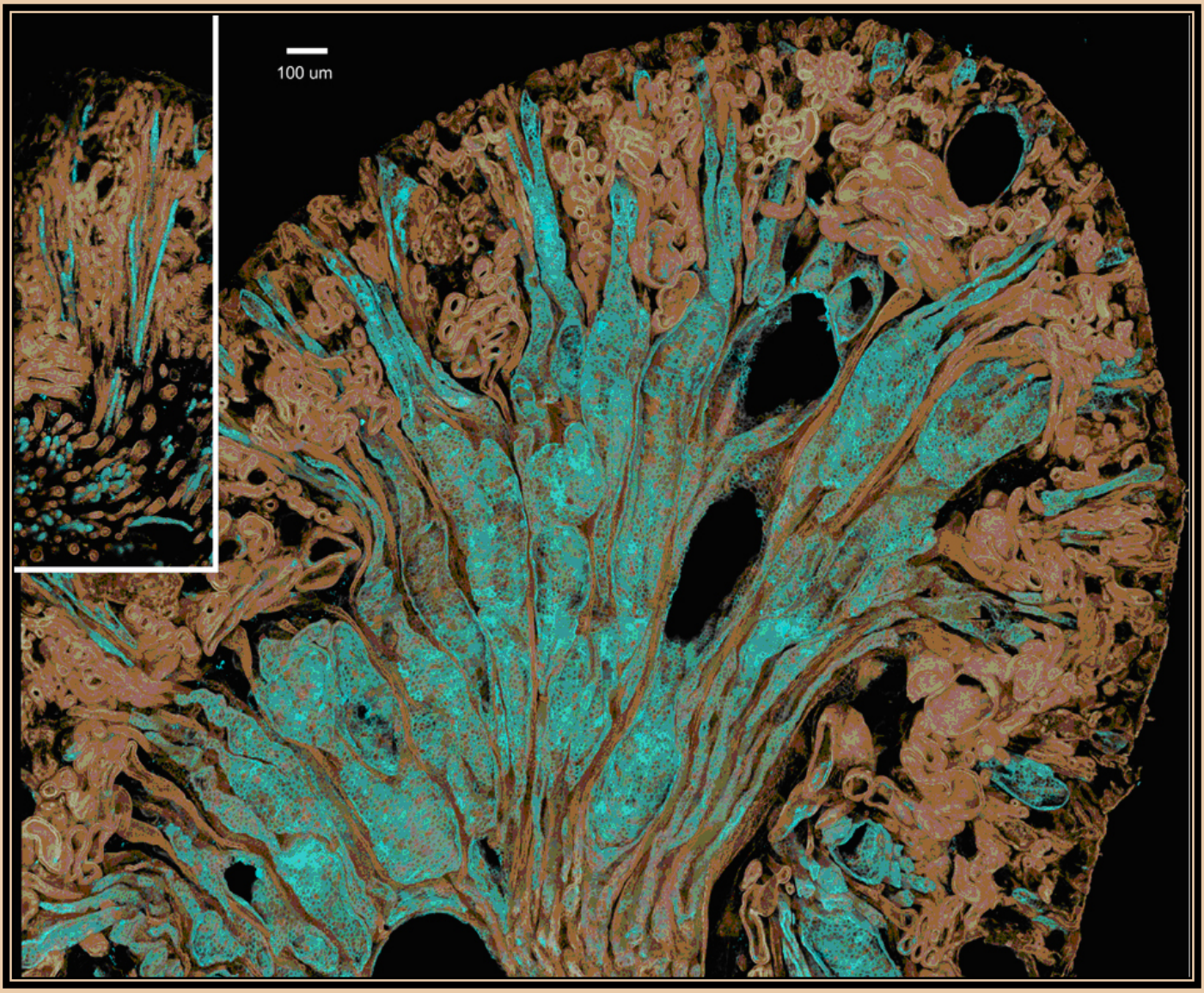
The Focal Point

Newsletter of the Indiana Microscopy Society
August 2005 Volume 1, Issue 2



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About the Cover

Dr. Carrie Phillips from the Division of Nephrology/Department Pathology at Indiana University School of Medicine took first place in the image contest at the first INMS spring meeting. A vibratome section of a cystic kidney (cpk mouse model) was imaged with a two-photon microscope. The larger image is a mosaic of 27 separate images and shows cystic collecting ducts labeled with DBA lectin (blue). The proximal tubules of the kidney are labled with PNA lectin (brown) and lack cysts. The image in the upper left corner shows a control kidney with no cysts.

