LSU Health - Shreveport

RESEARCH CORE FACILITY

The LSUHSC-S Research Core Facility (RCF) currently houses and maintains instrumentation for seven separate technologies. Each instrument is operated or supported by a Research Associate and is overseen by an LSUHSC-S Scientific Advisor. The role of the scientific advisors is to provide investigators with assistance in the application of each technology to his or her specific experimental needs. These advisors comprise the Scientific Advisory Board (SAB), the current membership of which includes Robert Chervenak (Chair), Rona Scott, Kevin Lin, Chris Kevil, and Nick Goeders. Deborah Chervenak is Manager of the RCF laboratories.

<u>**MICROSCOPY**</u>
Scientific Advisor: Dr. Hungwen (Kevin) Lin

Nikon A1R Confocal & N-SIM Super Resolution System
This is a high-speed Nikon A1R confocal microscope combined with a SIM (Structured Illumination Microscopy) super resolution system for fixed and live sample-imaging. The Nikon Eclipse Ti-E inverted microscope is built with fully automated objectives, stage, and filter turrets. The 5 objectives range from 10X to 100X. The confocal components include 5 detectors and 4 lasers: 405nm, 488nm, 561nm, and 640nm in wavelength. The SIM components include a Hamamatsu camera and 3 LED lasers: 488nm, 561nm, and 640nm in wavelength. DAPI images can still be taken under SIM mode. The microscope is capable for bright-field imaging, multichannel fluorescence imaging, large image stitching, multipoint acquisition, time-lapse acquisition, FRAP, FRET imaging, and 2D/3D deconvolution. The resolution for conventional confocal reaches 200-250nm, while the resolution for super resolution system reaches 85-100nm. To allow long hour live cell-imaging, the microscope is housed in an incubator with CO_2, O_2, and humidity controls.

Leica TCS SP5 Confocal Microscope This is a very flexible and fast confocal system for fixed and living samples. The system includes a Leica DMI 6000 CS fully automated inverted microscope with motorized stage, condenser, objective, and filter turrets. It is equipped with 5 lasers for excitation: violet diode (405 nm), multi-line Argon (458, 476, 488, 496, and 514 nm), green HeNe (543 nm), orange HeNe (594 nm), and red HeNe (633 nm); visualization is done using a mercury arc lamp. The spectral beam splitter has freely adjustable bandwidths for the collection of signal in 5 separate detectors simultaneously or sequentially. There is also a transmitted light detector for DIC. There are 9 available objectives, ranging from a 2.5x to a Plan Apo 100x/1.46 NA oil objective. The system runs on the newest version of LAS AF software (LAS AF 2.9), with FRAP, FRET, Mark & Find, 3D Visualization, Colocalization, and Live Data Modes. The microscope is housed in a Ludin full enclosure incubator with an internal Ludin Cube2 with CO_2, O_2, and humidity controls.

Zeiss AxioObserver/Apotome Microscope The system is capable for brightfield imaging (black and white) and fluorescence imaging for fixed samples and short time live sample imaging (no gas, temperature, and humidity control). It is built around a Zeiss AxioObserver Z1 inverted microscope, fully automated, with component recognition to minimize errors. System components include mercury arc lamp excitation, a Zeiss AxioCamMRm CCD camera with 12-bit

dynamic range, extended sensitivity in the near infrared, a fully automated XYZ stage, a complement of objectives ranging from 10x to 100x, and five installed filter sets for DAPI, FITC, narrow band GFP, Rhodamine, and Far Red. The Apotome attachment is designed for precise optical sectioning. The Apotome slides easily into the optical path and projects a grid onto the image plane, which is shifted laterally in three defined steps, with one image collected at each step. A software algorithm then removes any out-of-focus signal based on the three images. The acquisition software is AxioVision v.4.8, including plug-in options for Colocalization, Mark & Find, Mosaic (large image stitching), time lapse, and more.

