Immunology and Nano-Medicine

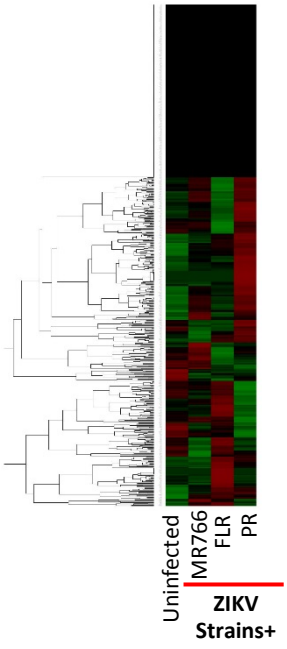
Research at Department of Immunology and Nano-Medicine is mainly focused on molecular and cellular aspects to investigate the interactive role of recreational drugs and HIV-1 infection on human immune system. The work involves unraveling the mechanism of immunopathogenesis and neuropathogenesis of HIV-1 infection, which will help to develop novel therapeutic strategies to reduce drug addiction and HIV disease in drug abusing HIV-1 infected patients. The department also conducts research on developing nanocarrier drug delivery systems to target therapeutic molecules across the blood-brain barrier to treat primarily drug addiction and NeuroAIDS. The department's goal is to promote translational research and training in immunological techniques related to drug use/abuse, HIV infection and drug targeting approach for graduate and postgraduate levels. The department's facilities is an integral part of the FIU Institute of Neuro-Pharmacology (INIP), which is a multidisciplinary translational research and training center focusing on the human immune system, and high-tech procedures for targeted drug delivery for patients with central nervous system disorders, including brain cancer and Neuro-AIDS. INIP collaborates with the Nano-Device Laboratory in the development of state-of-the-art nanotechnology approaches. The laboratory has the capability to formulated nanosilicone particles and test their potential effects on immune response, test nanosubstrate as used to facilitate neuronal growth and bone regeneration, as well as create magnetic nanoparticles to deliver nano-medications via external magnetic field.

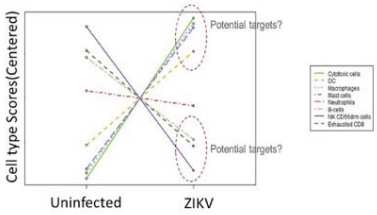
The following specialized and core labs of the department are as follows:

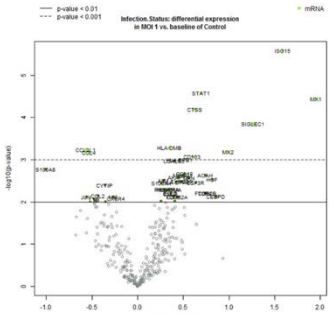
1. **<u>Gene Expression Profiling Core</u>: Andrea Raymond**

The gene expression core of the Department of Immunology and Nanomedicine houses the only NanoString Profiler at Florida International University capable of RNA, DNA, and protein profiling applications. The instrument is housed in Dr. Raymond's lab AHC1. The nCounter Sprint Profiler allows digital examination of multiple pathways in a single sample tube and requires only 10 minutes of hand-on-time (depending on application gene expression vs. miRNA profiling). Users will spend less time on sample preparation and are able to perform their own data analysis using the included nSolver Analysis Software. The barcode technology utilized by the nCounter Sprint Profiler allows for highly multiplexed analysis of deregulated pathways associated with basic Immunology/Cancer Biology/Neuroscience/Aging/Infectious Disease and the identification of biomarkers or expression profiles signatures associated with disease states.

