Advanced Microscopy Facility
Yalin Wang 434.924.2524

Bioinformatics Core
Stephen Turner 434.982.4208

Biomolecular Magnetic Resonance Facility
Jeff Ellena 434.924.3163

Biorepository and Tissue Research Facility
Craig Rumpel 434.982.0488

DNA Sciences
Yongde Bao 434.924.2553

Exercise Physiology Core Laboratory
Arthur Weltman 434.924.6191

Flow Cytometry Facility
Joanne Lannigan 434.924.0274

Genetically Engineered Murine Model Core
Wenhao Xu 434.982.6506

Lymphocyte Culture Center
Sallie Adams 434.924.1913

Mass Spectrometry
Nicholas Sherman 434.924.0070

Molecular Electron Microscopy Core
Kelly Dryden 434.982.0525

Molecular Imaging Core
Stuart Berr 434.924.5096

Molmart
Linda Beggerly 434.924.9211

Research Histology Core
Kenneth Tung 434.924.9205

Shared Instrumentation
John Shannon 434.243.9399

Tissue Culture Facility
Nena Fox 434.924.2501

RESEARCH CORES NEWS

UNIVERSITY OF VIRGINIA SCHOOL OF MEDICINE

From the Director of the School of Medicine Research Infrastructure and Office of Research Core Administration

Jay W. Fox, Ph.D.; Professor of Microbiology, Immunology and Cancer Biology

Fellow UVA School of Medicine Researchers: I hope you have enjoyed springtime in Virginia and are ready for summer! Many great things have been going on in the cores including fantastic science and acquisition of several new instruments. Of particular interest is the use of CRISPER/CAS by Drs. Shin and Farber conducted in the Genetic Engineered Mouse Models Core (GEMM). I believe you will see that this technology brought on-line by Laboratory Director, Dr. Wenhao Xu and the Faculty Director, Dr. Hui Zong have greatly enhanced the research capabilities for SoM investigators. Also, the acquisition of molecular interaction technology in the Shared Instrumentation Core is proving to be popular with a number of faculty. I encourage you all to read through the newsletter for details on these advancements.

The Lymphocyte Culture Core is redirecting its efforts toward advanced antibody technologies and these changes are reflected in its new name; the Antibody Engineering and Technology Core. To lead these changes we are in the process of hiring Dr. Bhupal Ban who has extensive experience in antibody engineering and moving novel antibody technologies into the market place. We expect Dr. Ban to assume the directorship of the core in late summer.

This month the SoM cores are submitting three S10 proposals for instrumentation (spinning disc microscope; high content cell screening instrument; and an Orbitrap mass spectrometer). I want to thank all the investigators who helped with these submissions and I wish them and the cores success! I would also like to remind faculty who use instrumentation acquired by S10 proposals that it is imperative that they reference those instrument grants in all publications. This is critical for future funding of shared instrumentation proposals.

Finally, I have seen a modest but significant increase in core activities which reflects the fact that our faculty have been enjoying increased success in their funding efforts. I applaud you efforts and remind all faculty that the SoM cores are ready to help however they can to strengthen your proposals and subsequently ensure you generate the best data possible for your research program. The SoM cores are ready and able to support your research and partner with you for our mutual success.

Have a great summer! Jay

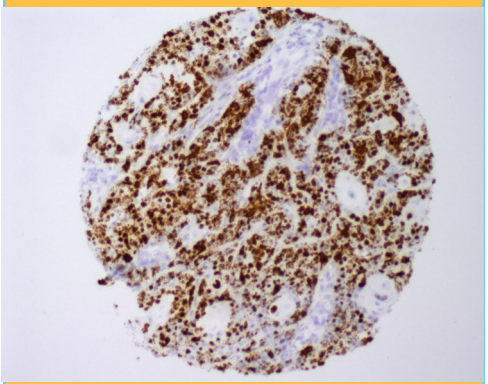
VOLUME 2, ISSUE 2 MAY 2015

The featured core for this quarter is the Genetically Engineered Murine Model Core, described on the following pages.