Nikon Widefield Microscope The system is built around a Nikon Eclipse TE300 inverted microscope with a range of objectives for brightfield, phase contrast, DIC, and high resolution epifluorescent imaging. The software package controls a Prior Optiscan™ XYZ stage with a full complement of stage inserts and a Prior filter wheel containing excitation filters from the Chroma 83000 filter set. This set includes single and multiband excitation filters for DAPI, FITC, GFP, Texas Red, Rhodamine, or PI. Fluorescent images are acquired with a black and white ANDOR Neo/Zyla camera; high resolution color images (brightfield) may also be acquired with the DS Fi2 color camera. The stage, filters, shutters, and camera are controlled by the NIS Elements software from Nikon.

Zeiss LSM 510 NLO Confocal/Multiphoton Microscope The Zeiss LSM 510 NLO system is configured to enhance living tissue research. The scanning system is connected to an upright Axioskop 2 FS MOT microscope equipped with a set of objectives selected for physiological measurements and live animal studies. The stage remains in a fixed position, and the objectives have motorized focus control. It is equipped with the following lasers and laser lines for excitation: Argon (458, 477, 488, 514 nm), HeNe (543 nm), HeNe (633 nm), and the Coherent Chameleon-XR Ti: Sapphire laser (tunable from 705 to 980 nm). The ultrafast-pulsed Chameleon laser emitting NIR radiation allows imaging up to 500 µm deep within tissue. There are three PMTs for visible wavelength detection, a transmitted light detector, and two non-descanned detectors for multiphoton imaging. The LSM 510 ZEN operating software includes the Physiology v3.5 and Image Visart v3.5 options that permit 2D, 3D, and 4D image collection and processing, 3D/4D animation, calibration and measurement of ion concentrations, time series analysis, and graphical mean-of-ROI analysis.

Off-line Image Analysis Stations There are two computer stations in the Core Facility Computer Lab reserved for microscope users, which are loaded with specialized imaging software packages. The RCFoffline1 workstation (Dell OptiPlex 3010), runs on Windows 7 Professional (64-bit) with 4GB RAM and is loaded with full off-line versions of Zeiss Axiovision 4.8.2 (for AxioObserver with Apotome), LAS AF 2.7.3 (for Leica confocal), and NIS-Elements AR 4.13.04 (for Nikon widefield). The RCFoffline2 workstation (HP Z440) runs on Windows 7 Professional (64-bit) with 64GB RAM, and is loaded with NIS-Elements AR 4.15 (for Nikon confocal/Super Res).

FLOW CYTOMETRY
Scientific Advisor: Dr. Robert Chervenak

BD Biosciences FACSAriaIII The FACSAriaIII cell sorter is capable of 17-parameter (Forward Scatter, Side Scatter, and 15 fluorescence detectors) analysis and cell sorting. It has four solid-state lasers for excitation at wavelengths of 405 nm, 488 nm, 561 nm and 633 nm. This is a

digital high-speed sorter, capable of sorting up to 70,000 events per second. It is capable of standard "bulk" sorting of up to four user-defined cell populations simultaneously, or can be used for direct deposition of a counted number of cells directly into tissue culture plates for cloning, frequency response assays, or other single cell analyses. This instrument uses BD Biosciences FacsDiva software for acquisition and analysis.

BD Biosciences FACSCalibur The RCF maintains a FACSCalibur flow cytometer available for investigator use. Documented training by staff is required for its use. The FACSCalibur is an ultra sensitive flow cytometer, capable of six parameter (two laser light scatter and up to four fluorescent colors) analysis. It uses two lasers for fluorochrome excitation: an Argon ion laser for 488 nm excitation and a Red Diode laser for 635 nm excitation. This instrument uses CellQuest Pro software for acquisition and analysis.

BD Biosciences LSRII-UV This LSRII is capable detecting up to 14 parameters (Forward Scatter, Side Scatter, and twelve fluorescence detectors). It has four solid-state lasers for excitation at wavelengths of 355 nm, 405 nm, 488 nm, and 633 nm. This instrument uses BD Biosciences FacsDiva software for acquisition and analysis.

