The Histochemical Society Newsletter
Vol. 30, No. 1

Session on Reproducibility scheduled for EB with The Histochemical Society

Joining us this year for a joint session at the annual meeting at Experimental Biology is the Histochemical Society (HCS). The joint session titled: "Reproducibility in Experimental and Preclinical Research" will feature four HCS members speaking on issues related to reproducibility in scientific research.

Reproducibility in Experimental and Preclinical Research

Guest session sponsored by The Histochemical Society

Chairs - Charles W. Frevert (University of Washington) & **Stephen M. Hewitt** (National Cancer Institute)

Denis Baskin (University of Washington)
Antibodies: The Good, Bad, and Ugly

Yvonne Reid (American Type Culture Collection [ATCC])
Cell Line Authentication for Biomedical Research

Charles W. Frevert (University of Washington)
Animal Models for Study of Human Disease: Increasing Reproducibility and Considerations for Translatability

Stephen M. Hewitt (National Cancer Institute)
Round Table Discussion: The Role of Journals and Societies in Improving Reproducibility in Research

We spoke to several of speakers to hear more about what they plan to present at EB and how they see the issue of reproducibility today as it affects research.

We asked the speakers a series of questions and share some of their responses below.

1. **Reproducibility is an increasingly popular topic facing researchers today. Why is this important to tackle this in a joint AAA/HCS workshop and what do you think the two societies can learn from the expertise of each other?**

"Overall, I think the critical issue is enhancing the discussion throughout the scientific community. The issues of reproducibility have been known, but not talked about, especially with post-docs and trainees. The demands to publish, and tighter budgets, are negative pressures on ensuring data is reproducible.

There are two other issues here, that are even more topical to AAA and HCS:

a) Immunohistochemistry requires special attention. "Not everything that is brown is real" would be one way to put it. The more a technique becomes a kit, the higher the risk of false results.

b) The quality of biospecimens has not been widely addressed in the past, and the HCS has played a unique role in this issue." – Stephen Hewitt

"Many members of the AAA use antibodies for probing tissues and cells for the localization and expression of gene products and, accordingly, benefit from methodological rigor and proper controls for interpreting findings derived from antibody-based methods. The HCS promotes the correct use of antibodies in immunohistochemical protocols. To this end, the HCS has published standards of practice for immunohistochemistry, emphasizing the importance of proper controls for the validity and reproducibility of research conclusions

based on immunohistochemical techniques. Together, the AAA and HCS represent a strong unified voice for the proper use of controls with antibody-based techniques that will benefit the larger research community." – *Denis Baskin*

"Increasingly, we are seeing more retractions of publications in peer-reviewed journals about the lack of reproducibility of scientific data. Becoming aware of the problem, which is inescapable in all disciplines, is an initial first step in addressing this issue. Funding agencies, editors of scientific journals, scientific societies have become more concerned about this issue. For example, NIH has issued Principles and Guidelines for Reporting Preclinical Research. These guidelines now impact publication and funding." - *Yvonne Reid*

2. Could you share some key highlights of the proposed topics to help give members a sense of the workshop and entice them to attend?

"Unfortunately, many investigators do not use appropriate controls when performing immunohistochemical procedures and seem to lack understanding of which controls are needed and how to interpret them. What's worse, many journals commonly do not require authors to report controls for immunohistochemistry (or accept inadequate controls as evidence for specificity). These negligent practices can lead to false findings and unreproducible data in the literature. The focus of the presentation on antibody-based methods will be on proper use of controls for valid interpreting the results of immunohistochemical methods." - *Denis Baskin*

" I will focus on three key areas: (1) Impact of best cell culture practices on reproducibility; (2)Issuance of new government guidelines that impact reproducibility; and (3) Use of consensus standards for reproducibility" - *Yvonne Reid*

3. What do you think is the #1 issue facing researchers today in terms of reproducibility?

"Reliance on published data. Researchers want to do good science, but they can't reproduce every experiment published before them. They have to rely on the published methods and data. Too often the methods are poorly described, and if there is a failure with validation in the previous papers it can ripple through the research. The phase "per standard protocol" is not acceptable." –*Stephen Hewitt*

"From a Biological Resource Center (BRC) perspective, - the suspecting and unsuspecting use of unauthenticated cell lines in basic and applied research." – *Yvonne Reid*