Indiana Microscopy Society Members:

The first meeting of the IMNS on May 20th, 2005 held at the IU School of Medicine in Indianapolis was a success. Approximately 40 microscopists enjoyed breakfast and morning coffee before attending excellent talks given by our guest speakers, Kent McDonald from UC Berkeley, CA and Robert Bacallao from the IU School of Medicine. The afternoon included tours of both the IU School of Medicine Electron Microscopy Center and the Indiana Center for Biological Microscopy. There was also a poster session with seven student posters entered and a micrograph contest. Prizes were awarded to the best student poster and micrograph. The winning micrograph can be seen on the front cover of our newsletter, The Focal Point. The micrograph of cystic collecting ducts in the kidney of a CPK mouse model was submitted by Dr. Carrie Phillips who is an Associate Professor in the Division of Nephrology/Dept. of Pathology. There was a tie for the winning student poster, Heather Ward, who is a student in Pathology at Indiana University and Whitney Miller, a student in chemistry at Butler University. The help of our corporate sponsors and memberships allowed our society to hold a great first meeting and still be ahead financially. Microscopists from all over the state and from different disciplines got together and shared their knowledge in a relaxed and informal setting. This meeting shows the interest of microscopy in the state of Indiana; what a great way to start our society! Thank you to everyone who helped me organize this meeting.

