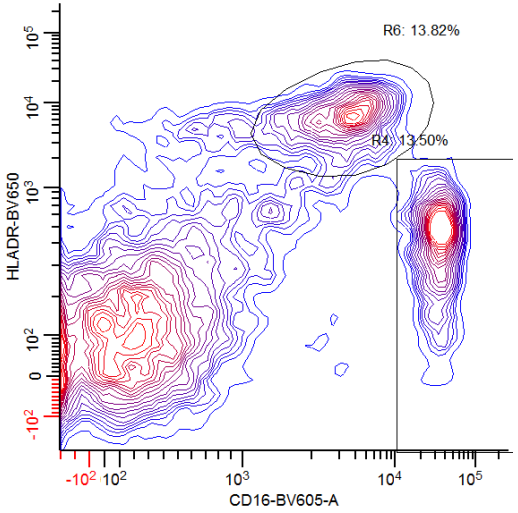
The UAB-UCSD O'Brien Center Workshop on Flow Cytometry for the Nephrology Researcher

May 31 – June 2 2017

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 Core Center for Acute Kidney Injury Research (DK79337)



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: Lisa Curtis, Travis Hull, Lingling Guo, James George

Introductory Lectures

8-9:45am

- Handling of rats and mice
- 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

Introductory Lectures

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

Practical Session

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

Data

- Software packages: FloJo and Winlist
- Gating
- Compensation
- Quantitation
- Common pitfalls

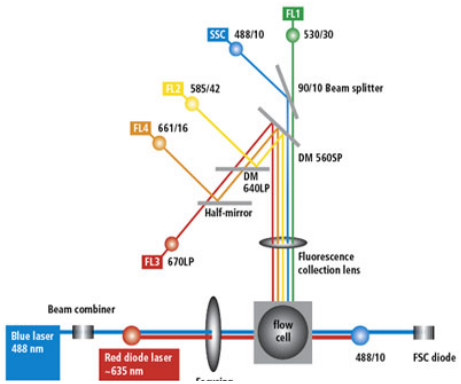
<u>Day 3</u> Flow Cytometric and magnetic sorting
Instructors: James George, Travis Hull

Introductory Lectures
Questions and Reviews

- FACS – sorting cells using a flow cytometer
  - How they work
  - Practical considerations for sorting experiments
- Magnetic sorting – principles and considerations
  - Bead based methods – eg. Dynal beads
  - The Miltenyi MACS system
  - Common pitfalls

Practical Session

- Reviews of data analysis and compensation
- Sorting cells from mouse spleen using the Miltenyi MACS system



Workshop Instructors

James F George, PhD Professor of Surgery
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.

Lisa M Curtis, PhD Assistant Professor of Medicine. Dr. Curtis focuses on cellular sources of epithelial repair in AKI, sex differences in the role of heme oxygenase-1 in kidney homeostasis and disease, and the pathobiology of AKI in the aging kidney. She is an expert in immunofluorescence techniques in renal tissue.

John D. Mountz, MD PhD. Professor of Medicine and Director of the UAB Comprehensive Flow Cytometry Core. Dr. Mountz directs UAB's largest flow cytometry core, equipped with state of the art instruments and sorters. His research interests include

Travis D Hull, PhD. Dr. Hull is an expert on renal leukocyte populations in the kidney and in techniques of renal leukocyte isolation and analysis.

Lingling Guo, MD. Is an expert microsurgeon with a focus on models of acute kidney injury and kidney transplantation. She has 15 years experience in working with animal models and is highly expert in animal manipulation, anesthesia, and surgery.

Jeremie Lever, BS. 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 19th Street.

Registration Deadline: April 24, 2017

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
1720 2nd Ave South, THT 638c
Birmingham, AL 35294
205-975-4812 / andringa@uab.edu

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.
https://goo.gl/maps/py4E789voPQ2

Double Tree by Hilton Birmingham
https://goo.gl/maps/6PNZSTmePUn

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

Venue

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 6th floor in room 644.

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