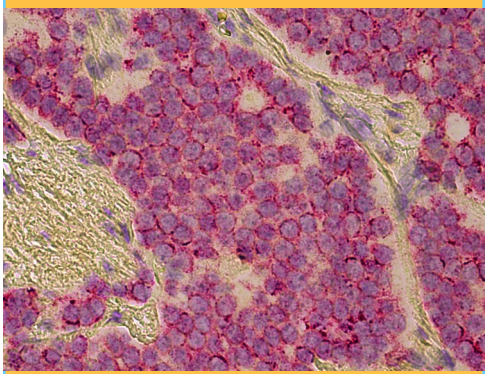
NEW SERVICE

The Biorepository and Tissue Research Facility now offers chromogenic RNA in situ hybridization (ISH) for histologic tissue sections. This powerful technique allows the specific localization of RNA targets in fixed tissues and cells, this providing correlation of specific gene expression with tissue compartments and cellular morphology. A novel probe design strategy and a hybridization-based signal amplification system (Branched DNA signal amplification) to simultaneously amplify signals and suppress background visualize single molecules in individual cells. The BTRF can analyze gene expression *in situ* FFPE and frozen sections of routine clinical and research specimens.

ACD RNAscope™ assay for High-Risk



Human Papilloma Viruses using DAB chromogen.



Affymetrix QuantiGene™ View RNA assay for chromagranin with Fast Red

Featured Core
Genetically Engineered Murine Model Core

"Building a Better Mouse Faster and Cheaper"

The GTTF (Gene Targeting and Transgenic Facility) has a new name – GEMM (Genetically Engineered Murine Model) Core. The "new" core will emphasize on creating human disease models and supporting translational research. The change is a necessary response to the challenges faced by traditional transgenic cores and to the evolving landscape in animal disease modeling. We believe this reorganization will open new directions for the core, make its expertise more integrated with other cores and create synergy with Center for Comparative Medicine to better serve UVa investigators. To celebrate this occasion in our 22 year history, we are very pleased to present two UVa labs that are pioneering the effort of adopting the Crispr-Cas9 technology in animals with the support of GEMM.

CRIPSR stands for "clustered regularly interspaced short palindromic repeats" and Cas9 is a nuclease. Both CRISPR and Cas9 are found in bacteria where they work together to function as a prokaryotic immune system. It was recently discovered that this system can be coopted and used in eukaryotic cells and organisms to generate mutations at specific DNA sequences. The system works by using a "guide RNA" to direct Cas9 to a certain region of the genome. Once targeted, Cas9 induces a double strand break, which is repaired by an error-prone process, leaving behind mutations at a high frequency. Using CRISPR-Cas9 the GEMM can generate mice harboring mutations at a particular gene by injecting fertilized mouse zygotes with a guide RNA complimentary to the targeted gene and Cas9. This has resulted in the ability of the GEMM to create mutant mice in approximately 5 weeks instead of months that it took using traditional approaches. In addition to global knockouts, the GEMM is also creating conditional knockouts and knock-in mutants in which specific mutations are precisely introduced with CRISPR-Cas9.

In a remarkably short time period, the CRISPR)-Cas system has emerged as the most efficient genome engineering tool to date. The ease of experimental implementation lead to an unprecedent-ed democratization of genome editing efforts, sweeping across all biological research areas and experimental model species.