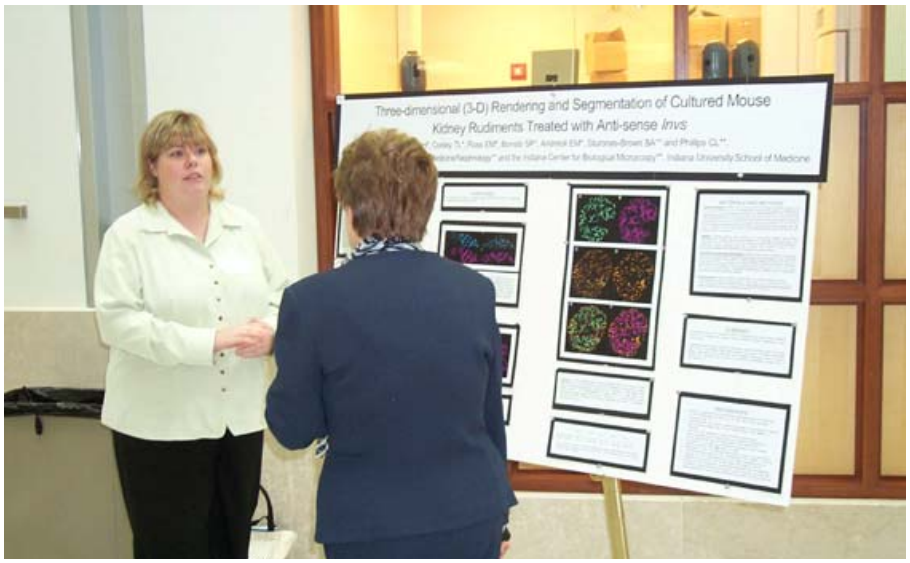
Caroline Miller
Program Chair

Dr. Kent McDonald

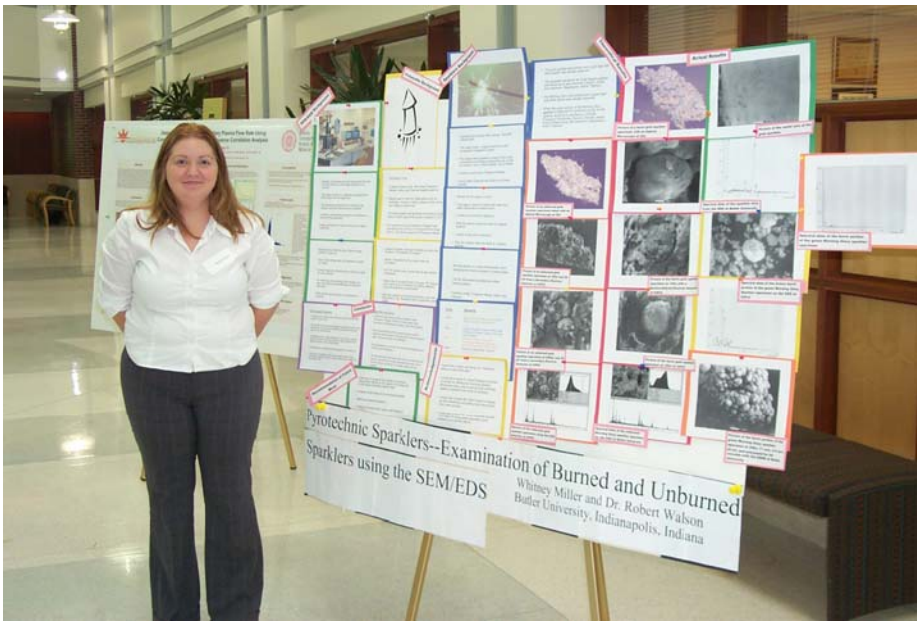
Dr. Robert Bacallao

Student Poster Winners

Heather Ward and Whitney Miller tied for first place



Heather Ward
Division of Nephrology, IU School of Medicine



Whitney Miller
Department of Chemistry, Butler University

Entries for the Image Contest

Kent McDonald, invited speaker, UC, Berkeley; Mike Boykin, Mager Scientific and Jeff Horn, Eli Lilly

Xin Li, Biomedical Engineering, Purdue; Emily Tung, BME, IUPUI; Yan Fu, BME, Purdue; and Zhong Wang, BME, IUPUI.

Clockwise: Janice Pennington, IUPUI(standing); Tetyana Shmyreva, Praxair; Weihong Lei, Mike Gohen (Pathology) and Caroline Miller (Anatomy), IUPUI; Robert Walson and Whitney Miller, Butler University, Peggy Harger-Allen, VA Hospital.

Sharon Ashworth, IUPUI, Nephrology, Caroline Miller and Janice Pennington, IUPUI, Anatomy

Mark Kelsey, Princeton Gama-Tech; Richard Peterson (PreClinomics) Anatomy and David Burr, Anatomy, IUPUI

Heather Ward and Jason Byars, Nephrology, IUPUI; PamYoung, Physiology, IUPUI

Mike Boykin, Jeff Horn and Carrie Phillips, Nephrology/Pathology, IUPUI

Mark Kelsey, Princeton Gama-Tech and Jim Little, Hitachi, display their brochures.

Abstracts

"High Pressure Freezing: Recent Developments and Applications"

Kent McDonald, PhD., Director Electron Microscope Lab, University of California, Berkeley

High pressure freezing (HPF) is one of the most important specimen preparation advances for biological electron microscopy since the development of glutaraldehyde fixation (Sabatini et al., 1963). HPF provides superior cellular preservation for morphological studies and better antigen retention for immunolabeling work than most conventional, room temperature methods. One powerful advantage of fast freezing is that it is extremely fast compared to conventional chemical fixation methods. Cells are immobilized in milliseconds instead of the seconds to minutes it takes fixatives like glutaraldehyde to diffuse into cells. Fast freezing is also non-selective, i.e., all chemical species are arrested equally whereas chemical fixative cross-linking is selective.

Despite obvious superiority over conventional methods, HPF has not emerged as a routine method for EM specimen preparation. Although the relatively high cost of the machines has had something to do with that, it is also because there have been few applications that absolutely required HPF. Now that is beginning to change as EM studies contribute to the effort to characterize cellular substructure down to the atomic level of detail (Sali et al., 2003).