4. Anything else you'd like to add?

"The journals play a critical role in addressing reproducibility. Good peer-review is a gate-keeper. If peer-review is the critical point, then the peer-review needs to be conducted by experts in the field. This is where society journals are important. The rigor in the society-sponsored field-specific journals is very high." – *Stephen Hewitt*

"Use of standardized test methods for cell line authentication will ensure consistency and reproducibility" - *Yvonne Reid*

FRET Workshop 2017 HCS Awardees

Article on FLIM & FRET Workshop 2017

by Andreas Alberti

The FLIM & FRET Microscopy Workshop 2017 gave me the unique opportunity to meet professionals in the field of microscopy as well as to learn the current state of the art on techniques based on Förster Resonance Energy Transfer (FRET) and Fluorescence Lifetime Imaging Microscopy (FLIM). Moreover, the structure and content of the workshop offered an optimal learning experience in terms of acquiring first the fundamental microscopy concepts through lectures led by the teaching faculty.

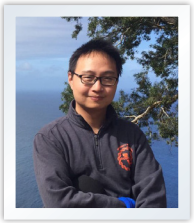
Second, an intense and interactive know-how sharing during the sessions on data acquisition & analysis permitted the consolidation of the theoretical learning (e.g. on the spectral characteristics of Acceptor-Donor fluorophore pair) to be able to perform later on useful quantitative FRET measurements during the guided cell's imaging sessions, which aimed at quantifying important parameters such as the Energy Transfer Efficiency and fluorophore pair's distance.

Furthermore, the data analysis sessions provided me a critical eye on how to interpret the acquired quantitative measurements, especially by considering signal corrections during image processing. It was inspiring to come in contact during the workshop with a set of microscopy techniques and related tools with interesting

FRET 2017 GROUP PICTURE

investigative applications such as in the field of protein to protein interactions.

I would like to thank the committee members of the Histochemical Society (HCS) for supporting my participation in the FLIM & FRET Microscopy Workshop 2017 as well as I would like to express my gratitude to the teaching faculty and staff at the W. M. Keck Center for Cellular Imaging (KCCI) from the University of Virginia for this precious learning experience.



Post-workshop article

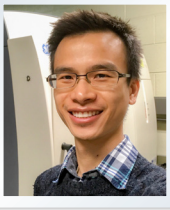
by Wenfan Ke

I am Wenfan from University of Virginia, Department of Biology. I had a great learning experience in 2017 FRET workshop and get to know the experts of the field.

As a first-year graduate student, I learned about basic concepts of FRET in my core course, however, I did not have any hand-on working experience on FRET and I did not have deep understanding on how to design a FRET experiment before the workshop. Throughout this workshop, I appreciated the development of all different types of FRET and the science behind these techniques. I also got to understand the logic behind the history and future direction of FRET, such that importance of shifting from intensity based FRET to lifetime based FRET has become widely accepted in the field. Further, I learned how to design FRET experiments in different scenario using the correct controls so I can apply what I learned in the workshop to my future studies.

The hand-on operation in the workshop was another amazing part of my experience. I got the chance to know not only FRET experiment design, but also the tools that could be used to achieve data collection and data analysis. It was a great opportunity for me to discuss with representative of venders from various of companies so I could catch up with their most recent techniques and potentially taking advantages on them in my future studies.

Overall, it was a very successful workshop and I hope it will continuously help more young scientist to get enrolled, understand and use FRET to advance their studies and promote the development of the field.



Post-workshop article

by Nattawut Sinsuebphon

I have been working with fluorescence lifetime imaging at Intes Molecular and Functional Optical Imaging Laboratory in Biomedical Engineering Department at Rensselaer Polytechnic Institute. My research focuses on FRET application in small animals using macroscopic fluorescence lifetime imaging (MFLI) platform. The FRET and FLIM Microscopy Workshop interested me because it would bring me FRET knowledge and application ideas in a different context.

