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Pediatric Research Alliance

The Core Report

The spot for news from the cores of the Pediatric Research Alliance

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Introducing New Director and New Name: Chris Porter, MD of the Children's Clinical & Translational Discovery Core

Clinical & Translational Discovery Core

The Pediatric Research Alliance Biorepository has undergone some changes: the appointment of a new director and a name change. The Biorepository is now the Clinical & Translational Discovery Core which more accurately reflects the wide capabilities of the lab. The CTDC is a tremendous resource for the pediatrics research community in Atlanta. Under the skilled technical direction of Bradley Hanberry, PhD, the CTDC can process samples for storage, preserve samples long-term for future use, and perform various assays such as ELISA, PCR and western blotting. As Core Director, my role is to ensure that the CTDC optimally meets the needs of pediatric researchers. Dr. Hanberry and I are readily available to investigators to discuss how the CTDC can enhance specific projects.

Check out the Clinical & Translational Discovery Core on www.pedsresearch.org

My Roles at Emory & CHOA

I joined the Emory Department of Pediatrics in September 2016, from Children's Hospital of Colorado, where I directed the Cell Bank for the section of pediatric oncology. In my lab at Emory, we study mechanisms of leukemogenesis and treatment resistance using a wide variety of models, including genome-scale shRNA screening. Our work is highly reliant on primary patient samples, so I appreciate the importance of careful collection and storage of human tissue. Clinically, I direct the Cancer Predisposition Program of the Aflac Cancer and Blood Disorders Center.

Outside the Lab

As a family, my wife, four children and I enjoy sports, travel and all things outdoors. When we don't have soccer, basketball or gymnastics events, we are often on a road trip, exploring the state of Georgia and surrounding region, ideally with a little bit of fly-fishing on the way.

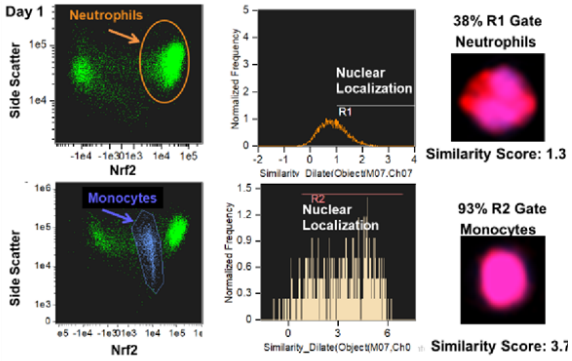
~submitted by Chris Porter, MD, PhD

Amnis ImageStream Mark II: A Hidden Gem in the Emory PEDS FACS Core

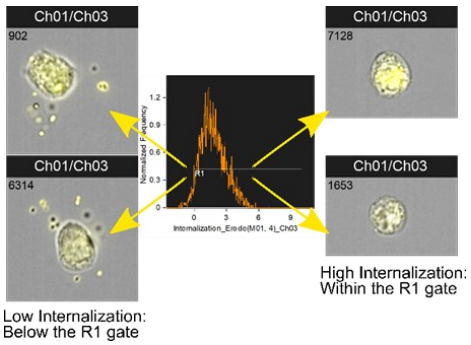
Get the detail of fluorescent microscopy with the robust data analysis of a flow cytometer!

When Jocelyn Grunwell, MD, PhD and Susan Stephenson, PhD wanted to determine if their neutrophil cell line was able to ingest bacterial particles, they ran into a problem. Contrary to the manufacturer's claims, they found that the particles fluoresced even though they were just outside of the cell. This made analysis of their samples on a regular flow cytometer impossible. They were going to have to mount the cells on slides and use fluorescence scope to do the analysis. This procedure, while accurate, is also time consuming and tedious. This was a perfect experiment for the ImageStream Mark II available in the Pediatrics Flow Cytometry Core.

"This was a perfect experiment for the ImageStream Mark II"



The ImageStream runs like a traditional flow cytometry, but a picture of every cell is captured for image analysis, thus the localization of probes to specific sites within the cell can be determined. The ImageStream analysis software is a user-friendly tool to help with standard image analysis. There are wizards that can aid in probe internalization, spot counting, co-localization, morphology, and apoptosis. The system in the Pediatrics Flow Core has 7 lasers and 12 available channels. "While the samples initially take a bit longer to run than traditional flow, the amount of data collected is enormous," states Susan Stephenson, "it takes us a few hours to determine the internalization factor of 10,000 cells per sample for 24 samples. Using traditional fluorescence microscopy, this experiment would have taken us a week of analysis and we would have only been able to look at about 300 cells per sample." Aside from using the ImageStream to confirm the internalization of bacterial particles, Grunwell and Stephenson have also assessed the nuclear localization of the transcription factor, Nrf2 in neutrophils and monocytes. "We wanted to see if there was a difference in the translocation of Nrf2 to the nucleus between neutrophils and monocytes. The ImageStream allowed for the robust analysis of multiple cell types simultaneously," notes Grunwell.



In addition to the user-friendly software and online tutorials, Amnis, the instrument's maker, also offers 3-day user seminars at its headquarters in Seattle. Susan will attend one of these seminars in September and is looking forward to honing her image analysis skills and getting ideas for new applications. If you have ever wished that you could have both the detailed images of a fluorescence microscope with the throughput and efficiency of a flow cytometer, then the ImageStream Mark II is the perfect instrument for your research. To book a training appointment and learn more about the ImageStream, contact Aaron Rae in the Emory Pediatrics Flow Cytometry Core.

~submitted by Susan Stephenson, PhD & Jocelyn Grunwell, MD, PhD

General Equipment Core

The General Equipment Core contains a selection of basic and specialized laboratory equipment available, at no charge, to all investigators affiliated with the Pediatric Research Alliance. Many of the core equipment items can be found in ECC lab 260, bench F. Currently, the Core supports Bio-Rad iCycler Thermal Cyclers, and Bio-Tek Synergy 2 Plate Reader, as well as two Konica X-Ray film processors.