nCounter® SPRINT Profiler







Gene	Log2 fold change	std error	P-value	Gene.sets
ISG15-mRNA	1.59	0.0408	2.62E-06	Interferon Signaling, Pathogen Response
STAT1-mRNA	0.693	0.0318	2.63E-05	Chemokine signaling, Cytokine Signaling, Growth Factor Signaling, Interferon Signaling, Th1 Activation, TLR signaling
MX1-mRNA	1.95	0.097	3.61E-05	Interferon Signaling
CTSS-mRNA	0.624	0.036	6.51E-05	Antigen Presentation, ECM remodeling, TLR signaling
SIGLEC1-mRNA	1.26	0.0887	0.000141	Cell Migration and Adhesion, Lymphocyte activation
HLA-DMB-mRNA	0.35	0.0341	0.000508	Antigen Presentation, Cell Migration and Adhesion
CCL3L1-mRNA	-0.567	0.0572	0.00058	Chemokine signaling, TLR signaling
MX2-mRNA	0.991	0.103	0.000643	Interferon Signaling
CCL4-mRNA	-0.535	0.0559	0.000662	Chemokine signaling, Pathogen Response, TLR signaling
CD169-mRNA	0.595	0.0663	0.000849	Differentiation and Maintenance of Myeloid Cells

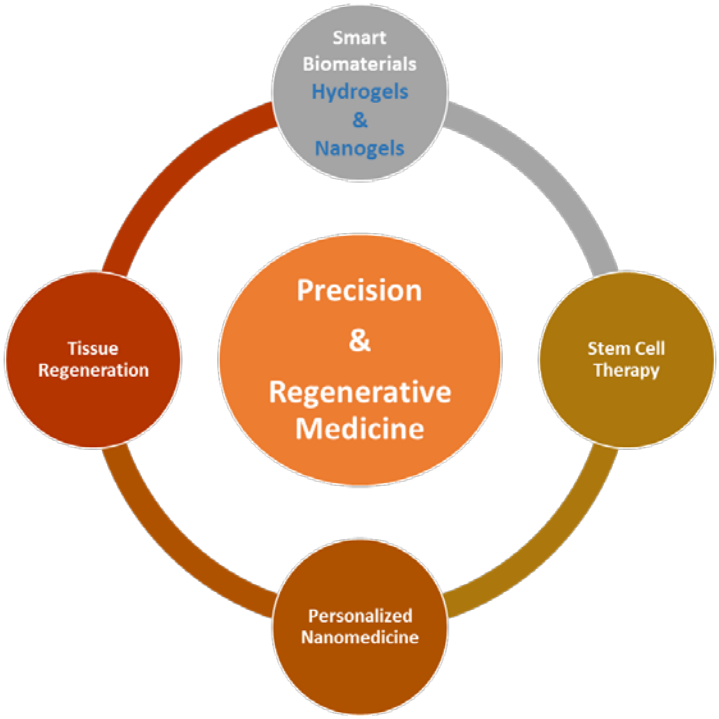
2. Drug Delivery and Pharmacokinetics Core: Nagesk Kolishetti

The Department of Immunology and Nano-Medicine at Herbert Wertheim College of Medicine Florida International University hosts Drug Delivery and Pharmacokinetics Core lead by Dr. Kolishetti at AHC-1 308. The services of the core available mainly through collaborations with the faculty and staff of department.

Core offers variety of services in developing drug delivery strategies for various therapeutics using polymeric, emulsion, metallic, magnetic and magneto-electric and liposomal based nano-formulations. The core also offers various services to understand the pharmacokinetics and biodistribution profiles of the nano-formulations through variety of techniques which include High-Performance Liquid Chromatography (HPLC), LC-MS, GC-MS and ICP-MS techniques. Institutional instruments such as ICP-MS and GC-MS usage will be charged as per user policy.

