

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

Gene Targeting and Transgenic Facility

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 Associate Dean for Research Infrastructure, Jay W. Fox

This newsletter represents a new approach to communicating with the faculty to inform them of the exciting advances being supported by the UVA School of Medicine Cores and the Office of Research Core Administration (ORCA). Our plan is to publish this newsletter quarterly highlighting upcoming workshops and training conducted by the cores, new instrumentation and staff in the cores as well as highlighting outstanding research being performed by a School of Medicine faculty member with the support of one of the cores.



Over the past year our organization has made great strides forward in providing a wider range of training opportunities to better utilize the services and technology offered in the cores. In addition, all the core staff have been diligent in seeking ways to hold down the costs so that your research dollars can go further in the cores. We have also been working on developing our nexus of core services with the aim of providing a seamless service across all the cores. In future communications we will highlight some of the successes of this approach to service provision.

Finally, we welcome any news you might wish to report in this communication as well and would be glad to highlight your research that was supported by the cores or core instrumentation. In line with this, I would like to take this opportunity to again reach out to the research community and ask that if you have suggestions for the cores or ideas for new instrumentation, training or services please contact me and I will be glad to discuss them with you and your laboratory staff.

VOLUME 1, ISSUE 1 AUGUST 2014

Introduction

This is the first newsletter to bring news from the research cores. Each quarterly issue will highlight how one of the research cores has enabled an investigator to advance a project; this quarter's core is the Flow Cytometry Core Facility (see below)

New Core

The Molecular Electron Microscopy Core opened in spring. The core specializes in electron cryomicroscopy for structural biology projects. Three electron microscopes are at the Fontaine Research Park location.

CORES Billing System

ORCA is investigating systems which may provide enhance administrative capabilities over our existing CORES billing system, including an instrument booking and billing system. We will make a decision in summer.

UPCOMING EVENTS IN RESEARCH CORES

Flow Cytometry Core Facility

[classes](#) August 11-15; October 27-31

Seminar: New Technologies for Live Cell RNA Detection and Profiling, Janell Rex, Ph.D. (EMD-Millipore), August 14th,

NEW SERVICES AND EQUIPMENT

Digital PCR system

The DNA Sciences Core now has a digital droplet PCR system providing greatly enhanced sensitivity and dynamic range over traditional real time PCR. See:

<http://www.medicine.virginia.edu/research/cores/biomolec/dna/rtpcr-page>

Cell Separation System

Thanks to the efforts of Dr. Janet Cross of Pathology, ETF funds were awarded for the purchase of an **autoMACSPro magnetic cell separation system**, which is available for a fee for service to all investigators. For more information please see <http://www.medicine.virginia.edu/research/cores/FlowCytometry/equipment/automacs-pro.html>

Mass Cytometer

The **CyTOF2 mass cytometer** is up and running and data are being generated! We are currently offering introductory runs for free on the instrument. If you are interested in pursuing this technology please contact Joanne Lannigan at 4-0274 or jl7fj@virginia.edu to set up a consultation. Please visit our website for more information and details <http://www.medicine.virginia.edu/research/cores/FlowCytometry/equipment/cytof-2.html>.