In addition, the Core is home to several XPN-100 Ultracentrifuge, J-26 XP High Speed Centrifuge, and ABI 7500 Real-time PCR system, as well as the Hidex 300 SL vial based liquid scintillation counter. Selected core pieces are described below.

Figure 1: XPN-100 is located in HSRB E497, E262, and ECC 510

XPN-100 Ultra Centrifuge

The XPN-100 Ultra centrifuge is capable of 1,000 to 100,000 rpm in 100-rpm increments and 0C to 40C in 1 degree increments. This centrifuge is paired with four rotors: SW 32 TI, SW 41 TI, Type 70 TI, and Type 70.1 TI.

Avanti J-26 XP High Speed Centrifuge

The Avanti J-26 XP High Speed Centrifuge is capable of 100 to 26,000 rpm in 10-rpm increments or equivalent RCF in 100xg increments and -10C to 40C in 1 degree increments. This centrifuge is paired with three rotors: JLA 10.500, JLA 16.250, and the JA 15.50.

Figure 2: J-26 is located in HSRB E497, E263, and ECC 510

Figure 3: Hidex 300 SL is located in HSRB E263

Hidex 300 SL

The Hidex 300 SL is a recent addition that provides researchers with a large volume, vial based liquid scintillation counter for applications such as cell studies and cytotoxicity measurement. User privileges requires initial training.

ABI 7500 Real-Time PCR

The ABI 7500 Real-Time PCR system offers five color dye flexibility in a 96 well format. The 7500 Fast Real-Time PCR System is optimized for use with standard dye sets: FAM™/SYBR®Green I , VIC®/JOE™, NED™/ TAMRA™/ Cy3™, ROX™/Texas Red®, and Cy5™dyes. User-friendly software includes plate setup wizards, and advanced analysis tools to make data processing simple and straightforward.

Figure 4: Located in ECC 260 Bench F and ECC 510 Bench A

How to Access

All affiliated Pediatric Research Alliance investigators are encouraged to use the General Equipment Core! Please be aware that the use of the Ultra and High Speed Centrifuges requires training as well as a username/password. Please email Kira Moresco to obtain centrifuge credentials as well as to ensure the appropriate access levels are granted on the user's proximity badge.

Please visit the General Equipment Core website for addition information. A full list of equipment details and user manuals can be found on the website, too.

~ submitted by Kira Moresco, MS

"The General Equipment Core contains a selection of basic and specialized laboratory equipment available, at no charge, to all investigators affiliated with the Pediatric Research Alliance."

Pediatric Heart Diseases Data Registry Core

The Pediatric Heart Diseases Data Registry (PHDDR) Core provides access to rich registry data from surgical, catheter-based and electrophysiologic interventions for pediatric heart diseases. Collection of these data was made possible by the Pediatric Cardiac Care Consortium (PCCC), a non-profit foundation whose mission is to improve the lives of patients with pediatric acquired and congenital heart disease (CHD) by answering key questions about treatments and outcomes, thereby empowering patients, families, clinicians, and policy-makers to make informed decisions and improve quality of care.

The PCCC is a North American, multi-center registry for interventions for pediatric heart diseases that collected patient-level data from 1982 until 2011 to support quality improvement in pediatric heart surgery. The data represent over 300,000 event outcomes and have been collected from over 140,000 patients operated on in 47 pediatric cardiac surgery centers from 27 US states and 10 international centers. The PCCC is the oldest and among the largest registries in this field in the world. The registry subjects are linked to the National Death Index (NDI) and the United Network for Organ Sharing (UNOS) to constitute a cohort that describes the long–term mortality experience of patients with repaired CHD.

Linking the PCCC registry with outcomes-focused national data repositories such as the NDI and UNOS leverages the strengths of both datasets to yield new understandings of these conditions. The linked registry data is available from the PHDDR core along with consultation assistance for research investigators wishing to perform outcome studies related to pediatric heart diseases.

Check out the PHDDR Core on www.pedsresearch.org

Established by Dr. Lazaros Kochilas, a pediatric cardiologist with dual appointments at CHOA and Emory University, the PHDDR Core is always eager for the opportunity to work alongside investigators with new project ideas and tailors efforts towards investigators' research needs. The core boasts also two full-time epidemiologically-trained employees and a database manager to meet all research needs.

Ongoing project topics:

- All-cause mortality in the PCCC
- Coronary artery bypass grafting (CABG) and non-CABG coronary reperfusion procedures in children with Congenital Heart Defects
- Transposition of the great arteries
- Tetralogy of Fallot
- Pulmonary atresia with Intact ventricular septum
- Long term survival after rescue of patients operated with CHD by use of extracorporeal membrane oxygenation
- Single ventricle physiology
- Patent ductus arteriosus
- Aortic valve replacement
- Genetic disorders (trisomies 13, 18 and 21, Marfan syndrome)
- Coarctation of the aorta
- Anomalous left coronary artery from the pulmonary artery

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~submitted by Lazaros Kochilas, MD & Amanda Thomas, MSPH

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Check out all cores at www.pedsresearch.org/research/cores

How to Acknowledge the Cores:

These cores are generously supported by Children's Healthcare of Atlanta and Emory University. When presenting or publishing work completed using the core, please include "Children's Healthcare of Atlanta and Emory University [core name]" in the acknowledgments.

This newsletter serves to highlight the activities of the cores supported by Emory University's Department of Pediatrics and Children's Healthcare of Atlanta. If you have a story idea for a future edition, please contact Karen Kennedy (kmurra5@emory.edu).