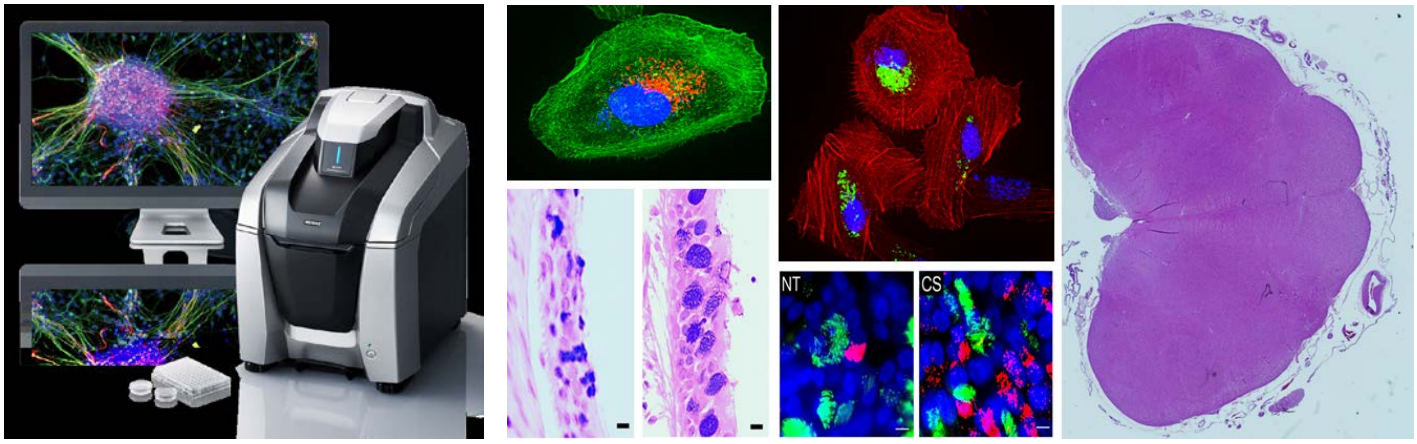
3. Precision and Regenerative Medicine: Arti Vashist and Asahi Tomitaka

This core facility Laboratory, AHC-1 #306, is involved in developing smart biomaterials for translational research in tissue engineering and personalized medicine. The facility's major objective is to develop smart materials such as Hydrogels, injectable gels, nanogels Polymeric nanoparticles, which are involved in the process of replacing, engineering or regenerating human cells, tissues or organs for the restoration or establishment of normal functioning of cells. The research focus is to develop the smart biomaterials for personalized nanomedicine and regenerative medicine. It also involve exploration of nanofillers such as MENP (Magneto electro nanoparticles), MNP (Iron oxide nanoparticles), gold nanoparticles within the smart materials for improved physical stability and enhanced functionality.



4. Microscopy & Imaging Core: Hitendra Chand

Microscopy helps us understand tissue architecture, cell structure and dynamics and cell organelle interactions. New age sophisticated microscopic instrumentations, ultra-sensitive digital cameras and specialized fluorescence probes has now made it possible to visualize cellular events in real time down to the RNA, DNA and protein levels.



Equipment

Department of Immunology & Nano-Medicine hosts an All-in-One microscope system that is a fully automated digital microscope that houses up to 6 high-quality lenses and 4 filter cubes in automated turrets. The compact, fully-enclosed design enables it to become its own built-in dark room, while all objectives and filters are controlled through the software. The unit uses a 14-bit monochrome CCD that is also able to take 24-bit true color images. The microscope is capable of stitching up to a 50,000x 50,000 pixel images in a combination of bright field, phase contrast, and/or fluorescence imaging modes (8 times faster than conventional methods). Stitched images of whole cross-sections or entire wells can be used within the "Navigation Window" to move the automated XY stage with precision to various areas of interest at higher magnifications (including the 100x oil lens). Stitching can be combined with the Z-stacking capability, which allows for capturing images within a range of focal planes that can then be projected as a single fully-focused image. The analyzer software combines image correction tools with powerful quantification functions, including the haze reduction tool which instantly removes fluorescence blurring and enables visualization of weak fluorescence signals.

