low-density lipoprotein, shedding light on potential therapeutic targets for mitigating cerebrovascular pathologies.

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