UPCOMING EVENTS IN RESEARCH CORES

Flow Cytometry training courses

August 17-21, 2015

Mid Atlantic Directors and Staff of Scientific Cores and Southeastern Association of Shared Resources

The University of Virginia SOM is hosting the *2015 Joint Conference of the Mid-Atlantic (MADSSCi) and Southeast (SEASR) Regional Chapters of the Association of Biomolecular Research Facilities (ABRF)* June 3rd-5th, at the Darden Conference Center.
A Pre-Conference Workshop entitled *"Business Skills for Core Managers, Directors, and Administrators"* is being offered through the Darden Executive Education Program(full).
Joanne Lannigan (FCCF), Julie Burns (ORCA), and Lesa Campbell (FCCF) are the local organizers for this conference.
A full description of the conference can be found here: http://madssci.abrf.org/. There are a limited number of "free" limited registrations (for attending scientific sessions and exhibitors only, no social events) available for this meeting, please contact Joanne Lannigan (jl7fj@virginia.edu) if interested.

Program highlights:

- Scientific sessions on research techniques and applications
- Vendor workshops on use of their instruments
- Meet cores offering services not currently available at UVA

Understanding the Genetics of Hearing

Dr. Jung-Bum Shin lab, Department of Neuroscience

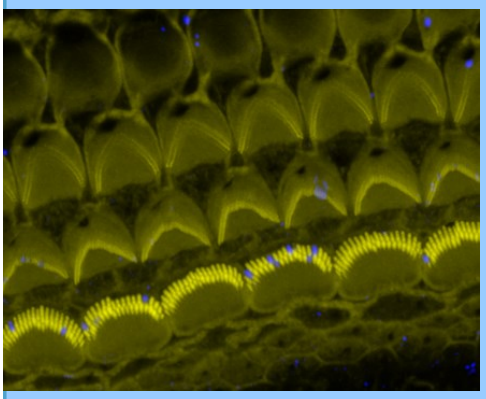
It did not take long for Dr Jung-Bum Shin, an assistant professor in the Department of Neuroscience, to realize the potential of CRISPR for his research. The Shin lab works on the molecular mechanisms underlying hearing and deafness. As the most prevalent sensory disorder, deafness has a strong genetic component. The Shin lab uses a proteomics-based method in conjunction with the Mass Spectrometry core lab to discover novel deafness genes, and creates mouse models to study these potential deafness genes *in vivo.*

"It used to be that grant applications, and often careers, depended on the successful generation and interesting phenotype of one mouse model. Now you can actually try out multiple mouse models without breaking the bank. And equally important, you can generate mice in a time span of a few months, which is significant considering the demands of short time cycles of NIH funding". For Dr. Shin, early adoption of CRISPR has paid off, leading to accelerated publications (1) and new NIH R01 funding (2). Under the leadership of Dr. Xu, Dr. Shin has assisted several labs in implementing CRISPR for their own research, and will also participate in future training sessions to enable implementation and development of this crucial technology at UVa.

Work published by the Shin lab this year using CRISPR, in collaboration with the GEMM.

(1)Francis SP, Krey JF, Krystofiak ES, Cui R, Nanda S, **Xu Wenhao**, Kachar B, Barr-Gillespie PG, **Shin Jung-Bum**. A short splice form of Xin-actin binding repeat containing 2 (XIRP2) lacking the Xin repeats is required for maintenance of stereocilia morphology and hearing function. *J Neurosci*. 2015 Feb 4;35(5):1999-2014

(2)NIH/NIDCD, R01, title: "Role of XIRP2 in hair cell function and degeneration".



Confocal microscopy immunofluorescence image from the Advanced Microscopy Facility of cochlear hair cells. F-actin labeled in green, novel deafness candidate protein labeled in blue.

USE OF NIH FUNDED EQUIPMENT

NIH requires investigators who use instruments funded by NIH SIG and HEI grants to acknowledge the use in publications.