The facility provides researchers to image samples and publish high profile research to:

- Elucidate molecular mechanisms of cancer, immunologic, infectious, neurologic and vascular diseases
- Evaluate therapeutic efficacy in cells and tissue samples
- Test nanotechnologies in cell based assays
- Quantitatively measure changes in cell and tissue morphology and pathology

Has resource staff who offer investigators:

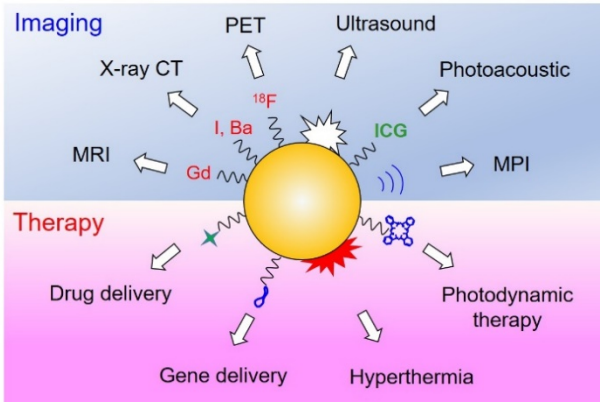
- Expert consultation on experiment design and specimen preparation
- Training
- Imaging & Ongoing Assistance

Possible Applications

- Fixed specimen mounted on slides
- Widefield fluorescence to capture 400-800 nM wavelength signal
- Stereology
- Live cell Imaging capability
- X,Y positioning, Z-stacks, and montage imaging
- DIC with both 20X and 40X objectives
- Brightfield – H&E, DAB, Giemsa, etc.

5. <u>Advanced Nano-Engineering and Theranostics</u>: Asahi Tomitaka and Roozebeh Nikkhah Moshaie

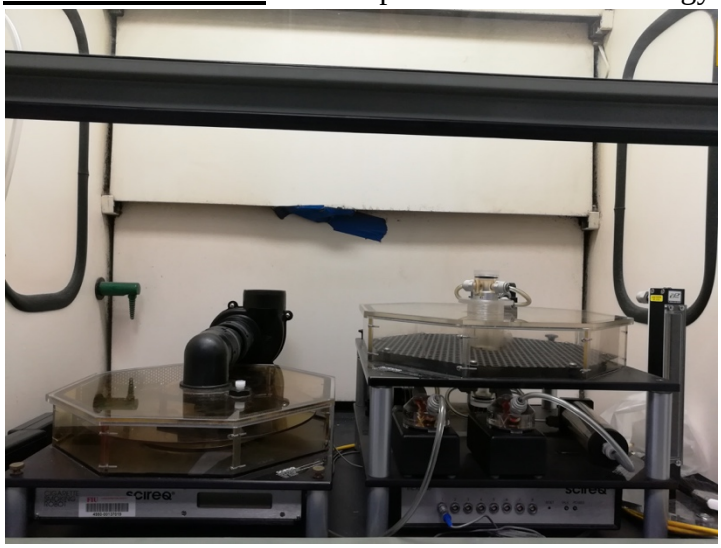
Nano-Engineering and Theranostics lab is located in AHC-1 #306 and #415. The focus of this research core is to develop multifunctional nanomaterials for biomedical applications and explore for theranostics which is an emerging approach combining therapeutics and diagnostics. We have developed various magnetic-based nanomaterials including magnetic nanoparticles, magneto-electric nanoparticles, and magneto-plasmonic nanoparticles for drug delivery system, image-guided therapy, and theranostics especially for HIV and brain disorder treatment. The objectives of this laboratory are 1) Synthesis of multifunctional nanoparticles, 2) Functionalization of nanoparticles with biological molecules, 3) Nanoparticle characterization, 4) *In vitro* evaluation of therapeutic efficacy. Our core lab is equipped with instruments including Argon gas connected synthesis line, Infrared laser, Zetasizer, UV-visible spectrometer, and magnetic field generator.



Schematic illustration of multifunctional nanoparticles and its applications for theranostics. (Reprint from Image-Guided Therapy. In Advances in Personalized Nanotherapeutics. Springer, Cham)

6. **Hoshang Unwalla Lab:**

Smoke robot core: The Department of Immunology and nanomedicine hosts a smoke robot for delivering cigarette smoke to surfaces of cultured cell lunes or primary cells. The Scireq InExpose smoke robot (SciReq) delivers cigarette smoke directly to the air-exposed surface of cells grown at the Air liquid interface or in culture dishes. The inExpose is a versatile, programmable, and compact exposure system that can be configured with smoke generation devices (e.g. cigarette smoking robot, single cigarette chamber, e-cigarettes), or nebulizers to generate a wide range of exposures consistently within and between studies, as well as across laboratories. The smoke robot utilizes research grade cigarettes from the University of Kentucky to generate cigarette smoke with defined number of puffs and puff volume.



