

Arizona Imaging and Microanalysis Society presents

# Microscopy Conference Program

March 13, 2015

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## President's Note

Dear Conference Attendees,

It is with great pleasure that we welcome each one of you to the 2015 Arizona Imaging and Microanalysis Society Conference. We are honored to have recruited an excellent group of speakers covering a wide range of imaging topics and look forward to learning more about their exciting research in the fields of biology, neurobiology and optical interferometry. We are thrilled to be hosting the meeting at Northern Arizona University as this is the first time in many years that the conference has been held in Flagstaff. We hope you enjoy all that the conference and Flagstaff has to offer while you're here. I'd like to thank all of those whose generous support have made this conference possible and each one of you for not only attending the meeting but for bringing your expertise to our gathering. It is through your commitment and knowledge that we are able to exchange ideas, collaborate and continue growing as a society. I would like to extend a special thank you to the Microscopy Society of America (MSA) for providing generous funding through their Tour Speaker Program which has allowed us to bring in remarkable speakers and to the Imaging and Histology Core Facility of Northern Arizona University for supporting and hosting this event. I would also like to thank all of our vendors for their continuing support of AIMS, we truly appreciate all of the funding they provide that allows us to cover meeting costs. I encourage you to take a moment to stop by their exhibits today to say hello and learn more about the cutting edge microscopy products they offer. Finally, I must thank Page Baluch, AIMS Secretary and Past President, for the enormous amount of time and effort she has put in to make this year's conference a success.

Thank you all for being an important part of the AIMS community, I hope you enjoy the meeting!

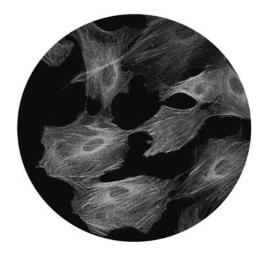
Sincerely,

Aubrey Funke, M.S.

Arizona Imaging and Microanalysis Society President

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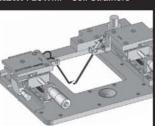
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look for us...







### AIMS 2015 Conference Program

## March 13, 2015

All sessions will be held in the Dubois Center Ballroom at Northern Arizona University

Check-In
Opening remarks
Snapshots of Nearby Stars: The Science of Optical Interferometry 11:45 - 12:45 p.m. Gerard van Belle, Astonomer, Lowell Observatory, Flagstaff Az
Buffet Lunch - Dubois Center Ballroom, NAU
Limits and Advantages of Light and Electron Microscopy When Studying Neural Circuits
Afternoon Break
Digital Imaging and Quantitative Morphometry to Evaluate Biomaterials for Tissue Applications
Student Awards and Announcements
Business Meeting



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#### Speaker Biographies

#### Gerard van Belle

Senior Research Scientist Lowell Observatory and Navy Precision Optical Interferometer, Flagstaff, Az

Dr. van Belle began working on optical interferometers during his graduate work at the University of Wyoming in the mid 1990's, while working with Mel Dyck on the IOTA interferometer on Mt. Hopkins, AZ, and collaborating with colleagues from lesser-known institutions such as Harvard and University of Massachusetts. Upon completion of his PhD at University of Wyoming in 1996, he has worked at NASA's Jet Propulsion Laboratory, Caltech, and the European Southern Observatory. In August of 2011, he took a position at the Lowell Observatory in Flagstaff, AZ, where he is Lowell's PI for the Navy Precision Optical Interferometer.

#### **Rob Diller**

Northern Arizona University, Department of Biological Sciences, Flagstaff, Az

Robert Diller is currently a Ph.D. student in biological sciences at Northern Arizona University. His current research interests focus on the use and evaluation of protein scaffolds to create medical devices which could be used to accelerate wound closure in diabetic patients. His Master's research focused on developing and implementing digital morphometric analysis of histology and immunohistochemistry samples to evaluate biocompatibility of medical devices. Robert previously was a lab manager for Flagship Biosciences, a biotechnology company that specializes in digital pathology. He currently is active in the bioscience industry as a consultant in digital histopathology analysis. Robert also teaches various Biology courses at Coconino Community College and Northern Arizona University. Feel free to contact him at robdiller@nau.edu

#### Jeanette Killius

Microscopy Society of America Past-President, Departpment of Anatomy and Neurobiology at Northeast Ohio Medical University (NEOMED)

Jeanette Killius is a Senior Laboratory Coordinator and an Instructor in Microscopic Anatomy in the Department of Anatomy & Neurobiology at the Northeastern Ohio Medical University (NEOMED) in Rootstown, Ohio. She oversees the EM lab and consults with researchers for their EM needs for various projects.

Ms. Killius has been a member of the Microscopy Society of America (MSA) since 1981 and has been involved in many committees during those years. She was elected to MSA Council in 2003 as a Biological Director. She served as Secretary from 2006 – 2011. She became President-elect in 2013, President in 2014 and is now Past-President.

