Animal facilities in the USA College of Medicine (USA-COM)

The University of South Alabama (USA) has an approved Animal Welfare Assurance, #A3288-01, on file with the NIH Office of Laboratory Animal Welfare. The College of Medicine was initially granted Full Accreditation by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) on March 2, 1999. Full Accreditation has been maintained to the present date. The USA COM animal program has been designated an "exemplary" program by AAALAC in 2021. USA has established and maintains its program and activities involving animals in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals (ILAR, 1996).

The USA-COM animal program is maintained on two sites, the main vivarium and the Laboratory for Infectious Disease (LID). The main vivarium has a variety of resources available to support biomedical research, including but not limited to: specialized imaging, clinical pathology, necropsy, histopathology, irradiation, and surgical areas. The main vivarium has been designated as ABSL-1/2 and ACL-1/2 and can accommodate ABSL-2 infectious disease studies. The LID has a small ABSL-3 suite which can accommodate ABSL-3 and/or ACL-3 studies.

Both research facilities are staffed 365 days per year. Weekend and holiday animal husbandry coverage is provided by full-time laboratory animal technicians. USA employs one full-time veterinarian with experience and training in laboratory animal medicine. This veterinarian is supported by two licensed veterinary technicians, two laboratory animal supervisors, five laboratory animal technicians (care staff), and one contract veterinarian. Weekend and holiday supervision and on-call veterinary coverage are provided by the facility supervisors and veterinary personnel on a rotational basis. A veterinarian and a supervisor are on-call at all times. Cellular telephone numbers for facility supervisors, veterinary technicians, and veterinarians are posted on the main entry doors and adjacent to facility telephones in the vivarium.

All animals are observed for signs of illness and/or injury at least twice daily, and are fed and watered daily. Routine animal husbandry tasks and research procedures are performed which include scheduled cage/pen changing and sanitation and research manipulations/treatments (such as post-operative care and weaning animal litters) as required by the experimental/breeding protocol. Clinical treatments are performed by vivarium personnel.

Veterinary care for the animals housed at the university is provided daily by veterinary staff. Although both prophylactic and therapeutic treatments are conducted, preventative medicine programs that include health surveillance (for rodents - through sentinel testing and annual monitoring), quarantine procedures, and environmental monitoring are emphasized to prevent the introduction of infectious diseases and other health problems.

Biobank

The USA Health Biobank is a core facility operating to meet the University Health System and the University biomedical research community's needs for tissues and bodily fluids from patients diagnosed with different types of diseases and conditions. Located at the Mitchell Cancer Institute, the Biobank occupies three laboratories and is staffed by a full-time manager and histotechnologist. Services include collecting, processing, and storing biospecimens, DNA/RNA extraction for biospecimen quality control, and complete histological services. All patient information and de-identified biospecimens are kept in a restricted access area with authorized personnel's keycard entry.

The Biobank is equipped with four -80ºC freezers and two cryogenic liquid nitrogen freezers for storage of biospecimens, and cabinets for storage of tissue paraffin blocks. The biospecimen processing and quality control analysis laboratory is equipped with a QIAGEN TissueLyser II system for biospecimen disruption, a QIAGEN QIAcube Connect instrument for DNA and RNA extraction, a QIAGEN QIAxpert unit for DNA and RNA quantification, and a QIAGEN QIAxcel Advanced system for RNA quality control analysis. The histology laboratory is equipped with a tissue processor, an embedding station, a cryostat, a microtome, a staining

station, and an immunohistochemistry autostainer. All associated patient and biospecimens data are stored securely using a Labmatrix™ Laboratory Information Management System software.

Flow Cytometry Shared Resource

The Flow Cytometry facilities within the College of Medicine are located in the Medical Sciences Building (MSB 2264, 2228) and the Mitchell Cancer Institute (MCI 3085) and both serve as fee-for-service shared resources. The MSB facility is operated by Dr. Domenico Spadafora (over 5 years of experience in flow cytometry) while the MCI facility is operated by Steve McClellan (over 20 years of experience). Dr. Robert Barrington (over 20 years of experience) serves as overall director of both facilities.