Featured Core

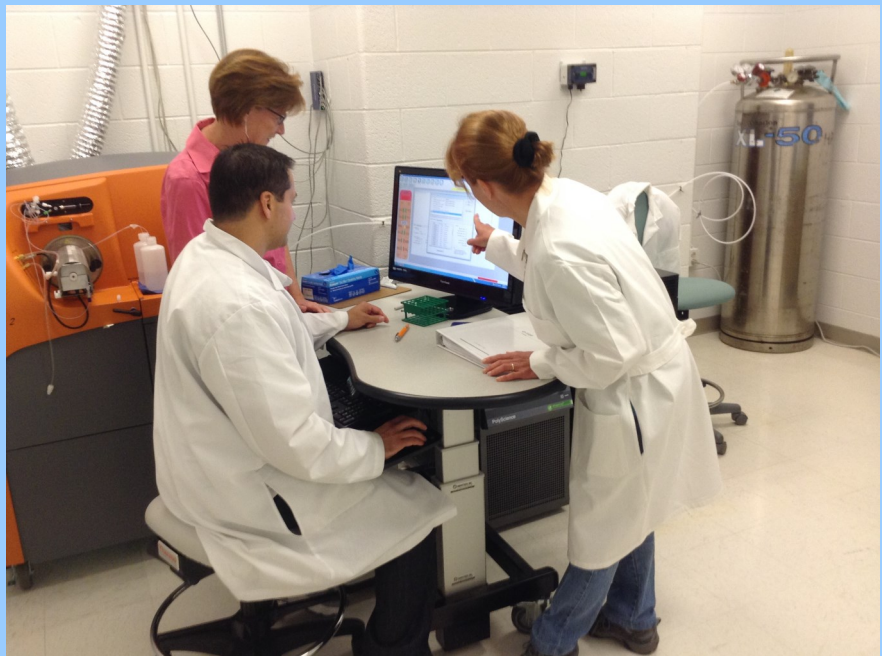
Flow Cytometry Core Facility

Coleen McNamara, M.D., Edward W. and Betty Knight Scripps Professor of Internal Medicine; Professor of Medicine/ Cardiovascular Medicine and Molecular Physiology and Biological Physics



In addition to being a clinician in Cardiology, Coleen has an active and productive research lab in the Cardiovascular Research Center (CVRC). Coleen's research interest is in understanding the role of the Id3 transcription factor as a major regulator of atherosclerosis and obesity. In fact, her initial work studying the role of Id3 in regulating the immune response in atherosclerosis lead her lab to become one of the largest users of the FCC. Initially the work revolved around finding the right digestion protocols for quantitating immune cells in the aorta (and other tissues) without destroying the typical surface markers used to identify the immune subsets.

Amanda Doran, a graduate student in the McNamara lab, spent many



Coleen McNamara (left), Mike Solga (center) and Chantel McSkimming (right) at the CyTOF2 mass cytometer.

hours in the core facility to develop the protocols to be able to phenotype and enumerate the different immune subsets present in tissues from both normal and atherosclerotic mice. With use of the CyAn ADP flow cytometer and 9 fluorescent markers, Amanda was able to demonstrate Id3 regulated predominantly the number of B cells in the aorta.

CORE STAFF CHANGES

Sallie Adams is interim director of the Lymphocyte Culture Center. She has many years experience in hybridoma production at the University of Virginia and Vanderbilt University. In recognition of her efforts, she has been chosen Outstanding Employee-see page 5

The Flow Cytometry Core Facility is pleased to welcome the newest addition to our staff, Victor Guo, Ph.D. Victor comes to us from MSKCC where he worked as a Flow Cytometry Specialist in the Dept. of Pathology since 2011. Prior to this Victor was an avid user of flow cytometry during his graduate and post doctoral training in Immunology & Microbial Pathogenesis at Weill Medical College of Cornell University. Victor has over 7 years of experience performing 12 or more color analytical flow cytometry and more than 3 years of cell sorting experience.

CORE EMPLOYEE RAISING FUNDS FOR PATIENT CARE RESEARCH

R. Jack Roy (Molecular Imaging Core) initiated and organized the *Blues for a Cure* concert June 29 with world class musicians and multiple genres of the blues to raise funds to support pancreatic cancer research. The audience, ranging from 5 to over 80, wanted to support the outstanding work of Dr. Todd Bauer and help prevent the losses that some of them have experienced.



Photo by Ken Lane

Following studies using flow cytometry, analysis of aorta after adoptive transfer of B cells to a B cell deficient host revealed that Id3 regulated homing of B cells to the vasculature and that the loss of Id3 resulted in loss of B cell-mediated atheroprotection. This work was published in *Circ. Res.* 2010 Apr 16;106(7):1303-11. Ongoing research continues to explore how these B cells regulate atherosclerosis and whether similar subsets of B cells identified in mice are applicable to human disease.



Beckman Coulter CyAn ADP LX

In addition, Coleen is co-PI of a Human Core lab for a PPG in collaboration with Dr. Angela Taylor. This Human Core also services their PPG colleagues, Drs. Lynn Hedrick, Klaus Ley, Yury Miller, Joe Witztum and Sam Tsimikas at La Jolla Institute of Allergy and Immunology and UCSD. Critical to these investigations is the

need to evaluate many different markers on these cells in addition to markers of function, such as cytokine/chemokine production, proliferation and cell signaling. It is also important to identify the presence of other immune cell subsets in addition to B cells that may be playing a role in this process. To do this in humans requires the use of minimal amounts of sample from the blood of highly characterized patients with known degrees of atherosclerosis.