The workshop consisted of lecture, hands-on, and discussion sessions, all of which were seamlessly organized. The lecture session covered many interesting topics in FRET from fundamental to pre-clinical applications. The topics were efficiently organized for participants with any level of background knowledge in FRET. There was much new and updated information to learn from this session. The hands-on session allowed participants to work with both intensity-based and lifetime-based measurements. For intensity-based measurement, the workshop provided many types of microscope platform, including

widefield, confocal and spectral microscopes. For fluorescence lifetime measurement, which has been increasingly employed for FRET applications, both frequency-domain and time-domain imaging systems were provided in this hands-on sub-session. Participants practiced FLIM-FRET data analysis using phasor plot and exponential fitting. Fixed and live samples, which were well prepared, were provided to participants to learn to acquire FRET data in different scenarios. There was also data analysis sub-session, in which participants learned and practiced intensity-based FRET analysis using PFRET, ratiometric and acceptor photobleaching algorithms. Participants could bring their own data or use the data collected from other sub-sessions. The discussion session was organized in a roundtable format. Participants, staff, and faculty could bring any FRET topics to the table. Everyone actively engaged the discussion, and the faculty provided great feedback and suggestions.

Overall, the workshop provided comprehensive and updated information on FRET application. Everyone can participate the workshop regardless his or her background knowledge. This workshop has fulfilled my knowledge and experience in microscopic FLIM and FRET, and guided me to achieve my goal, which is to translate microscopic FRET to macroscopic FRET in pre-clinical settings.

FASEB
Federation of American Societies for Experimental Biology

FASEB Public Affairs: Progress toward Strategic Goals (1st Quarter 2017)

Promote Optimal Funding for Biological and Biomedical Research

Advocated for research funding increases

- FASEB signed onto Ad Hoc Group for Medical Research letter on FY 2017 appropriations (February 7)
- FASEB released FY 2018 federal funding recommendations (March 1)
- FASEB submitted testimony to the House LHHS Appropriations Subcommittee on FY 2018 NIH funding (March 3)
- FASEB Capitol Hill Day had 55 participants and meetings with 112 congressional offices (March 9)
- In addition to Hill Day, FASEB staff held 18 meetings with congressional offices this quarter
- FASEB issued an e-Action alert on FY 2017 funding that generated 7,500 messages to Congress (March 9)
- FASEB signed onto Energy Sciences Coalition letter on FY 2017 appropriations (March 17)
- Jennifer Zeitzer met with senior staff in the Office of Management and Budget to discuss the President's FY 2018 budget (March 23)

FASEB GOALS con't

- FASEB signed onto a multi-society letter on FY 2017 and FY 2018 budgets (March 31)
- FASEB signed onto Coalition for National Science Funding letter in support of $8 billion for NSF in FY 2018 (March 31)

Strengthened liaisons with Congress and federal agencies

- Hud Freeze, Howard Garrison, and Yvette Seger participated in NINDS workshop on rigor and reproducibility in conference presentations (February 6)
- Tom Baldwin met with Jim Olds (NSF BIO Director) and Rich Derksen (Acting Director, Office of the Chief Scientist, USDA) (March 28)

Strengthened strategic partnerships

- OPA staff serve in leadership capacities in major coalitions and partner organizations: Jennifer Zeitzer is on the Steering Committee of the Ad Hoc Group for Medical Research and the Board of Directors for the Friends of VA, Yvette Seger is Vice President of the National Postdoctoral Association, and Anne Deschamps is on the Board of Directors of Americans for Medical Progress

Improve the Climate for Research

Developed and presented advocacy priorities and perspectives

- FASEB shared instrumentation survey released (January 5)
- FASEB responded to NIH RFI on Peer Review and Privacy Act Implementation (February 3)

Promoted training and sustainable careers

- Yvette Seger was an invited speaker at the MD Anderson Presidential Career Symposium (February 16)
- Educated and engaged scientists, the public, and policy makers
- 2017 FASEB BioArt Winners featured in January 12, January 19, January 26 and February 2 NIH Director's Blogs
- Winners of FASEB BioArt Competition on display by Cville Biohub in Charlottesville, VA (February 2)
- FASEB coordinated a Capitol Hill briefing on rare diseases and basic research (March 9)
- Senator Schumer's office contacted FASEB for state and district factsheets (March 16)
- Representative Mark Pocan's office contacted FASEB for suggestions about questions to ask at a future House Appropriations Committee hearing on the FY 2018 NIH budget (March 16)
- Hud Freeze was interviewed on KUSI TV about effect of President's Budget on NIH (March 22)

Expand the Community that FASEB Represents

Developed strategies in response to the increasing globalization of science

- FASEB signed onto multi-society letter on immigration policy (January 31)
- FASEB signed onto AAMC letter on immigration policy (February 1)