Both facilities house a BD Biosciences FACSAria-SORP cell sorter and a BD FACSCanto II cell analyzer. The **FACSAria-SORP cell sorters** are capable of 12 fluorescence parameters. The sorter has 4 laser lines, including a 60 mW 350 nm UV line, 100 mW 488 nm, 100 mW 561 nm and 40 mW 640 nm lines. The sorters can be used to isolate cells in multiple formats, including 4-way tube sorting and 96-well plate sorting via the Automated Cell Deposition Unit. **The BDFACSCanto II cell analyzers** are equipped with 3 lasers (30 mW 405 nm, 20 mW 488 nm, and 17 mW 640 nm) and are capable of 8 fluorescence parameters.

The MCI Flow Cytometry facility also offers the following:

Nexcelom CeligoS microplate-based imaging cytometer: Designed for performing assays directly in multi-well plates (6-384 wells). There are many advantages to this approach, including lower demand for cells (allowing for the use of rare primary cells isolated from patient samples) and reagents; as well as analyzing the cells in their native state without the need to enzymatically remove them from their growth substrate, as is required for traditional flow cytometry. It can image cells in Brightfield and 3 fluorescent channels: blue (377/50 nm excitation; 470/22 nm emission) green (483/32 excitation; 536/40 emission) and orange/red (531/40 excitation; 629/53 emission). Advanced image recognition software provides over 10 specific analysis modes for a wide variety of applications.

Celsee Prep100 CTC isolation system: This uses size exclusion microfluidic filtration to capture CTC from whole blood using a label free method. The system is based on disposable microfluidic chips and with manual processing, isolate very rare cells can be isolated from blood (100-1000 in 10 ml of whole blood).

On-Chip Biotechnologies Microfluidic Cell Sorter (3 laser 6 color): This is world's first in its class microfluidic cell sorter, developed in Japan and USA MCI was one of the first centers in the US to obtain this new technology. Benefits over traditional sorting include the ability to analyze extremely low starting sample volumes/number of cells. The sorting is much gentler and can accommodate a wide range of particles from exosomes to cell spheres using two different size microfluidic chips. The sorter is aerosol free and placed in a BSL-2 cabinet for safe isolation of cells infected with lentivirus or other pathogens. The 3 excitation lasers (405, 488 & 561 nm) are collinear such that fluorochromes excited by any laser can be detected in any of the following channels: FL1 (445/20 nm), FL2 (543/22 nm), FL3 (591.5/43 nm), FL4 (607/36 nm), FL5 (676/37 nm), and FL6(732/68 nm).

On-Chip Biotechnologies Single Cell Plating System (SPiS): As a companion instrument, the SPiS performs single cell or sphere deposition into 96 or 384 well plates in a far more gentle and reliable manner than traditional sorters.

The MSB Flow Cytometry facility also offers the following:

ZetaView® TWIN - NTA Nanoparticle Tracking - Video Microscope PMX-220: The 2-laser platform (488 & 640 nm) was installed in the core lab in May 2019. Nanoparticle Tracking Analysis (NTA) captures the Brownian motion of each particle in the video. Based on the different diffusion movements of large and small particles in the surrounding liquid, the hydrodynamic

diameter of the particles is determined. Pattern parameters, such as intensity fluctuations, surface geometry and shape of the particles, as well as particle concentration, are documented at each recording and can be used to distinguish sub-populations. In addition, the charge state of the particle surface (zeta potential) can be measured via the movement of the particles in an applied electric field. Depending on the type of sample and the measuring mode, the measuring range is between 15 nm and 5 µm.

Agilent Seahorse XFe24 Analyzer: Supported by an S10 award and installed in November 2019, the Seahorse measures the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of live cells in a 24-well plate format. Seahorse XF technology measures the flux of oxygen and protons in the transient microchambers created by specially designed tissue culture microplates in real time. The software calculates the rates of oxygen consumption and extracellular acidification from its measurements of oxygen and proton flux.