What are the applications and uses for HPF? First and foremost, it is a technique for preserving high resolution ultrastructural information in biological samples. This makes it ideal, if not essential, for sectioning and tomography of frozen hydrated material (Hsieh et al., 2002; Al-Amoudi et al., 2004) as well as resin-embedded material (McIntosh, 2001; McEwen and Marko, 2001). But it is also emerging as an essential fixation technique for EM studies of the important model organisms such as *Drosophila, C. elegans, E. coli, S. cerevisiae, and Arabidopsis* among others. These are organisms that are difficult to fix well by conventional methods and HPF facilitates accurate phenotype characterization as well as EM immunolocalization studies. In this presentation we will cover these applications of HPF as well as consider the types of high pressure freezing machines, how they work, and how to optimize their use. We will discuss artefacts of HPF and some of the processing problems that are encountered following HPF.

Al-Amoudi, A., J.-J. Chang, A. Leforestier, A. McDowall, L.M. Salamin, L.P.O. Norlén, K. Richter, N. Sartori Blanc, D. Studer, and J. Dubochet. 2004. Cryo-electron microscopy of vitreous sections. EMBO Journal 2004:1-8.

Hsieh, C.-E., M. Marko, J. Frank & C.A. Mannella. 2002. Electron tomographic analysis of frozen-hydrated tissue sections. J. Struct. Biol. 138:63-73.

McEwen, B.F., and M. Marko. 2001. The emergence of electron tomography as an important tool for investigating cellular ultrastructure. J. Histoch. Cytoch. 49:553-563.

McIntosh, J.R. 2001. Electron Microscopy of cells: a new beginning for a new century. J. Cell. Biol. 153:F25-F32

Sabatini, D.D., Bensch, K., and R.J. Barnett. 1963. Cytochemistry and electron microscopy. The preservation of cellular structures and enzymatic activity by aldehyde fixation. J. Cell. Biol. 17:19-58.

Sali, A., R. Glaeser, T. Earnest & W. Baumeister. 2003. From words to literature in structural proteomics. Nature 422:216-225.

"Peering into the Future of Light Microscopy"

Robert L. Bacallao, MD, Associate Professor of Medicine,
Associate Professor of Anatomy and Cell Biology, Indiana University

As more genomes are sequenced, the challenge of characterizing how gene products interact becomes the compelling mission of biological sciences. The melding of whole organ physiology with transgenic animal models, gene transfer methods and RNA silencing will form the next wave of scientific inquiry. A host of new microscopy imaging technologies will allow researchers to directly visualized gene products, probe alterations in cell function in transgenic animals and map tissue organization. This talk will explore the optical technologies available currently and show how light microscopy methods will allow an advance from semi- quantitative to quantitative approaches.

Student Poster Abstracts

1. Intravital Microscopy of the Kidney in Real-time. Xin Li, [1] Weiming Yu.[2] Department of Biomedical Engineering, Purdue University, West Lafayette, IN 47907[1], Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, 46202[2].

Two-photon microscopy technique possesses compelling advantages for deep tissue imaging, and it has become a powerful means for intravital imaging of animal organs. One of the challenges of imaging kidneys *in vivo* is associated with the fast physiological processes, such as blood filtration and endocytosis of renal tubular cells, happened within the kidney. The dynamics of these processes tightly connect with kidney functions and diseases. To be able to image these fast events in real-time is critical. We used a simple method of scanning multiple focal points using a microlens array and galvo scanners to achieve real-time imaging rate. Kidney images of healthy and ischemic injured rats infused with fluorescently labeled dextrans were obtained and the results were discussed. This work was supported by the INGEN grant from Eli Lilly and Company Foundation and the NIH-George M. O'Brien Kidney Research fund.

2. Differences in Troponin Exchange for Cardiac and Skeletal Troponin in Psoas Rigor Myofibrils. Zhenyun Yang and Darl R. Swartz, Department of Anatomy and Cell Biology, Indiana University, Indianapolis, IN, 46202.

