#### The UAB-UCSD O'Brien Center Workshop on Flow Cytometry for the Nephrology Researcher

# Oct 1 – Oct 3. 2018

A 3-day Training Course for Nephrology Researchers on Isolation and Analysis of Intra-Renal Cell Populations Fees: \$500 (\$1,000 for industry)

## **Division of Nephrology** UAB

## Presented by the UAB-UCSD O'Brien Center for Acute Kidney Injury Research (DK079337)



**Program Goals** 

This workshop is designed to provide researchers in nephrology with a basic working knowledge of primary methods of recovery and analysis of intra-renal leukocytes with an emphasis on flow cytometry. By the end of the course, it is expected that participants will be able to isolate viable intra-renal leukocytes and analyze them by multi-color flow cytometry. They will have acquired the basic knowledge necessary to design a workable panel of antibodies, have familiarity with the operation of a flow cytometer and how to analyze data from an experiment. Methods of cell sorting will also be addressed.

#### **PROGRAM**

**Day 1 Introduction and Basic Procedures** Instructors: Jeremie Lever, James George

#### **Introductory Lectures**

8-9:45am

- Animal regulations, obtaining clean tissue • specimens for single cell preparation and microscopy
- Principles of Immunofluorescence

### **Practical Session**

10-5pm

- Handling of rats and mice
- Gaseous (isoflurane) and injectable anesthesia
- Perfusion •
- Drawing blood
- Harvesting intact kidneys
- Kidney digestion for single cell suspensions

- Preparations of tissue for immunofluorescence
- Staining of cells with labeled antibodies (titering antibodies)

### Day 2 Flow Cytometry analysis of renal tissue

Instructors: James George, John Mountz, Vidva Sagar Hanumanthu, Jeremie Lever

#### Introductory Lectures

- Cytometry Basics •
  - Types of information to be gained 0
  - Cytometer construction/layout Ο
  - Fluorescence/fluorophores 0
  - Spectral Compensation (revisit in data Ο analysis)
- Antibodies and indicator dyes
  - Antibody basics isotypes, primary 0 and secondary antibodies, biotinylated antibodies.
  - How to obtain the antibodies you 0 need
  - Designing a panel, controls, FMO's Ο (design simple 4 color panel with students to use that afternoon in laboratory)
- Current and upcoming flow cytometry technology - high color and CYTOF instruments.
- Flow cytometry quality control.

### **Practical Session**

- Examination of LSRII and benchtop flow cytometers
- Operation and maintenance of a flow cytometer
- Data acquisition using the FACSDiva • software
  - o How to name and organize data files

#### Data

- Software packages: FlowJo
- Gating
- Compensation
- Quantitation
- Common pitfalls

#### Day 3 Flow Cytometric and magnetic sorting

Instructors: James George, Jeremie Lever

#### Introductory Lectures

Questions and Reviews

- FACS sorting cells using a flow cytometer
  - How they work
  - Practical considerations for sorting experiments
  - Advanced data analysis techniques. T-SNE.

#### **Practical Session**

• Reviews of data analysis and compensation



# Workshop Instructors

James F George, PhD Professor of Surgery (jgeorge@uab.edu) Research interests include iron and heme metabolism in acute kidney injury and chronic kidney disease and the role of intrarenal inflammatory cells in renal disease and homeostasis. Dr. George has 30 years experience in flow cytometry and leukocyte biology.

John D. Mountz, MD PhD. Professor of Medicine and Director of the UAB Comprehensive Flow Cytometry Core. (jdmountz@uab.edu) Dr. Mountz directs UAB's largest flow cytometry core, equipped with state-of-the-art instruments and sorters. His research interests include the development of germinal centers, DR5 apoptosis in arthritis and autoimmune disease, and immunosenescence.

# Vidya Sagar Hanumanthu MBBS, MS

Flow Cytometry Core Manager. Mr. Hanumanthu is an expert in flow cytometry analysis, sorting, instrument maintenance, and quality control. He has extensive hands-on experience with a variety of instruments from different manufacturers.

Jeremie Lever, BS. (<u>ilever@uab.edu</u>) Is experienced in flow cytometry and antibody panel design. He has extensive experience in renal leukocyte isolation and analysis.

## Registration

The workshop is being held at the UAB Medical Center, 915 Zeigler Research Building, 703 So. 19<sup>th</sup> Street

Registration Deadline: Sept 15th, 2018

Course Fees: \$500 (\$1000 industry) The registration includes course material, lunches, coffee, one evening meal.

#### Name

Institution
Department
Address
Phone
Email

Please submit registration and a brief paragraph on experience and career goals by email to:

Kelly Andringa, PhD O'Brien Workshop Coordinator THT 638C 1720 2<sup>nd</sup> Ave South Birmingham, AL 35294 205-975-4812 Dr. Andringa will then communicate the details of remitting payment, which is required to reserve a place in the course.

#### Accommodations and Travel

Participants are responsible for their own travel and housing arrangements. Dr. Andringa, the O'Brien Center Workshop coordinator can provide assistance if needed.

NOTE THAT ONLY 6 PEOPLE CAN BE ACCOMMODATED IN THIS WORKSHOP, SO PLEASE REGISTER EARLY.

#### **Nearby Hotels**

Residence Inn by Marriott Birmingham Downtown at UAB. <u>https://goo.gl/maps/py4E789voPQ2</u>

Courtyard by Marriott Birmingham Downtown at UAB. <u>https://goo.gl/maps/zyWZKWbgDCt</u>

#### Venue



The UAB Campus

A view of the Zeigler Research Building where the workshop is to take place can be found at

https://goo.gl/maps/aD11g5R2KnL2

The conference will begin on the 6<sup>th</sup> floor in room 644.





Funded by the

National Institutes of Health

(P30 DK079337)