The need to measure many more markers than is reasonably feasible using traditional fluorescence probes has led the McNamara Lab to pursue the mass cytometry technology, which allows measurement by mass spectrometry up to 40 different tags using heavy metal isotopes instead of fluorescence. These types of analyses with traditional flow would require up to 50 different tubes, with many markers needing to be repeated between tubes, creating

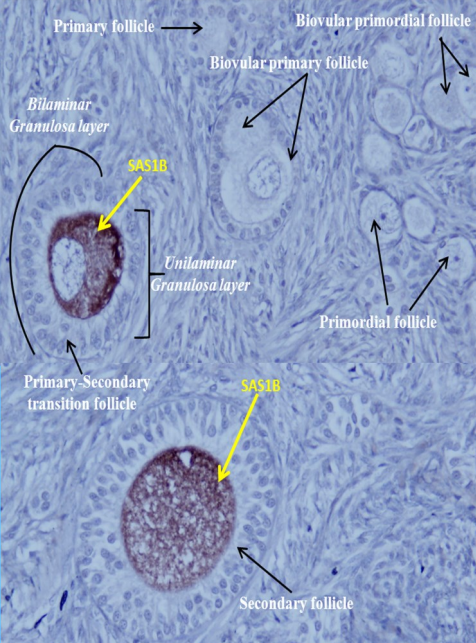


duplication of reagents. Mass cytometry will provide better-correlated data, less tube-to-tube variability while utilizing less sample. The McNamara lab is currently measuring 27 markers and will be adding another 7 to the panel shortly. This will be a critically important factor as the McNamara Lab pursues funding to study samples from the

Framingham Project (<http://www.framinghamheartstudy.org/>), which has a cohort of well-characterized (genome/phenome) cardiovascular patients. Previous studies from this cohort showed a strong association with B cell related genes and cardiac disease, a key driver for the more in depth study using CyTOF. The presence or absence or increase/decrease of certain immune subsets may lead to an important biomarker for predicting disease. The availability of the CyTOF has also peaked the interest for studies with Astra-Zeneca for evaluating “on target” and “off target” effects of compounds with regard to pro-atherosclerotic activity, anti vs. pro inflammatory responses, regulation of immune phenotypes, etc.

ADVANCED MICROSCOPY FACILITY IMAGE OF THE QUARTER

AMF invites users to submit images taken in the facility for consideration as Image of the Quarter. The user submitting the image selected will be awarded a week of free use of AMF equipment. The facility will post the image on the AMF website and in a gallery in the AMF hallway. Images can be submitted anytime and will be considered for the corresponding quarter depending on the time of submission. See more of the [first image](#) on the AMF website.



Immunolocalization data of oocyte specific protein SAS1B by Eusebio Pires in laboratory of John Herr.

Send your images to Yalin Wang
yw6x@virginia.edu

In addition to traditional polychromatic flow cytometry and mass cytometry, the McNamara Lab has taken advantage of other services provided by the FCC such as cell sorting for further down stream assays, i.e. Elispot, adoptive transfer and proliferation assays, as well as Luminex bead based assays for screening chemokine panels to determine which are regulated by Id3.

Data from the FCC has provided support for three RO1s, one PO1, a Clinical Translational Research Award and an Astra-Zeneca Research Award, funding totaling \$16 million.