The myofibril is an excellent model system for the study of signal transduction along the thin filament. It can be manipulated to give three states on thin filament within a half sarcomere. A leftward shift of calcium sensitivity and decreased cooperativity of calcium activation of force occurred when cTn was exchanged into psoas myofibril (Piroddi et al., J. Physiol. 522.3: 917-931). To determine how cTn influences myofibrillar ATPase and labeled Tn exchange, cTn was exchanged into psoas myofibrils. Similar results were observed with ATPase activity of myofibrils as a function of pCa as observed with force measurement. Labeled cTn then was exchanged into cTn myofibrils at pCa9 and 4. The pattern was different from sTn myofibrils (Swartz er al., Bophy. J., 86:218a). The exchange rate in the non-overlap region at pCa9 was faster than sTn. At pCa4, there was little difference in the exchange rate between the non-overlap and overlap region in cTn myofibrils, while it was faster in the non-overlap region for sTn myofibrils. These observation show the relative rates of cTn exchange are c+M>B regions, while those of sTn are C>M>>>B. To test how a less cooperative system responds to calcium, labeled cTn was exchanged into cTn myofibrils at different pCa. Exchange in the non-overlap region graded wth pCa differing from sTn myofibrils. The greatest amount of exchange was in the non-overlap region, just adjacent to the overlap region in the cooperative rage of calcium activation (around pC6) in sTn myofibrils (Swartz et al., Biophys. J., 86:219a). This unique pattern was not displayed in cTn myofibrils and could be the result of the less cooperative nature of cTn myofibrils. Supported by AHA(DRS and ZY 0315241Z

3. Pyrotechnic Sparklers – Examination of Burned and Unburned Sparklers Using the SEM/EDS. Whitney Miller, Department of Chemistry, Butler University, Indianapolis, IN, 46202.

Turning sparklers the specific color seen is caused by different chemical compositions. The colors can be created by elements such as Barium, Aluminum, Strontium, and Carbon. When looking at sparklers two main groups can be observed: dipped sparklers and Morning Glories. Dipped Sparklers are sparklers that are on a metal wire that are created by dipping the wire into a chemical solution. Morning Glory sparklers are mini torches that are wrapped in paper. Chemical analysis was done with an Energy Dispersion System upon both burned and unburned specimens of a Gold dipped sparkler and the green section of a tri-color Morning Glory.

4. Three-Dimensional (3-D) Rendering and Segmentation of Cultured Mouse Kidney Rudiments Treated with Anti-Sense *Invs*. Heather H. Ward[1], Tameka L. Conley[1], Elizabeth M. Ross[2], Sawyer P. Bonsib[1], Eric M. Andreoli[2], Barbara A. Sturonas-Brown[2], Carrie L. Phillips[1], [2]. [1]Pathology, Indiana University School of Medicine, Indianapolis, IN; [2]Medicine, Division of Nephrology, Indiana University School of Medicine, Indianapolis, IN

Renal organogenesis involves the reciprocal induction of ureteric bud (UB) and metanephric mesenchyme resulting in formation of mature nephrons. We hypothesize that the *Invs* gene is important for maturation of renal tubules since we have shown that *inv/inv* mutant pups are born with diffuse corticomedullary cysts (*J Am Soc Nephrol* July 2004). The purpose of our study was to determine the direct role of the *Invs* gene product, inversin, in normal kidney development and how targeted knockdown of the *Invs* gene alters renal morphology. We cultured murine embryonic day E11.5 to E12.5 kidney rudiments on transfilters with anti-sense oligodeoxynucleotides (ODN) targeted to *Invs* or scrambled ODN controls for 6 days. Rudiments were fixed with 4% paraformaldehyde and incubated with *Dolichos biflorus (DBA)*-fluorescein to identify the UB. Z-stacks were collected using a two-photon system with a 20X water objective. Images were rendered using Voxx2 software to examine 3-D morphology or Amira 3.1 and Neurolucida to objectively segment and measure volume, length or branching pattern of UB. Rudiments incubated with control ODN showed the expected pattern of UB branching with long limbs and blunt ampullary tips. Rudiments treated with *Invs*-ODN showed altered arborization of UB with wispy, back-arching ampullary tips and shortened distances between branch points. Two-photon microscopy combined with 3-D rendering and segmentation software provides us with powerful tools to study rudiment kidneys subjected to gene knockdown and highlights the direct role that *Invs* plays in renal development.