Electrophysiology Core: The Department of Immunology and nanomedicine has a Physiological Instruments six-channel voltage current clamp with 4 temperature regulated EasyMount Ussing chambers and data acquisition hardware and software for studying transport across epithelial barriers. Transepithelial voltage, short-circuit current and resistance. The EasyMount Ussing Chamber Systems consisting of individual Ussing chambers, a heating block for temperature control, needle valves for adjustment of gas flow for oxygenation and stirring, and Ag/AgCI reference electrodes for measuring transepithelial voltage and passing current. Cell Cultures and a wide variety of tissue types may be mounted on special inserts that simply slide into position in the chamber. Electrodes insert from the front permitting unrestricted access for fluid sampling.

Applications: Electrophysiology, Pathophysiology, Nutrition, Flux Studies, Permeability Studies, Drug Transport, Drug Screening, Toxicology, Pharmacology, Immunology, Parasitology, Cell Biology, & Many More.

7. **<u>Gene Editing Core Lab</u>: Sudheesh Pilakka-Kanthikeel and Venkata Atluri**

The Genome Editing Core Facility (GECF) focuses on providing expertise and technical services required to manipulate the genomes of various cell lines including human cells that can serve as *in-vitro* models for addressing a wide variety of biological questions. The GECF utilizes state-of-the-art technologies such as CRISPR/Cas9 to perform gene editing and continually explore new technologies to further enhance the facility's services. GECF's mission is to help labs/ PIs to (1) incorporate CRISPR/Cas9 genome editing into their existing research programs, (2) perform gene editing procedures to generate customized cell lines, and (3) innovating new genome editing technology. The center can provide design and assistance for genome engineering project. The investigators can get advice on the best way to generate the desired cell lines.

8. **<u>Smart Diagnostics and Nanomedicine</u>: Ajeet Kaushik and Vidya Sagar**

Smart Diagnostics Laboratory, AHC-1 #306, explored miniaturized diagnostics systems for early stage diseases diagnostics at point-of-care (POC) application. The focus of this research core is to explore smart-electro-active sensing nanostructures, optical/electrical biosensing system, and miniaturized sensing system for the diseases detection and therapy efficacy assessment. The research objectives of this laboratory are 1) exploring chip based electrochemical infection diseases like HIV-infection monitoring, 2) developing miniaturized electrochemical sensors to detect targeted biomarkers for cancer, Alzheimer's disease, Ebola, Zika-virus etc., at pM level, 3) exploring sensing system for POC applications, 4) exploring electrochemical analytical tools to assess therapy efficacy, 5) bio- informatics for epidemic management, and 6) exploring sensor for personalized health care

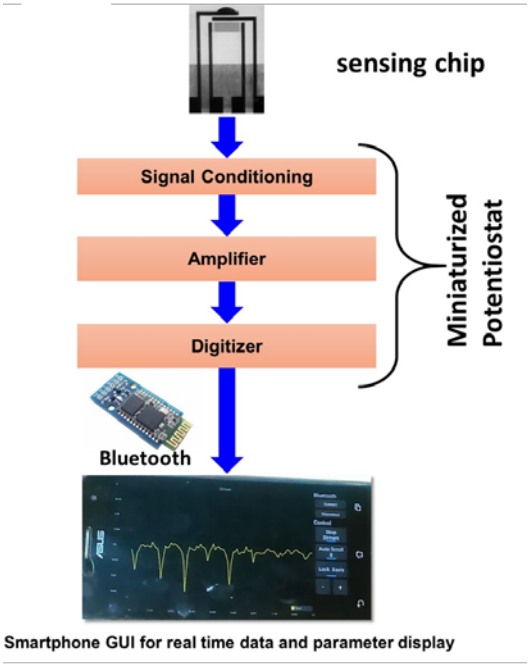
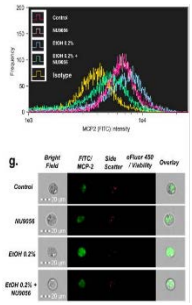