A list of equipment in the research cores funded by these grants is at:

http://www.medicine.virginia.edu/research/cores/orca/nih-funded-equipment-list.html

NEW EQUIPMENT

Microscopy

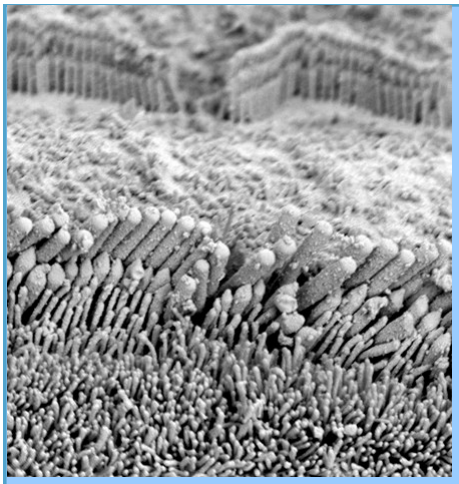
The Advanced Microscopy Facility has replaced the Zeiss LSM 510 in MR4 with the 880 model, which is more sensitive, has six lasers, can read 10 dyes simultaneously, fast data handling and has a heated environmental chamber for live cell imaging. Funded by Equipment Trust Fund.

http://www.medicine.virginia.edu/research/cores/microscopy/equipment/lsm-880.html

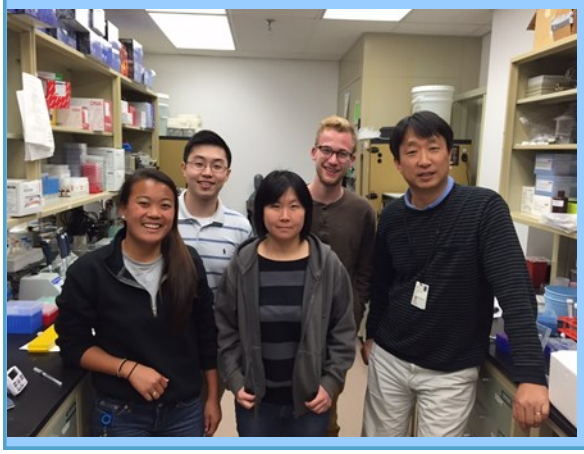
Molecular Interactions

The fortéBIO Octet molecular interactions instrument in the Shared Instrumentation Core is in operation. The instrument dips immobilized proteins or other ligands into a solution of analyte to measure association and dissociation rates through Bio-Layer Interferometry. It can also measure dissociation constants in system which reach rapid equilibrium and perform quantitation instead of using an ELISA assay. Because of the low cost of probes, finding regeneration conditions may be unnecessary. First users are happy with the instrument and prefer it to SPR type instruments. Funded by Equipment Trust Fund.

http://www.medicine.virginia.edu/research/cores/biomolec/Shared_Instrumenation/molecular-interactions



Scanning electron micrograph from Advanced Microscopy Facility of mouse cochlear organ of Corti, inner hair cell bundles in the front, outer hair cell bundles in the background.



Shin laboratory, left to right: Brianna Kim, Sibo Zhang, Tingting Du, Will Butler, Jung-Bum Shin

Mouse Models of Human Disease

Dr. Charles Farber Lab, Center for Public Health Genomics

Charles R. Farber is a resident faculty member in the Center for Public Health Genomics. Charles' research is focused on identifying genetic differences that influence an individual's risk of developing common diseases. Though he has an interest in a number of diseases, his lab primary works on osteoporosis, a condition of weak and fragile bones that affects millions of individuals. Susceptibility to bone fragility is primarily determined by an individual's genetic makeup and by identifying the contributing genes they hope to be able to develop new approaches to prevent and treat this devastating disorder.