She has a BS in Microbiology from the Ohio State University and an MS in Cell Biology from Kent State University, Kent, OH. She is currently engaged in the study of neural circuits in the brain relating to the translation of sound frequencies.

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### SPEAKER ABSTRACTS

## Snapshots of Nearby Stars: The Science of Optical Interferometry

Gerard van Belle

A brief introduction to the concepts of longbaseline optical interferometry (LBI) will be presented, followed by a review of fundamental stellar parameters as directly determined using LBI. Special attention will be paid to the progression of precision over the years of the observables of linear radius and effective temperature, with the current state-of-the-art measures approaching sub-percent levels for hundreds of stars (and being limited primarily by the ancillary data products of distance and bolometric flux, not measured angular size). Discussion will also be presented on the diminishing meaning of these gross parameterizations of stellar atmospheres, as higher-order surface details such as shapes, limb darkening, gravity darkening, and spotting are beginning to be imaged with LBI.

#### Limits and Advantages of Light and Electon Microscopy When Studying Neural Circuits Jeanette Killius

The neural pathways associated with hearing are complex. Sounds reach the ear and are translated into frequencies by the cochlea. These frequencies are transmitted along the ascending auditory pathway through the inferior colliculus (IC) in the midbrain, then on to the auditory cortex (AC) of the brain. The AC sorts the frequencies and then alters nerve cell responses to the sounds in the IC through AC neural projections of the descending auditory pathway. Thus the IC is a very important component in hearing as it integrates ascending auditory input AND descending input.

In this talk, I will discuss light microscopy techniques used to identify AC axons and to classify IC neurons as excitatory or inhibitory.

TEM ultrastructural studies will show further identification of synapses and neurons. Connectional studies like these are a major reason behind the development of President Obama's collaborative BRAIN Initiative, which aims to detail the connections in the brain.

## Digital Imaging and Quantitative Morphometry to Evaluate Biomaterials for Tissue Engineering Applications Rob Diller

Repairing damaged tissues and organs often requires the use of replacement cells, tissues or biomaterials. In the case of biomaterials and tissue engineering, they must first undergo biocompatibility testing prior to their end clinical use. For example, biomaterials must appropriately interact with living cells as well as mimic the native biology and mechanics of the recipient tissue or organ. There are several different established methods and instruments for evaluating engineered biomaterials and their resulting biocompatibility. These methods and instruments have the ability to measure structures in the nanometer range, which can increase the ability of the investigator to understand cellular influences on biocompatibility. The recent advancements in digital histology and quantitative morphometry afford investigators the ability to simultaneously evaluate and characterize multiple factors (e.g. histology stains and immunohistochemistry) across numerous sections of tissue with high throughput. The use of digital imaging of histology and subsequent quantitative morphometry is a powerful tool for evaluating biocompatibility.

In this talk I will discuss many of the microscopic techniques used to evaluate biomaterials. While this list is not all inclusive these are some of the common techniques used in industry today.

#### Student Abstracts

## Using Immuno-labeling and TEM to Test the Winding Filament Hypothesis

Cutler, B., Funke, A., Hessel, A., Baker, E and Nishikawa, K.

Northern Arizona University, Flagstaff, Az

In the sliding-filament theory for muscle contraction, myosin translates actin protein filaments in muscle through hydrolysis of ATP, resulting in overall shortening of muscle sarcomeres. This theory, however, fails to explain some commonly observed phenomena in muscle contraction. To accommodate these phenomena, a new model for muscle contraction has been proposed, dubbed the "winding filament" hypothesis (WFH). In the WFH, the N2A region of the protein titin is thought to bind to actin upon muscle activation. As the muscle contracts, actin rotates and the PEVK region of titin is wound like a spring enhancing the contractile force. To support this hypothesis, we are immuno-labeling the N2A region of titin so that its location within the sarcomere may be observed with transmission electron microscopy. Should this binding occur, we expect to see the ratio between the distance to the N2A region and the overall sarcomere length to change. If binding does not occur, we expect this ratio to remain constant.

#### Mechanical Characterization of Freshly Excised and Decellulariazed Murine Dermis

Geier, R.P<sup>1</sup>., Diller, R.B<sup>2</sup>., and Kellar, R.S.<sup>1,2,3</sup>
<sup>1</sup>Department of Mechanical Engineering,
<sup>2</sup>Department of Biological Sciences,<sup>3</sup> Center for Bioengineering Innovation, Northern Arizona University, Flagstaff, Az

Medical products that are developed for end clinical use first must pass rigorous in vitro (bench top) and in vivo (pre-clinical) testing before human clinical trials can be conducted. Therefore, the desire exists to more appropriately develop in vitro test methods and conditions that

can help screen prototypes or provide indications on how prototypes may function in living systems. In the case of developing novel biomaterial scaffolds as skin replacements (for wound healing applications), determining the mechanical properties of the skin can provide design inputs for the bioengineering of a suitable architectural replacement.