BD Biosciences LSRII-SORP This LSRII is capable of detecting up to 17 parameters (Forward Scatter, Side Scatter, and fifteen fluorescence detectors). It has four solid-state lasers for excitation at wavelengths of 405 nm, 488 nm, 561 nm and 633 nm. This instrument uses BD Biosciences FacsDiva software for acquisition and analysis.

Workstations and Software There is one Macintosh MacPro and two Dell PCs for off-line analysis of data. The off-line Macintosh computer is loaded with Cellquest Pro, while the off-line PCs are loaded with the latest version of FacsDiva software and FCS Express for data analysis. In addition, FlowJo is available on the Macintosh and Dell workstations for specific data analysis needs. For the analysis of cell cycle data, ModFit LT is available on both the Macintosh and PC workstations.

GENECHIP
Scientific Advisor: Dr. Rona Scott

Affymetrix GeneChip System This system is suitable for global gene expression studies using the Affymetrix GeneChip Probe arrays. Oligonucleotide arrays, prepared on glass, are hybridized to biotinylated probes prepared from biological samples and detected with a fluorescent label. Probes for these experiments are derived from a single source, and differentially expressed genes are identified by comparing the results of experiments performed with different chips. A major advantage of this approach is the ready availability of pre-prepared arrays representing a large number of sequences from a number of species.

This system consists of the following components:

1. A GeneChip Hybridization Oven 640 for automated control of hybridization to the GeneChip arrays.
2. A GeneChip Fluidics Station 450 for automated washing of chips and labeling of hybridized probes. This station can wash and stain four arrays simultaneously.

3. A GeneChip Scanner 3000 7G for obtaining high-resolution images of hybridization signals. The scanner can scan 64 array simultaneously.
4. A GeneChip Workstation that controls the operation of the system, data collection, and processing of initial raw data.
5. A bioinformatics system, including Expression Console, Transcriptome Analysis Console, and Ingenuity Pathway Analysis.

NEXT GENERATION SEQUENCING (NGS)
Scientific Advisor: Dr. Rona Scott

Illumina MiSeq The Illumina MiSeq is a next-generation DNA sequencer with a single-lane flow cell. It is capable of generating up to 25 million reads with up to 15GB of output in a single run. It can produce 2 x 300 paired end reads. It has the output to accommodate targeted gene sequencing, metagenomics, small genome sequencing, targeted gene expression and amplicon sequencing. Sample libraries are loaded directly onto the MiSeq where amplified clusters are generated on the flow cell, followed by sequencing by synthesis. Samples can be barcoded and multiplexed or pooled together in the same lane.

Illumina NextSeq 550 The Illumina NextSeq 550 is a high-throughput sequencer that uses sequencing by synthesis technology, a highly accurate reversible-terminator technology. This technology uses fluorescently labeled nucleotides to sequence hundreds of millions of clusters on a flow cell surface in parallel. This method greatly reduces the number of errors and missed calls associated with homopolymers. The NextSeq 550 integrates cluster generation and sequencing into a single instrument, generating base calls and quality metrics in real time. Data are automatically transferred to the BaseSpace environment for simplified analysis, storage, and sharing. The NextSeq 550 is flexible, offering tunable read lengths and output options to support individual research needs. This system has a maximum output of up 120GB with up to 400 million sequencing reads. Applications include genomic DNA sequencing, exome sequencing, mRNA sequencing, small RNA/microRNA sequencing, epigenetics, small genome sequencing and targeted resequencing.