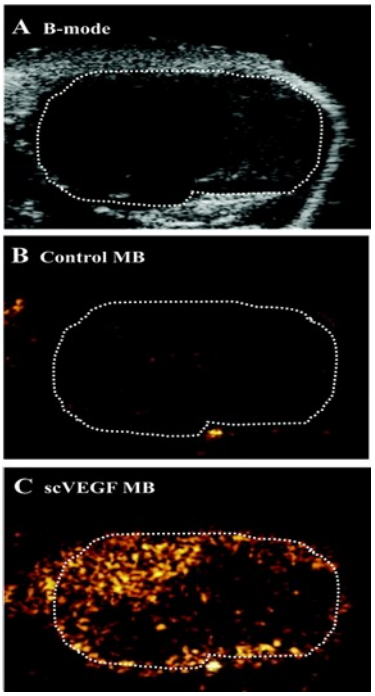
A major component of Charles' research is the development and characterization of knockout mice. Mouse "knockouts" lack a functional copy of a specific gene and by characterizing knockout mice, Charles' lab is able to determine if a particular gene plays an important role in osteoporosis-related bone traits. Since arriving at UVa in the fall of 2008, all of the knockout mice characterized by Charles' lab have been developed by the GEMM. The GEMM specializes in the development of mouse models and serves a large number of investigators across many departments in the School of Medicine. Historically the GEMM has created knockout mice using homologous recombination in embryonic stem cells to replace a normal gene with a defective copy. This approach is effective, but laborious, costly and slow. Recently, through the hard work and dedication of its director,

NEW SERVICE- ULTRASOUND IMAGING

The Molecular Imaging Core recently acquired an ultrasound scanner to perform imaging for anatomy, tumor sizing, tissue perfusion and imaging with targeted contrast bubbles. Some applications are quick visualization and sizing of tumors, monitoring and quantifying tumor blood flow, and providing information on the molecular biomarkers of tumor neovasculature (e.g., neuropilin, avb3, VEGFR2). This system is also suitable for imaging perfusion in other organs .

Ultrasound imaging provides unsurpassed detection sensitivity for microbubble contrast agents: a single microbubble (~1-2 µm in diameter and <1 pg in mass) can be visualized in real time. Due to the rapid clearance of circulating microbubbles from the bloodstream, one imaging session (with one contrast type) takes only ~15 min.

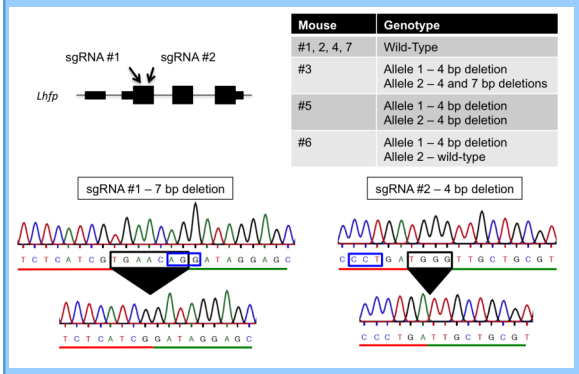
Ultrasound studies will be performed in a small operating room inside the MR4 vivarium .Contact Dr. Sasha Klibanov (sklib1@gmail.com) to use this service.



Ultrasound Molecular Imaging of VEGFR2 with scVEGF-decorated microbubbles in a subcutaneous colon adenocarcinoma model. A: grayscale (anatomy) image of tumor mass. B: contrast US image of non-targeted microbubbles after 6-minute dwell time. C: contrast US image of adherent scVEGF-microbubbles.

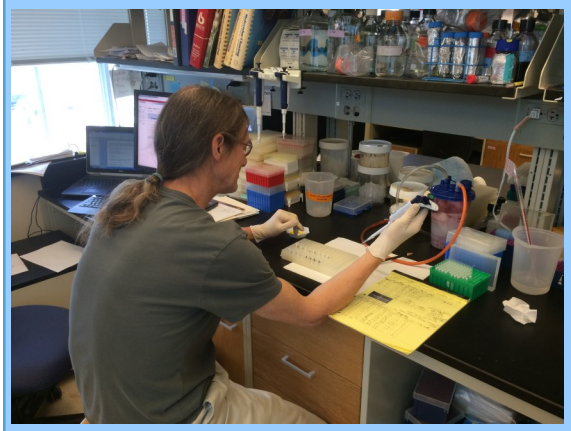
Dr. Wenhao Xu, the GEMM began investigating new ways generate knockout mice in a quicker and more cost effective manner and adopted CRISPR-Cas9 technology.