In the current study, the mechanical properties of mouse skin were evaluated and characterized using a newly designed and built uniaxial tensile testing device. Freshly excised and decellularized murine dermis was tested and the tensile strength, peak strain, and elastic modules values were determined. These data will allow for mechanical design inputs to be established for the development of replacement (bioengineered) skin scaffolds that match the mechanical characteristics of native skin. The ability for "mechanical matching" of targeted diseased or damaged tissues affords scientists the opportunity to more appropriately design replacement bioengineered substitutes such as skin replacements for wound healing applications.

#### Using Digital Histopathyology to Determine Biocampatibility of Tropoelastin and Collagen Implants

McMinimy, K<sup>1</sup>., Diller, R.<sup>2</sup> and Kellar, R.<sup>1,2</sup>
<sup>1</sup>Department of Biological Sciences, <sup>2</sup>Center for Bioengineering Innovation, Northern Arizona University, Flagstaff, Az

Biomaterial implants are used throughout the body to treat or replace damaged tissues or organs. These implants must undergo extensive biocompatibility testing prior to their clinical use in humans to avoid complications. Electrospun tropoelastin and collagen scaffold implants are promising biomaterials for wound healing research because they can be manufactured/generated to mimic the extra cellular matrix (ECM).

Before implantation, however, these protein scaffolds must be cross-linked in order to prevent early degradation of the materials' mechanical properties, which may have a direct effect on the scaffolds' biocompatibility in living tissues. Native protein biomaterials (non-cross-linked) are known to degrade quickly under physiologic conditions, thus demanding the necessity for cross-linking. In the current study, various biomaterials including tropoelastin and collagen were surgically implanted, subcutaneously, into a murine model for two weeks. Post-explant, the resulting fibrous capsule and neovascularization surrounding the implanted materials were evaluated using digital histopathology with quantitative morphometry to determine the effects of gluteraldehyde scaffold biocompatibility differences.

Electrospinning as a Method to Create Tunable Scaffolds for Tissue Engineering Applications Muller, J.R.<sup>1</sup>, Diller, R.<sup>1</sup>, Watson, J.R,<sup>3</sup> and Kellar, R.S.<sup>1,2</sup> <sup>1</sup>Department of Biological Sciences, <sup>2</sup>Development Engineering Sciences, Northern Arizona University, Flagstaff, Az, <sup>3</sup>University of Arizona, Tucson Az

Customizable electrospun biomaterials can be used throughout the body to treat pathologies or replace damaged or compromised tissues. Novel "templates" can offer unique characteristics for therapeutic applications that can be tuned or altered through varying parameters in the process of electrospinning. Protein matrixes were electrospun in order to examine different parameter effects on the creation of biomaterial scaffolds. Three different protein solution concentrations were created and exposed to varying flow rates and electric fields to produce unique electrospun prototype scaffolds. The ability to create tunable scaffolds will allow researchers to vary template characteristics to represent the extracellular matrix of various native tissues. These biomimetic scaffolds may assist in the healing process, thus increasing implant biocompatibility.

## Augmented Microcopy with Near-Infrared Fluorescence Detection

Watson, J.R.<sup>1</sup>, Martirosyan, N.<sup>2</sup>, Skoch, J.<sup>2</sup>, Lemole, G.M..<sup>2</sup>, Anton, R.<sup>2</sup> and Romanowski,M.<sup>1</sup> <sup>1</sup>Department of Biomedical Engineering, <sup>2</sup>University of Arizona Medical Center, University of Arizona, Tucson, Az

Near-infrared (NIR) fluorescence has become a frequently used intraoperative technique for image-guided surgical interventions. In procedures such as cerebral angiography, surgeons use the optical surgical microscope for the color view of the surgical field, and then switch to an electronic display for the NIR fluorescence images. However, the lack of stereoscopic, real-time, and on-site coregistration adds time and uncertainty to image-guided surgical procedures. To address these limitations, we developed the augmented microscope, whereby the electronically processed NIR fluorescence image is overlaid with the anatomical optical image in real-time within the optical path of the microscope.

In vitro, the augmented microscope can detect and display indocyanine green (ICG) concentrations down to 94.5 nM, overlaid with the anatomical color image. We prepared polyacrylamide tissue phantoms with embedded polystyrene beads, yielding scattering properties similar to brain matter. In this model, 194 µM solution of ICG was detectable up to depths of 5 mm. ICG angiography was then performed in anesthetized rats. A dynamic process of ICG distribution in the vascular system overlaid with anatomical color images was observed and recorded. In summary, the augmented microscope demonstrates NIR fluorescence detection with superior real-time coregistration displayed within the ocular of the stereo-microscope. In comparison to other techniques, the augmented microscope retains full stereoscopic vision and optical controls including magnification and focus, camera capture, and multiuser access. Augmented microscopy may find application in surgeries where the use of traditional microscopes can be enhanced by contrast agents and image guided delivery of therapeutics, including oncology, neurosurgery, and ophthalmology.



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