Illustration of a biosensor towards point-of-care art for personalized health care

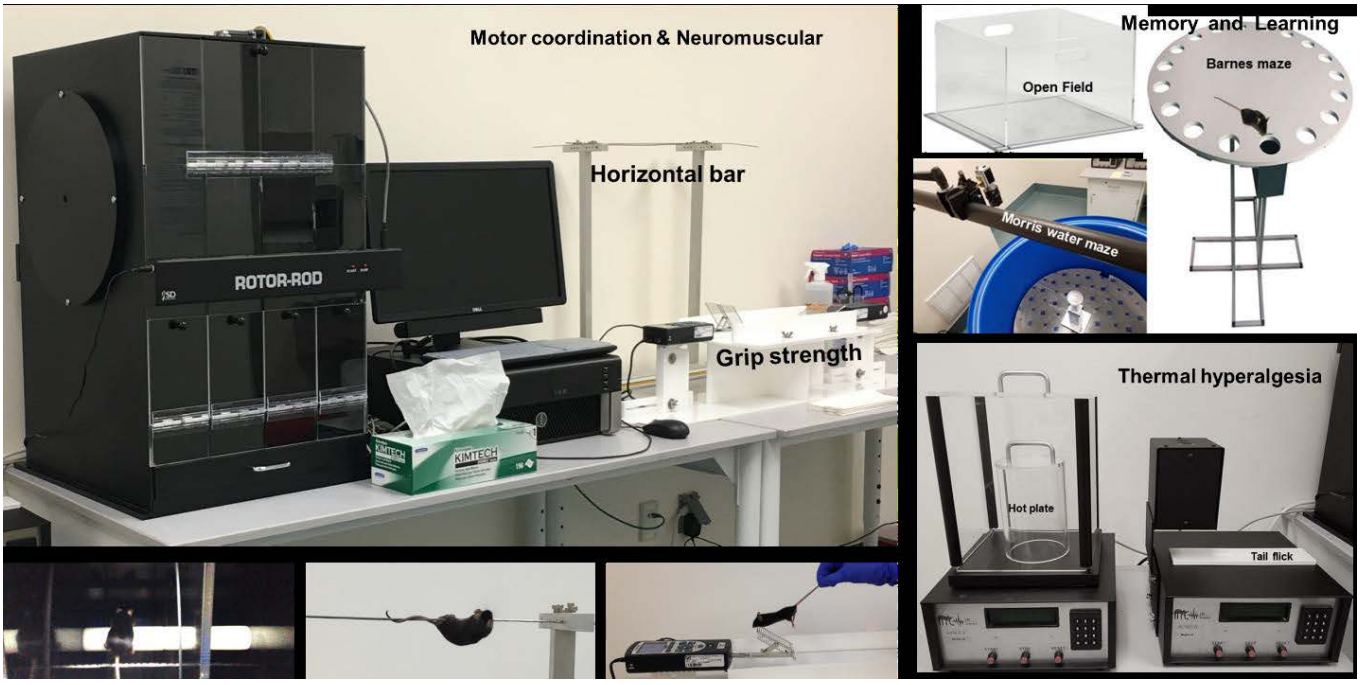
9. <u>Flow Cytometry Core:</u> Marisela Agudelo and Boris Castillo