The Farber lab has benefited from the adoption of the CRISPR-Cas9 technology by the core. Using CRISPR-Cas9 the GEMM created a knockout mouse for the lipoma HMGIC fusion partner (*Lhfp*) gene for Charles' lab. *Lhfp* was a gene that the Farber lab identified as involved in the function of bone-forming cells called osteoblasts. In vitro attempts to characterize the function of *Lhfp* in osteoblasts had proven problematic, but now that lab has a robust model they can use to determine the role of *Lhfp* in osteoblast in vivo. Importantly, CRISPR-Cas9 provided a way to generate this model at a fraction of the time and cost of traditional approaches. To date, the GEMM has generated 6 (of 13 total) founder mice that harbor mono- (2) or biallelic (4) mutations, all of which resulted in truncated *Lhfp* proteins.



Strategy and result of the first successful attempt to generate frameshift mutation in the *Lhfp* gene by using simultaneously two small guide RNAs (sgRNA #1 and #2).

For the Farber lab, the GEMM has provided support for one R01 and three pending R01 applications. In addition, the data generated from the models created by the GEMM will be included in three upcoming publications.



Larry Mesner in Farber lab is setting up the assay for screening the potential founder mice.

NEW BILLING AND RESERVATION SYSTEM FOR CORES

The research cores have switched to the iLab billing system for billing and some reservations.

Log into iLab using your computing ID and eServices password at:
https://uva.corefacilities.org
Windows users should use Firefox or Chrome browsers. Internet Explorer may work poorly or not work at all.
Instructions at: http://www.medicine.virginia.edu/research/cores/orca/cores-billing-system/ilab

The head of a lab must register so that lab members can be associated with a lab. The head of lab or designated person (lab manager, fiscal administrator) then needs to assign PTAEO(s) to lab members.
The Molmart product core will switch to iLab later.
As common with a complex system, there have been delays and problems. If you encounter a problem, you can report it to the core you are using, ORCA business manager Paul Shin or iLab.

NEW EQUIPMENT REQUESTS

The Mass Spectrometry Laboratory has submitted an NIH S10 grant requesting an Orbitrap Fusion Tribrid mass spectrometer. This instrument operates at higher resolution and mass accuracy than the current instrument. New, more efficient fragmentation modes analyze lower abundance samples, give more complete data from each peptide to make more confident identifications, and reduce false positive identifications of proteins. The high accuracy increases confidence in peptide identification. The improvements in instrument performance give much improved label free quantitation of proteins in complex mixtures, and improved quantitation using labels. Sensitivity is higher and performance is ten or more times faster than the current Orbitrap.

PAPERS AND GRANTS CO-AUTHORED WITH RESEARCH CORES

FLOW CYTOMETRY

Asya Smirnov, **Michael D. Solga**, **Joanne Lannigan** , Alison K. Criss *An improved method for differentiating cell-bound from internalized particles by imaging flow cytometry*, published online May 09, 2015 in Journal of Immunological Methods. http://dx.doi.org/10.1016/j.jim.2015.04.028. This manuscript is scheduled to be published in a Special Issue of JIM dedicated to Imaging Flow Cytometry.

Graham M. Richardson, **Joanne Lannigan** and Ian G. Macara "Does FACS Perturb Gene Expression?" *Cytometry A*. **87(2)**:166-175 Feb 2015

Joanne Lannigan, in collaboration with Loren Erickson, Scott Commins, Judith Woodfolk, Coleen McNamara, Ani Manichaikul, was awarded a Transformative, Collaborative Science Pilot Grant for the proposal entitled: "High-dimensional immune profiling of diverse inflammatory diseases using mass cytometry"