FASEB GOALS con't

- Foundation for Vaccine Research reposted FASEB graphics on Trump NIH budget (March 21)
- The Society for Nuclear Medicine and Molecular Imaging used FASEB materials in their meetings with Senator Blunt (March 21)

Educated scientists to become more effective advocates

- Yvette Seger spoke about FASEB activities at the Biochemistry Chairs meeting (January 12)
- Howard Garrison spoke about FASEB activities at the Pharmacology Chairs meeting (January 28)
- Yvette Seger gave a science policy lecture at SUNY Stony Brook (February 24)
- Hud Freeze and Yvette Seger gave presentations on science communication at the annual meeting of the National Postdoctoral Association (March 18)
- Jennifer Zeitzer co-presented a workshop on "Advocacy at Home" at the COSSA Science Policy Conference (March 29)

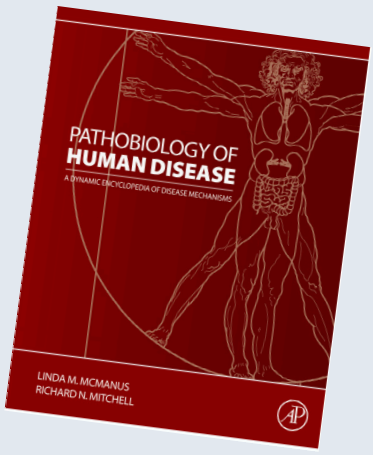
Provide Value for the FASEB Societies and Their Members

- FASEB helped the Teratology Society develop a commentary on Zika (January 18)
- FASEB issued a call for nominations for FASEB Excellence in Science Award (January 18)
- Jennifer Zeitzer trained society representatives for ASBMR Capitol Hill Day (January 31)
- OPA provided co-branded state and district factsheets for AAI (March 15) and Endocrine Society (March) Capitol Hill Days
- APS used FASEB's canine research factsheet in its letter to GAO (March 2)

HISTORY OF IMMUNOCYTOCHEMISTRY

Enjoy this detailed piece on the History of Immunocytochemistry now posted on the HCS website!

CLICK HERE TO READ

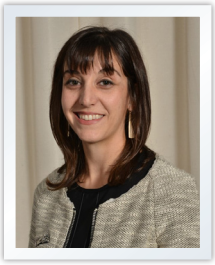


FASEB GOALS con't

- OPA created a slide set on the benefits of biomedical research for the American Society for Human Genetics (March 5)
- Jennifer Zeitzer and Ben Krinsky assisted AAI with its Capitol Hill Day (March 15)
- OPA reproduced its "Teach Evolution, Learn Science" buttons for SDB and ASHG (March 20)

Strengthen Effectiveness and Sustainability of the Federation

- OPA staff participated in FASEB Governance and Membership Reviews (February 7-8 and March 10-11)
- Hud Freeze spoke to the USHUPO Board (March 20)
- Hud Freeze gave the keynote address at the ABRF annual meeting (March 25)



2017 New Investigator Awardee – Amy Spinelli

Dr. Amy Spinelli received her Ph.D. in Biomedical Science from the Cardiovascular Sciences Department at Albany Medical College in Albany, NY. During this training she examined the role of altered smooth muscle calcium signaling in the induction of allergen induced airway hyperresponsiveness (AHR). She currently is a postdoctoral scholar in the Pediatrics Department at Pennsylvania State University College of Medicine in Hershey, PA.

Amy's present research focus is on examining sex-dependent differences in airway responsiveness to ozone, using a mouse model. This model recapitulates many symptoms of obstructive airway disease including airway neutrophilia and AHR. Structural remodeling of ozone challenged lung tissue was also observed using the label free two-photon microscopy techniques, second harmonic generation (SHG) and multiphoton excitation fluorescence.

In addition to her postdoc responsibilities, Amy is an intern in the college's Office of Technology Development.

HCS Honorary Lifetime Member – Shon-Rong Shi

Dr. Shi received his MD degree from West China University of Medical Sciences, Chengdu, China, in 1957. The early years of his career were spent as an ENT doctor at the same university in China. Because of his research project in nasopharyngeal carcinoma, he was sent to the Department of Pathology of the same university to have extensive pathology training for one year. This experience provided the solid foundation for his future study in pathology.