CyTOF 2 Mass Cytometer

In addition, the following publications have resulted from data generated in the FCC:

1. Perry HM, Oldham SN, Fahl SP, Que X, Gonen A, Harmon DB, Tsimikas S, Witztum JL, Bender TP, McNamara CA. Helix-loop-helix factor inhibitor of differentiation 3 regulates interleukin-5 expression and B-1a B cell proliferation. *Arterioscler Thromb Vasc Biol.* 2013 Dec;33(12):2771-9.
2. Doran AC, Lipinski MJ, Oldham SN, Garmey JC, Campbell KA, Skafien MD, Cutchins A, Lee DJ, Glover DK, Kelly KA, Galkina EV, Ley K, Witztum JL, Tsimikas S, Bender TP, McNamara CA. B Cell aortic homing and atheroprotection depend on Id3. *Circ Res.* 2012 Jan 6;110:e1-12. PMID 22034493.
3. Lipinski MJ, Campbell KA, Duong SQ, Welch TJ, Garmey JC, Doran AC, Skafien MD, Oldham SN, Kelly KA, McNamara CA. Loss of Id3 increases VCAM-1 expression, macrophage accumulation, and atherogenesis in Ldlr^{-/-} mice. *Arterioscler Thromb Vasc Biol.* 2012 Dec;32(12):2855-61.
4. Doran AC, Lehtinen AB, Meller N, Lipinski MJ, Slayton RP, Yeboah J, Rich SS, Bowden DW, and McNamara C A. Id3 is a Novel Atheroprotective Factor Containing a Functionally Significant SNP that is Associated with Intima-Media Thickness in Humans. *Circ Res.* 2010 Apr 16;106(7):1303-11. PMID: 20185798. No PMID
5. Harmon DB, Oldham SN, McSkimming C, Perry HM, Kirby JL, Kaplan JL, Hollowell P, Schirmer B, Tsimikas S, Taylor AM, McNamara CA. Protective role for B-1b B cells and IgM in obesity. Under Revision for *Circulation Research* 2014.

ACKNOWLEDGEMENT OF NIH FUNDED EQUIPMENT USE

NIH rules require investigators who use equipment funded by Shared Instrumentation Grants and High End Equipment grants in the last five years to acknowledge the equipment in papers. See: <http://www.medicine.virginia.edu/research/cores/orca/nih-funded-equipment-list.html>

We also ask you to acknowledge use of cores in papers to show how they contribute to School of Medicine research.

OFFICE OF RESEARCH CORE ADMINISTRATION

P.O. Box 800741
University of Virginia
Tel: 434.982.6565
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>

All current projects in the McNamara lab utilize the FCC. One might wonder with all the flow cytometry this research lab does why they have never pursued buying their own instrumentation. When asked, Coleen stated that working with the core provides more than just the data, and that the real value was in the education, networking, seminars, consultative guidance and the expertise and direct involvement of the core staff in the various projects.

The FCC has enjoyed working with the McNamara Lab and congratulates all of the students (Amanda Doran, Mike Lipinski, Heather Perry, Jennifer Kaplan, Dan Harmon, Sam Morris-Rosenfeld), post docs (Angela Taylor (now her own research PI collaborating with Coleen), Prasad Srikakulapu) and Lab Specialists (Chantel McSkimming, Melissa Marshall, and Lab Manager Jim Garmey) for their outstanding scientific contributions to the field of Cardiovascular Research.

BIANNUAL OUTSTANDING EMPLOYEE SCHOOL OF MEDICINE RESEARCH CORES

Sallie Adams,
Interim Director, Lymphocyte Culture Center

Sallie Adams has been employed at UVa for over 30 years in several laboratories. In all of her positions she has been actively involved in various types of cell culture techniques conducted with numerous cell types. 17 years ago Sallie joined the staff of the Lymphocyte Culture Core and over the years she has been involved with 400 fusions supporting the investigations of 150 researchers at UVa, other academic institutions, companies and the U.S. Government. According to Sallie each project and investigator is different and have different aspects that must be considered. From Sallie's experience the key to success is developing a partnership with the investigator and having clear and precise communications to ensure that all parties understand the goals and expectations of the project.



The hybridomas and monoclonals that Sallie and the LCC have produced over the years have been cited in many publications, many have been patented or licensed and some have ultimately found application in diagnostic kits. Furthermore, some of these reagents have brought substantial financial returns to the University and the PI. The LCC staff firmly believes in providing the highest quality service and reagents to University investigators and Sallie is delighted that the community of core staff directors has recognized her dedication and service and thanks everyone for this recognition.

WHAT DID YOU THINK?

Go to <https://www.surveymonkey.com/s/CoresNews> to complete a short survey on this newsletter and become eligible for a drawing for \$1000 of service* in a research core of your choice.

*not valid for product cores, must be used by 12/31/2014.