BIOINFORMATICS

James C. Cronk, NoelC. Derecki, Emily Ji, Yang Xu, Aaron E. Lampano, Igor Smirnov, Wendy Baker, Geoffrey T. Norris, Ioana Marin, Nathan Coddington, Yochai Wolf, **Stephen D. Turner**, Alan Aderem, Alexander L. Klibanov,Tajie H. Harris, Steffen Jung, Vladimir Litvak,and Jonathan Kipnis *Methyl-CpG Binding Protein 2 RegulatesMicroglia and Macrophage Gene Expression in Response to Inflammatory Stimuli* Immunity 42, (4), 21 April 2015, 679–691

Carlos E. Barbery, Frank A. Celigoj, **Stephen D. Turner**, Ryan P. Smith, Parviz K. Kavoussi, Brian H. Annex and Jeffrey J. Lysiak "Alterations in microRNA Expression in a Murine Model of Diet-Induced Vasculogenic Erectile Dysfunction" *Journal of Sexual Medicine*. **12 (3):**621-30, 2015

Hurney CA; Babcock SK; Shook DR; Pelletier TM; **Turner SD**; Maturo J; Cogbill S; Snow MC; Kinch K. "Normal table of embryonic development in the four-toed salamander, Hemidactylium scutatum". *Mechanisms of Development*. **136**:99-110, 2015

Mathers AJ; Stoesser N; Sheppard AE; Pankhurst L; Giess A; Yeh AJ; Didelot X; Turner SD; Sebra R; Kasarskis A; Peto T; Crook D; Sifri CD. "Klebsiella pneumoniae carbapenemase (KPC)-producing K. pneumoniae at a single institution: insights into endemicity from whole-genome sequencing". *Antimicrobial Agents & Chemotherapy*. **59 (3):**1656-63, 2015

DNA SCIENCES, EXERCISE PHYSIOLOGY CORE, BTRF

Argo CK; Patrie JT; Lackner C; Henry TD; de Lange EE; **Weltman AL**; Shah NL; Al-Osaimi AM; **Pramoonjago P**; Jayakumar S; Binder LP; Simmons-Egolf WD; Burks SG; **Bao Y**; Taylor AG; Rodriguez J; Caldwell SH. "Effects of n-3 fish oil on metabolic and histological parameters in NASH: a double-blind, randomized, placebo-controlled trial". *Journal of Hepatology*. **62(1)**:190-7, 2015

MOLECULAR IMAGING CORE AND MOLECULAR ELECTRON MICROSCOPY CORE

Anna B Banizs, Tao Huang, Kelly Dryden, Stuart S Berr, James R Stone, Robert K Nakamoto, Weibin Shi, Jiang He "In vitro evaluation of endothelial exosomes as carriers for small interfering ribonucleic acid delivery" *Int J Nanomedicine*. 2014; **9**: 4223–4230

SHARED INSTRUMENTATION

Andarawewa KL, Moissoglu K, Sup Lee C, Ando Y, Yu M, Debnath P, **Shannon JD**, Sirinivasan S, Conaway MR, Weber MJ, et al. "Integrin adjunct therapy for melanoma". *Pigment Cell Melanoma Res.* 2015 Jan;**28(1):**114-6

MASS SPECTROMETRY

Granato DC; Zanetti MR; Kawahara R; Yokoo S; Domingues RR; Aragao AZ; Agostini M; Carazzolle MF; Vidal RO; Flores IL; Korvala J; Cervigne NK; Silva AR; Coletta RD; Graner E; **Sherman NE**; Paes Leme AF. "Integrated proteomics identified up-regulated focal adhesion-mediated proteins in human squamous cell carcinoma in an orthotopic murine model." PLoS ONE [Electronic Resource]. 9(5):e98208, 2014

NEW EQUIPMENT REQUESTS
(CONTINUED)

DNA Sciences has requested a NextSeq 500 benchtop genome sequencer which can sequence a human genome in one lane in 30 hours with 30X coverage. The core has requested funding from Equipment Trust Fund.

Advanced Microscopy has three requests.