5. A Microscopic Examination of *Equisetum hyemale*, Scanning Electron Microscopy, Transmission Electron Microscopy, and Electron Dispersive Spectroscopy. Peggy Harger-Allen, Department of Chemistry, Butler University, Indianapolis, IN, 46202.

The study was undertaken to learn how to operate an environmental scanning electron microscope (ESEM) fitted with an electron dispersive spectroscopy(EDS) system. A species of plant, *Equisetum hyemale*, was chosen for the study. In addition, conventional transmission electron microscopy (TEM), and scanning electron microscopy (SEM) was done on the material to supplement the study. *Equisetum* was utilized because it is easily prepared for viewing in the ESEM or SEM by air-drying, and contains a high silicon content for demonstration by EDS.

6. Determine *in vivo* Capillary Blood Flow Rates Using Component Frequency And Fluorescence Correlation Analysis. Zhong Wang[1], Weiming Yu[2]. [1]Department of Biomedical Engineering, Indiana University Purdue University at Indianapolis, IN, [2]Division of Nephrology, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, 46202.

With the recent development of intravital two-photon fluorescence microscopy techniques, subsurface information of vital tissues and fluid activities can be obtained. This research involved the detection and analysis of blood flow in kidney microcapillaries within live animals. Capillary blood flow is tightly associated with diseases and the efficiency of drug delivery, and yet its rate is difficult to measure quantitatively. Blood flow rate measured in a capillary vessel is a convolution of a number of component signals such as the blood plasma flow, cellular flow and heart beat. It is practically difficult using classical correlation spectroscopy approach to directly analyze such data. We have developed a new signal processing algorithm based on the component frequency and correlation analysis that is capable of recovering flow signals and distinguishing different characteristic components. We have tested this algorithm with model data and blood flow data measured in live animals. Results were presented and discussed. This research was supported by the INGEN grant from Eli Lilly and Company foundation and the NIH-George M. O'Brien Kidney Research Fund.

7. Novel aspects of natriferic responses in high resistance epithelia: Transient insulin-induced volume changes accompanying ENaC membrane insertion.

Jason Byars*, Charity Nofziger[+], Heather Ward*[#], Robert Bacallao*, and Bonnie L. Blazer-Yost[+]

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[+]Biology, Indiana Univ. – Purdue Univ. Indianapolis, 723 W. Michigan, Indianapolis, IN 46202, USA.

Insulin stimulates reabsorptive Na^+ flux in amphibian and mammalian cell culture models of renal principal cells. The epithelial Na^+ channel (ENaC) is the rate-limiting step in the transepithelial Na^+ transport. Recently we have elucidated a novel membrane de-limited signaling pathway whereby insulin binding to a receptor on the basolateral membrane causes the insertion of ENaC into the apical membrane. ENaC insertion is mediated via the formation of PIP3 (phosphatidylinositol 3,4,5, trisphosphate) in the basolateral membrane and diffusion of the lipid into the apical membrane. The response to insulin is accompanied by a striking but transient increase in cell volume which returns to original size within 4 minutes. These observations were made possible by rapid acquisition confocal microscopy of live, confluent cell monolayers. The present studies were designed to address the questions of whether ENaC insertion into the apical membrane precedes the swelling event and whether the formation of PIP3 is a requirement of the volume change. Pretreatment with LY294002, a specific inhibitor of PIP3 formation also blocked cell swelling. Thus, these studies confirm that the changes in cell volume are dependent on apical insertion of ENaC.

NON STUDENT POSTER ABSTRACT

8. High Pressure Freezing and Freeze Substitution: Advances in Renal Research.

JG Pennington*, CA Miller**, R Sandoval*, VH Gattone**, B Molitoris*, RL Bacallao*

Division of Nephrology in the Department of Medicine*, Department of Anatomy and Cell Biology, Electron Microscopy Center**, Indiana University School of Medicine, Indianapolis IN, 46202

Cryofixation using High Pressure Freezing (HPF) should be the best way to preserve biological specimens with minimal artifact. This technique is still not widely utilized as a research tool by electron microscopists because of the expense of equipment and technical expertise needed to process samples. Recent improvements in equipment along with new and innovative techniques may advance the use of HPF as a routine application.