REAL-TIME QUANTITATIVE PCR
Scientific Advisor: Dr. Rona Scott

ABI 7900HT Fast Real-Time PCR System The Applied Biosystems 7900HT Fast Real-Time PCR System is the only real-time quantitative PCR system that combines 96- and 384-well plate compatibility and the TaqMan® Low Density Array. With optional Fast Real-Time PCR capability, this system reduces run time to about 35 minutes in a standard 96-well plate, or about 55 minutes in a 384-well plate. Key applications include gene expression quantification and the detection of single nucleotide polymorphisms (SNPs) using the fluorogenic 5' nuclease assay. To induce fluorescence, the 7900HT system distributes light from an Argon laser excitation source to all sample wells via a dual-axis synchronous scanning head. It then directs the resulting fluorescent emission through a spectrograph to a CCD camera. Emission wavelengths from 500 to 660 nm are monitored, allowing the simultaneous detection of multiple fluorophores. The system is compatible with FAM/SYBR Green I, VIC/JOE, NED/TAMRA/ Cy3, ROX/Texas Red, and Cy5 fluorescent dyes. The Sequence Detection Software for the 7900HT system runs on the Windows XP operating system and is used for instrument control, data

collection, and data analysis. The software includes a plate set-up wizard for easy experimental design.

Bio-Rad CFX96 The RCF currently houses three Bio-Rad CFX96 instruments. The CFX96 is a six-channel real-time PCR system that combines advanced optical technology with precise thermal control to deliver sensitive, reliable detection. The system's solid-state optical technology (six filtered LEDs, each with a corresponding filtered photodiode) maximizes fluorescence detection for specific dyes in specific channels, providing sensitive detection for quantification and target discrimination. Data are collected from all wells during data acquisition. At every position and with every scan, the optics shuttle is reproducibly centered above each well, so the light path is always optimal and there is no need to sacrifice data collection on one of the channels to normalize to a passive reference. Users can select multiple data acquisition modes, including a one-color fast scan for SYBR green. Thermal gradient features can be used to optimize reactions in a single run. The new CFX Manager software has advanced analysis tools for performing normalized gene expression. In addition, this system does not require fluorescein or ROX for instrument normalization.

LASER CAPTURE MICRODISSECTION
Scientific Advisor: Dr. Rona Scott

Arcturus XT The Arcturus XT instrument performs Laser Capture Microdissection from heterogeneous tissue samples simply, quickly, and precisely. In minutes the investigator can locate a single cell or large groups of cells and, using a simple aim-and-shoot method, extract them for subsequent molecular analysis. LCM preserves the exact morphologies of both the captured cells and the surrounding tissue. The Arcturus XT transfers cells from paraffin-embedded and frozen tissue sample stained slides. The entire process can be monitored and documented, and the images stored in an archiving workstation. Research applications include genomics (differential gene profiling, loss of heterozygosity, microsatellite instability, and gene quantification) and proteomics (two-dimensional protein gels, Western blotting, and immuno-quantification of proteins).

MASS SPECTROMETRY
Scientific Advisor: Dr. Chris Kevil

Waters SYNAPT High-Resolution Mass Spectrometer This instrument is capable of operating in both quadrupole time-of-flight (Q-TOF) and ion mobility mode and is primarily used for unbiased detection of peptides and metabolites for proteomics and metabolomics applications, respectively. A NanoAcquity UPLC system with 2D technology is directly interfaced to nanospray ionization source of SYNAPT HDMS for proteomics applications. An Acquity UPLC system is interfaced to ESI/atmospheric pressure chemical ionization (APCI) source for metabolomics applications. An atmospheric pressure photoionization (APPI) source is also available for Synapt.

Waters Q-TOF Micro This is a quadrupole/time-of-flight mass spectrometer for MS/MS-based sequencing of peptides with a mass resolving power of ~8,000$_{FWHM}$. The Q-TOF is interfaced to a Waters CapLC HPLC system with integrated auto sampler for automated unattended nLC-MS/MS. Determination of peptide sequences from MS/MS spectra will be through a suite of software tools including ProteinLynx Global Server (2.2), SEQUEST and MASCOT.

Thermo Finnigan Deca XP Max Nanospray LC/MS Mass Spectrometer This ion trap mass spectrometer is capable of extremely rapid MS/MS experiments. Ultra-high sensitivity coupled with high resolution and fast scan speeds allows for exceptional peptide sequence coverage and metabolites quantification. This instrument is utilized for sensitive bio molecular identification and experiments requiring MS^n. It is coupled to a Michrom Paradigm MG4 multidimensional LC system with integrated auto sampler.