For the new Zeiss LSM 880, they have requested fund from ETF for an AiryScan detector which increases resolution to 140 nm lateral, 400 nm for higher resolution scanning.

With Paula Barrett, Adavnced Microscopy Facility is submitting an S10 grant application for an Andor Technology Revolution™ XDb-H spinning disk confocal imaging system whose rapid scanning allows live cell imaging and photomanipulation of tissues, small organism and plated cells.

With John Lazo, Adavnced Microscopy Facility is submitting an S10 grant for a high content imaging systm. The Operetta platform delivers fully automated image acquisition, analysis, and data management for robust phenotypic fingerprinting. It's a spinning disc confocal system configured for both fixed and live cell imaging in the format of plate or slide. A robotic plate handler and an extension incubator allows unsupervised imaging of up to 42 plates of live or fixed samples. It comes with sophisticated image analysis software for identifying and quantitating a wide variety of cellular parameters.

BIANNUAL OUTSTANDING EMPLOYEE

SCHOOL OF MEDICINE RESEARCH CORES

Ms. Linda Beggerly, MS, have been voted by the Office of Research Core Administration Core Directors as the first 2015 biannual Outstanding Core Staff Member. Linda has been employed within the SoM core system for approximately 31 years. Linda began her service within the core system in the Biomolecular Research Facility working with Dr. Jay Fox performing amino acid analyses. She then continued with Edman protein sequencing and peptide synthesis. Currently Linda operates "MolMart" providing supplies and reagents to SoM investigators. Linda's dedication to supporting our faculty as well as her ability to adapt to the changing demands of research support has made her a natural candidate for the ORCA a 2015 Biannual Outstanding Core Staff Member. Congratulations Linda.

NEW EXPERTISE

Claude Chew, B.S. CCy, Laboratory Research Specialist Senior in the Flow Cytometry Core Facility, is now a Certified Cytometrist after passing the rigorous International Cytometry Certification Exam.

ACKNOWLEDGEMENT OF USE OF RESEARCH CORES

If you use data generated by a research core in a paper, please acknowledge the core and we would be delighted if you tell us that you have published data from our cores.

The School of Medicine supports the cores financially. Publications that use the cores show that support from the School results in research that would otherwise not be feasible .

OFFICE OF RESEARCH CORE ADMINISTRATION

P.O. Box 800741
University of Virginia

Fax: 434.982.6963
E-mail: ps4sf@virginia.edu

Links to research cores
www.medicine.virginia.edu/research/cores/orca

Services in research cores
http://www.medicine.virginia.edu/research/cores/orca/services-in-research-cores

Equipment in research cores
http://www.medicine.virginia.edu/research/cores/list-of-equipment-in-research-cores.html

Training in research cores
http://www.medicine.virginia.edu/research/cores/orca/training-opportunities-in-research-cores.html

Outstanding Core of The Year

Most years, one research core is chosen as the Outstanding Core of the Year. The selection may be for continued excellence or a major accomplishment during the year.

2003 Flow Cytometry Core Facility
For excellence of services and cost recovery

2004 Advanced Microscopy Facility
For procuring state of the art equipment to continue and expand excellent services

2005 Molecular Imaging Core
For receiving one of 10 national major equipment grants for a cyclotron

2006 Mass Spectrometry Facility
For advances in instrumentation and informatics

2007 Gene Targeting and Transgenic Facility
For increase in business and funding

2008 Lymphocyte Culture Center
For many years of solid business and science even in times of tight funding

2009 Biorepository and Tissue Research Facility
For expansion of services and activity in recent years

2011 Biomolecular Magnetic Resonance Facility
For creation and efficient operation of a campus wide NMR spectroscopy facility

2012 Advanced Microscopy Facility
Awarded for adding two major pieces of equipment and providing outstanding service to many investigators

2013 Bioinformatics Core
Rapidly established a source of customized solutions to bioinformatics problems.

2014 Molecular Imaging Core