Our laboratory used high pressure freezing and freeze substitution for cultured MDCK cells grown on sapphire discs, and needle biopsies taken from an anesthetized rat using Leica's EM PACT high pressure freezer, Microbiopsy system and Automatic Freeze Substitution Unit (AFS). Kidneys from an anesthetized, live, rat were imaged with a two-photon confocal microscope. Cortex and outer medulla from the kidney were taken with the Microbiopsy system. The transfer station allowed samples to be quickly moved from the biopsy needle to slotted specimen holders, then quickly to the EM PACT. An additional rat was anaesthetized and samples taken from kidney as well as other organs using the Microbiopsy system. Additional samples were excised from the animal and placed in flat specimen carriers. MDCK cells were grown on sapphire discs for 3 days, high pressure frozen and freeze substituted according to Hess. Before the cells were plated, the discs were cleaned in ethanol and then carbon coated to allow easy release from the resin after polymerization. The discs with cells were transferred to flat specimen carriers in buffer before freezing. Any remaining spaces in all types of specimen carriers used were filled with hexadecene and high pressure frozen. Samples were freeze substituted in 1%OsO4 in acetone or 1%OsO4, 0.1%GA, 0.1%UA in acetone and embedded in Embed 812 for morphology. The rat kidney was freeze substituted in 0.01%OsO4, 0.1%GA and 0.25%UA and embedded in LR Gold for immunocytochemistry.

Many people have found High Pressure Freezing to be a formidable procedure. The EM PACT used a minimum amount of LN2, occupied a small space, was very mobile and easy to start up and shut down. The compact design of the EM PACT allowed us to move the High Pressure Freezer to a room by the confocal microscope for correlative light and EM studies. With minimal experience, adequate instruction, technical support and some trial and error, well preserved tissue was reliably and consistently obtained. We feel confident that HPS with AFS can become a routine method of tissue preparation in our service facility.

SPECIAL THANKS TO ALL OF OUR CORPORATE MEMBERS AND SPONSORS FOR THEIR CONTRIBUTIONS WHICH HELPED MAKE OUR FIRST MEETING OF *THE INDIANA MICROSCOPY SOCIETY* A GREAT SUCCESS!

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Dear INMS Members

The Audit Committee met June 30th and performed the annual audit required by our Bylaws. The total income this year was $2,790.00 derived from membership fees and corporate sponsors for the Spring Meeting. Expenses totaled $1,651.75 ($1,535.83 for the meeting and $115.92 for the website and checks). Our balance in checking and savings together are $1,138.25. We are carrying forward a healthy balance, which should make our efforts for next Spring's meeting easier.

Vincent H. Gattone II, Ph.D.

President, Indiana Microscopy Society

Indiana Microscopy Society

Logo

The logo for the Indiana Microscopy Society was developed by the Executive Council. Each council member submitted a logo and a vote was taken. The logo is a combination of the logos submitted by Vince Gattone and Jason Byars. Jason Byars put together the final image you see here. The logo shows the blue and gold colors of the state flag of Indiana.

Fall Meeting of the

The Indiana Microscopy Society

hosted by

Eli Lilly

October 31, 2005

The meeting will include:

Two Invited Speakers

Lunch

Social Hour

Full details of the meeting will be announced soon

Dear Indiana Microscopy Members,

The first meeting of the Indiana Microscopy Society has come and gone. Thanks to all the members who helped make this meeting a great success! One of the main priorities of this society is to create an inviting setting for microscopist of different disciplines from around the state to get together. Mark your calendars for October 31, (Halloween!!) when Eli Lilly will host the fall meeting at their Indianapolis campus. This will be a short meeting with two invited speakers and plenty of time to socialize with other microscopists. Faculty, students and technicians are encourages to attend. Full details of the meeting will be posted soon.

The website for INMS has not been linked to the MSA website yet. The web page may be found at www.indianamicroscopy.org. Check here for updates on the meeting, membership forms and bylaws of the organization. Also, if you have any news, short techniques, or announcements you would like to have published in the newsletter please send them to me. The newsletter will be published on our web site two to three times a year. A third newsletter will be published this year after the fall meeting. I look forward to seeing you all in October.

Janice Pennington
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