In early 1980, he passed a national examination and obtained a scholarship provided by Chinese government to study abroad. He was sent to Massachusetts General Hospital as a Visiting Scholar from 1982 to 1984. It was during this time that he started his life-long interest in the application and development of immunohistochemistry (IHC).

Shon-Rong Shi article con't

He returned to China in 1985 and published his first book entitled "Techniques of IHC" which was also the first IHC book in China. In early 1990s, he successfully developed the antigen retrieval (AR) technique which expands the application of IHC from largely frozen tissue only to formalin-fixed, paraffin-embedded (FFPE) tissue. AR has been recognized as a milestone in IHC and a revolutionary breakthrough in pathology.

Dr. Shi worked as a faculty member at Department of Pathology, University of Southern California since 1992 and retired in 2012. He published more than 70 articles and three books in AR. Because of his contribution and endeavor in the development of AR technique and IHC, he received the George Gomori Award on March 31, 2011 at the 62nd Annual Meeting of The Histochemical Society (HCS). Dr. Shi served as an Associate Editor of Journal of Histochemistry and Cytochemistry (JHC) (2003-2012), Editorial Board members of JHC, Applied Immunohistochemistry and Molecular Morphology, and Chinese Journal of Pathology. He was a Councilor of HCS from 2006 to 2010.

His major research interest is focusing on AR techniques as well as extending application of this AR technique in extraction of proteins and nucleic acids from FFPE tissue sections, and tissue proteomics.

Histochemical Society Capstone Grants

The purpose of the Histochemical Society's Capstone Research Grant is to provide assistance to further the research projects of undergraduate students who propose to use immunohistochemistry or other histochemical techniques in their projects. We aim to encourage undergraduate students to effectively utilize histochemistry as an analytical method for biological research. The Histochemical Society will award $500 to successful applicants. Preference will be given to students who hold a junior or senior academic standing. Recipients will be given approximately one year to perform the research. Grant recipients will be required to report their findings to the Histochemical Society Education Committee upon the completion of the project.

Applicant Requirements: Applicants must be undergraduates and current members of the Histochemical Society. Ideally, applicants will have conducted research previously and this award will be used to help the students to collect remaining data to prepare the project for publication or presentation. Preference will be given to applicants who will be juniors or seniors during the proposed research.

Evaluation: Applications will be evaluated on the effective and necessary use of immunohistochemistry or other histochemical techniques toward completion of the project. Likelihood of successful completion of the project (based on availability of appropriate laboratory space, equipment and institutional support) and significance of the question being addressed will also be considered.

Reporting: Successful applicants should submit a summary of their project in the form of a scientific manuscript. This should be submitted within 2 months of the project's completion date.

Timeline:
March 1 to May 15: Application site open
May: Applications reviewed
June: Applicants notified; Funds disbursed
August of following year: Project completion
October of following year: Report submitted to the HCS Education Committee

Acceptance of funding:
The Histochemical Society will work with awardees and their institution to determine the best mechanism for delivering the funds. The HCS reserves the right to publish names and photographs of the awardees on the HCS website and other informational forums, including social media sites such as Facebook and Twitter.

Contact Information:
Please contact Education Committee Chair, Scott Tanner (stanner@limestone.edu; 864-488-4500) or HCS Executive Director, Jennifer Holland (jholland@faseb.org) regarding any questions.

To Apply: https://hcsslb.wufoo.com/forms/srh7kqh0vq0sgj/

Revealing life in full color.

The Histochemical Society

Meet the Editor-in-Chief

Journal of Histochemistry & Cytochemistry

Join us at the Histochemical Society Booth #1537 for an opportunity to speak one-on-one with the newly appointed Editor-in-Chief of *JHC*.

Stephen Hewitt, M.D., Ph.D.

Monday, April 24th, 12 – 1 pm

Tuesday, April 25th, 12 – 1 pm

Stephen M. Hewitt, M.D., Ph.D. is a Clinical Investigator within the Laboratory of Pathology, National Cancer Institute and serves as head of the Experimental Pathology Laboratory. Stephen received his BA from the Johns Hopkins University, and his MD and PhD degrees from the University of Texas Health Science Center, Houston. He completed his residency in Anatomic Pathology at the NCI. Dr. Hewitt is Editor-in-Chief of the *Journal of Histochemistry &Cytochemistry*. Dr Hewitt has co-authored over 250 articles and servers on the editorial board of four peer-reviewed journals.