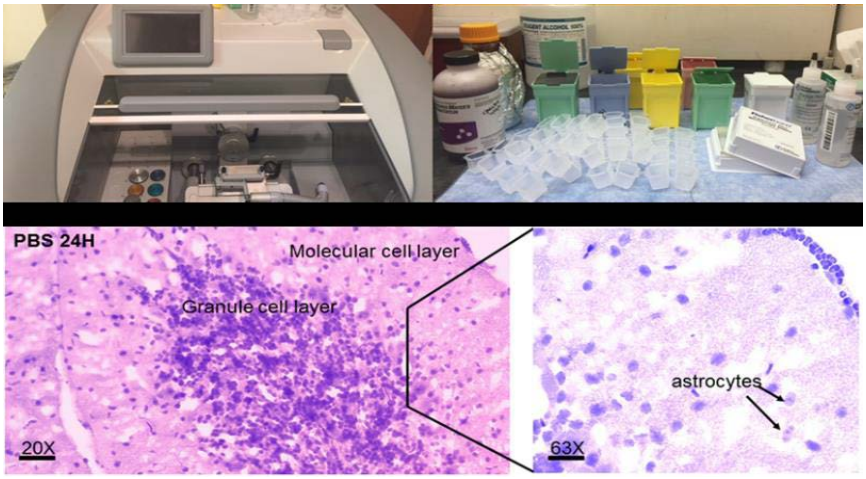
The Department of Immunology hosts a state of the art flow cytometry instrument in Dr. Agudelo's Lab located in AHC-I 433, which is available free of charge and on collaborative basis to the faculty and staff of the Department of Immunology and Nano-Medicine at the Herbert Wertheim College of Medicine (HWCOM) and other collaborators from HWCOM and FIU. The FlowSight® instrument has the capability of integrating flow cytometry and microscopy by combining the speed and sample size of flow cytometry with the resolution and sensitivity of microscopy in a single instrument. Some of the applications relevant to the proposed studies include: cell signaling, internalization, co-localization, micronucleus counting, cell-cell interaction, morphology, cell death, autophagy, cell cycle and mitosis, DNA damage and repair, spot counting, and other emerging applications such as detection of nanoparticles.

10. <u>Animal Neurobehavioral Core Lab</u>: Nazira El-Hage and Zaohua Huang

Animal Neurobehavioral Core facility of the Department of Immunology and Nano-medicine is located in AHC-4 animal vivarium and under the supervision of Dr. Nazira El-Hage (core director) and Dr. Zaohua Huang. The facility is available based on a pay-for-fee service. The facility offers the following state-of-the-art behavioral tests to measure: (1) memory and learning: Morris water maze, open field and Barnes maze, (2) motor coordination and endurance: rotor-rod, and horizontal bar (3) neuromuscular functions: grip strength and horizontal bar, and (4) Thermal hyperalgesia: tail flick and hot plate. The facility is also equipped with a digital stereotaxic instrument with anesthesia mask, ear and bite bars, a remote infuse/withdraw syringe pump, an electrical heating pad, bead sterilizers and surgical tools for small animal surgery. For more information, please contact Dr. Nazira El-Hage (nelhage@fiu.edu) or Dr. Zaohua Huang at (zahuan@fiu.edu).



11. <u>Histology Core Lab</u>: Nazira El-Hage and Myosotys Rodriguez



Histology Core facility is located in the Department of Immunology and Nano-medicine in AHC-1 422 and under the supervision of Professor Nazira El-Hage. The facility is available free of charge and on a collaborative basis to the faculty and staff within the Department. The CryoStar NX50 Cryostat offers standard features well suited to the routine clinical histology laboratory. The NX50 utilize a rapid response cooling system that actively cools all areas of the cryochamber that come in contact with the sample, including the specimen clamp, cryobar and blade. The result is precise temperature control and half the energy consumption of other routine cryostats. Low energy LED lighting with user-adjustable intensity is also included. Dr. El-Hage's laboratory is also equipped with an inverted phase contrast microscope with xenon epifluoresence Hoffman optics and 37°C / CO2 environmental chamber and cell observer video system with (2) 506 Axiocam camera system (one mono and one color for histology imaging). For more information, please contact Dr. Nazira El-Hage or Dr. Myosotys Rodriguez at 305-348-4346 or 305-348-4524.