10x Genomics Chromium Controller: Installed in February 2020, this instrument uses advanced microfluidics to perform single cell partitioning and barcoding in minutes. The controller combines a single cell with a single gel bead that is coated with a unique oligonucleotide barcode to form a single cell emulsion droplet, which is referred to as a "GEM" (Gel Bead-in-emulsion). Within each GEM, the unique barcoded oligonucleotides are used as primers to generate cDNAs, which can then be traced back to cell of origin. The barcoded amplified fragments from hundreds to thousands of cells are pooled to create short read sequencer compatible libraries. Sequencing of libraries enables analysis of single cells for gene expression, cell surface proteins, immune clonotype, antigen specificity, and chromatin accessibility.

High-Throughput Compound Screening Core

Located in the Mitchell Cancer Institute and housed within a designated 300 sq. ft. laboratory, this core provides researchers expertise and resources for assay development and high-throughput screening against large compound libraries. The laboratory contains a fully integrated Beckman/Coulter Industrial Robotic Integrated System (IRIS) automated platform for high throughput screening of small molecule libraries fully contained in a HEPA-filtered clean enclosure. The 6-axis Motoman robotic arm and all screening instrumentation are coordinated by the SAMI Workstation EX scheduling software system. Integrated devices include a Molecular Devices ImageXpress Ultra confocal high throughput imager (HCS instrument) capable of rapid, high resolution fluorescent microscopic cellular assays. Automated image acquisition and analysis are controlled by the MetaXpress and Acuity Xpress software packages along with MetaXpress PowerCore Parallel Processing system. An additional Molecular Devices ImageXpress Micro widefield high throughput imager is available for both fluorescent and bright field automated microscopy assays. A BioMek FXp Hybrid Dual Arm automated liquid handler enables transfers of up to 96 samples at once or individual "cherry picking" of active samples and sample titration. A BioTek EL406 microplate washer / dispenser is available for dispensing cells, detection reagents, or processing of immunoassays. The Molecular Devices Paradigm multimode microplate reader enables homogenous endpoint and kinetic detection in absorbance, fluorescent intensity, fluorescent polarization, luminescence, HTRF, and AlphaScreen-based assays. A chemical diversity library of over 50,000 unique chemical entities from Life Chemicals, as well as smaller libraries of known bioactive compounds, and novel focused compound libraries contributed by medicinal chemist collaborators are available for high throughput screening projects.

BioImaging Core Facilities

There are two bioimaging core facilities in the College of Medicine operating on a fee-for service basis: one in the Medical Sciences Building (MSB) on the main campus and one at the Mitchell Cancer Institute (MCI).

BioImaging Core Facility at the Medical Sciences Building:

i. **Nikon A1R spectral confocal microscope:** Procured in 2010, this system has become the primary imaging tool for multi-fluorophore and spectral applications in live cell and tissue preparations. This system has four laser lines, a 32-bin spectral detector and NIS Elements acquisition/analysis software. This system is capable of 4D (x,y,z,t) time lapse and 5D (x,y,z,λ,t) spectral time lapse imaging in live-cell and tissue studies.

ii. **Andor WD Revolution spinning disk confocal microscope:** This dual camera system allows deep penetration for thick tissue preparations (>300 μm) and multi-channel live-cell/tissue imaging. It is now the primary system for imaging fast Ca^{2+} dynamics in cells and tissues. A hyperspectral module is currently being implemented to allow high speed scans of the excitation spectrum (rather than the emission spectrum).

iii. **Zeiss LSM 980 Airyscan2 spectral confocal microscope:** Following an NIH S10 instrument grant (S10OD028606, Rich PI) a Zeiss LSM 980 Airyscan2 spectral confocal microscope was purchased recently and installed in December 2020. This system features Airyscan2, high sensitivity emission scan spectral image acquisition via an array of 32 GaASP detectors, an environmental chamber, and a wide range of quantitative image analysis software. Airyscan2 dramatically increases the sensitivity of the imaging system, allowing extended time lapse imaging of cells and tissues with minimal photobleaching or photodamage. Airyscan2 also allows enhanced/super resolution imaging of live cell preparations. The use of 32 GaASP detectors in the spectral detector allows a marked increase in the sensitivity of spectral detection, discrimination, which we anticipate will lead to a ~5-fold decrease in scan time.

iv. **Computer workstations:** Three dedicated computers are available for image analysis and mathematical modeling. Analysis software includes Zeiss Zen, NIS Elements, Matlab, Image J, and S8.

vi. Other instrumentation in the core: **MMI laser capture microdissection system** and **Photon Technology International QuantaMaster 40 spectrofluorimeter**.

BioImaging Core Facility at the Mitchell Cancer Institute, University of South Alabama:

i. **Nikon A1rsi with TIRF and N-STORM:** a fully-featured laser scanning spectral confocal microscope system with seven laser lines (355, 405, 441, 488, 514, 561, 647 nm), 32-bin spectral detector with variable bin size, Bruker XY Mini-scanner for UV laser applications, Tokai-Hit stage-top incubator for live-cell imaging, anti-vibration table, and featuring TIRF and N-STORM modules for super-resolution imaging. STORM (Stochastic Optical Reconstruction Microscopy) is a single-molecule super-resolution technique allowing for lateral resolutions of ~35 nm. NIS Elements software is used for control, image acquisition, and extension/customization of the system. Extensions include the MIDAS laser micro-irradiation system for inducing and studying DNA damage.

ii. **The Nikon A1r resonant laser scanning confocal microscope:** This has four laser lines (405, 488, 561, 647 nm), two high-sensitivity GaAsP detectors, Tokai-Hit stage-top incubator for live-cell imaging, and anti-vibration table. GaAsP detectors provide improved signal-to-noise over standard PMT detectors, reducing photobleaching, improving sensitivity, and allowing for extended live imaging. NIS Elements software is used for control, image acquisition, and extension/customization of the system. Extensions include the MIDAS laser micro-irradiation system.

iii. **Nikon N-SIM Structured Illumination Super-Resolution Microscope:** this utilizes structured illumination to resolve structures below the traditional diffraction limit of light microscopes (maximum lateral resolution ~100 nm). The system is equipped with two laser

lines for SIM imaging (488 and 561 nm), light source and filters for epi-fluorescence imaging, automated stage, and features multiple SIM modalities, including 2D SIM, 3D SIM, and TIRF-SIM. NIS Elements software is used for control, image acquisition, and image processing.

iv. **Nikon TE-2000E automated wide-field epi-fluorescence microscope:** This features a computer-controlled automated microscope base, Pathology Devices stage-top incubator for live-cell imaging, Prior automated stage, external emission and excitation filter wheels for FRET imaging, and an anti-vibration table. NIS Elements software is used for control and image acquisition.

v. **MMI Cell Cut Plus laser micro-dissection microscope system:** this features an integrated touch-screen monitor, automated stage, 355 nm cutting laser, dedicated software and computer-controlled sample retrieval for precision micro-dissection of prepared tissue slides.

vi. **Computer workstations:** Three dedicated analysis computers are available at MCI for image analysis. Available software includes NIS Elements, Zeiss Zen, ImageJ/FIJI, and MIDAS.

Mass Spectrometry Research Facility

Located in the Mitchell Cancer Institute and operating on a fee-for-service basis, the mass spectrometry facility provides a central resource of metabolomics and proteomics measurements to identify, characterize, and quantify metabolites and proteins present in different biological and biomedical samples by using state-of-the-art mass spectrometry. The facility is equipped with the following mass spectrometers: a Thermo LTQ Orbitrap XL, Thermo Q-Exactive Plus and Waters Ultima™ QTOF. It is directed by Dr. Marie Migaud operated by two dedicated research staff: Dr. S. Tadi, a senior research scientist who has extensive expertise in metabolomics and proteomics, and Mrs. L. Schambeau, the lab manager who has been managing the facilities and performing most of the experimental fee-for-service tasks over the last 10 years.

X-ray crystallography

Under the direction of Dr. Aishwarya Prakash, facilities for X-ray crystallography are available in the Mitchell Cancer Center. This comprises (a) new state-of-the-art sealed-tube D8 Quest X-ray generator with PHOTON 200 CMOS detector (Bruker) with ancillary computers for data collection and data processing; (b) an Oxford 800 liquid nitrogen cryo-system for flash cooling crystals; (c) a Formulatrix NT8 dropsetter; (d) a Rock Imager I system; (e) four Thermo-Scientific incubators maintained at different temperatures 4 – 24 °C for crystal growth; and (f) two Zeiss stereomicroscopes.