The mission of the **MSU Flow Cytometry Core Facility (MSUFCCF)** is to provide investigators with access to cutting-edge analytical flow cytometry instrumentation and cell sorting services, as well as training and experimental consultation

The MSUFCCF South Hub is located on the second floor of the IQ Bioengineering Building (Room 2521 and 2522). Instrumentation available in IQ include a BD FACSAria IIu cell sorter, a Cytek Aurora spectral cytometer, a BD Accuri C6, a 10x Chromium controller, a ParticleMetrix ZetaView fNTA small particle analyzer, and a Luminex 200. The MSUFCCF North Hub is located on the fourth and fifth floor of the Biomedical and Physical Sciences Building (BPS; Room 4120 and 5115). Instrumentation available in BPS include a BD Influx cell sorter, a SONY MA900 cell sorter, a ThermoFisher Attune CytPix imaging cytometer, a BD LSR II, and a Yokogawa CQ1 spinning disk high-content imaging system. Laboratory space for the North and South Campus Hubs are approximately 885 sq. ft. and 300 sq. ft., respectively. The MSU Flow Cytometry Core is directed by Dr. Matthew Bernard who both provides professional oversight of the Core Facility and assay development expertise, as well as technical support and guidance for general education in flow cytometry principles. Dr. Daniel Vocelle, Assistant Director, oversees instrument and data analysis training, assay/application development, as well as technical support and guidance for general education in flow cytometry principles.

**Benchtop Analyzers**

**Cytek Aurora:** The Cytek Aurora is a cutting-edge spectral flow cytometer equipped with 5 lasers (UV 355nm, violet 405nm, blue 488nm, yellow-green 561nm, and red 640nm) and has the capability of detecting up to 64 fluorescence channels with additional light scatter detectors off of the violet (SSC) and blue lasers (FSC; SSC). The optics and state-of-the-art low-noise electronics provide excellent sensitivity and resolution. The instrument is also equipped with an auto-sampler for sample acquisition in 96-well plates. The Cytek Aurora is located in the MSUFCCF South Campus Hub (IQ Room 2521).

**ThermoFisher Attune CytPix:** The Attune CytPix is equipped with 4 lasers (violet 405nm, blue 488 nm, yellow-green 561 nm, and red 637nm) and has the capability of detecting up to 14 fluorescent parameters in addition to light scatter (FSC/SSC). This cytometer is additionally equipped a brightfield camera and AI driven automated image analysis software to derive single cell morphological parameters. The Attune CytPix is equipped with a CytKick Max autosampler, which is capable of automated sampling in 96- and 384-well plates, as well as a variety of tubes. The Attune CytPix is located in the MSUFCCF North Campus Hub (BPS Room 4120). ***The* *Attune CytPix is supported by the Equipment Grants Program, award #2022-70410-38419, from the U.S. Department of Agriculture (USDA), National Institute of Food and Agriculture (NIFA).***

**BD Accuri C6:** The BD Accuri C6 is a compact flow cytometer that uses a low-pressure peristaltic pumping system to drive instrument fluidics allowing for the derivation of sample volume and calculation of absolute counts or sample concentration per microliter. The instrument is capable of running up to 10,000 events per second at sample concentrations of over 5 x 106 cells/mL. It is equipped with 2 lasers: a blue 488nm (3 detectors + FSC/SSC; FL1 [530±15 nm], FL2 [585±20 nm], FL3 [>670 nm]) and a red 640nm (1 detector; FL-4 [675±12.5 nm]). The optical configuration allows multi-parameter detection of up to 4 fluorescent parameters and 2 light scatter parameters. The system is operated using CFlow Plus software. Optical filters allow for detection of commonly used fluorescent markers, including, but not limited to: FL1- FITC, AF488, GFP, CFSE, YFP; FL2- PE, PI; FL3- PerCP, PerCP-Cy5.5, PE- Cy7, RFP, mCherry; FL4- APC, AF647, APC-Cy7, APC-H7. The BD Accuri C6 is located in the South Campus Hub (IQ Room 2521).

**BD LSR II:** The BD LSR II can detect up to 11 parameters, including 9 fluorescent parameters, and is used for acquisition/analysis only. It is equipped with 3 lasers: a violet 405nm (2 detectors), a blue 488nm (4 detectors + FSC/SSC), and a red 640nm (3 detectors). The system is operated using BD FACSDiva software. The BD LSR II is located in the MSUFCCF North Campus Hub (BPS Room 5115).

**ParticleMetrix ZetaView fNTA:** The ParticleMetrix ZetaView fNTA is a small particle analyzer that can quantify and measure the size of small particles through analysis of Brownian motion. The ZetaView is also equipped with a 520 nm excitation laser and 550LP filter, allowing for assessment of fluorescence on nanoparticles, extracellular vesicles, viruses, etc. The ZetaView is located in the MSUFCCF South Campus Hub (IQ 2500 laboratory).

**Cell Sorters**

**BD Influx:** The BD Influx is equipped with 5 lasers: UV 355nm, violet 405nm, blue 488nm, Y-G 552nm, and red 633nm. The optical configuration allows multi-parameter detection of up to 14 fluorescent parameters and 2 light scatter parameters. The system is operated using BD FACS Sortware. The instrument is capable of performing both acquisition/analysis of samples, in addition to its main cell sorting function. The BD Influx is equipped with Sweet Spot technologies that increase sort accuracy and stability. The sorter is capable of high-speed sorting through over 50x106 cells per hour with up to six populations simultaneously and ability to sort for single- cell applications. An Automated Cell Deposition Unit, ACDU, is integrated to allow for sorting cells into custom devices and microtiter plates up through 384-well layouts. The sorter is fitted with 70, 100, 140 and 200 μm nozzles to allow for sorting a wide variety of cell types. The instrument is installed in a Baker BioProtect Class II Biosafety Cabinet to allow for appropriate sorting of BSL-2+ samples. The Biosafety cabinet provides a means to evacuate the sort collection chamber and traps aerosols that may be generated during the sorting process. The BD Influx is located in the MSUFCCF North Campus Hub (BPS Room 5115).

**SONY MA900:** The MA900 contains 4 lasers in co-linear pairs (488nm & 561nm, and 405nm & 634nm) and can detect up to 12 fluorescent parameters and 2 light scatter parameters.The system is operated using SONY Cell Sorter Software. The instrument is capable of performing both acquisition/analysis of samples, in addition to its main cell sorting function. The SONY MA900 is a unique instrument that provides unparalleled workflow automation, a modular fluidics system, and advanced biosafety features, making it the perfect instrument for independent use in Core facilities. The instrument is capable of high-speed sorting through over 43x106 cells per hour with up to four populations simultaneously into tubes (12x75mm or microfuge tubes) or a plate deposition system (6-well through 384-well plates). The MA900 can operate with 70, 100, and 130 μm nozzles to allow sorting for a wide variety of cell types. The instrument is contained in a Baker Biosafety Cabinet to allow for appropriate sorting of BSL-2+ samples. The Biosafety cabinet provides a means to evacuate the sort collection chamber and traps aerosols that may be generated during the sorting process. The SONY MA900 is located in the MSUFCCF North Campus Hub (BPS Room 5115).

**BD FACS Aria IIu:** The BD FACS Aria IIu is equipped with 3 lasers: a 405nm violet (4 detectors), a 488nm blue (6 detectors + SSC), and a 633nm red (3 detectors). The optical configuration allows multi-parameter detection of up to 13 fluorescent parameters and 2 light scatter parameters. The system is operated using BD FACSDiva software. The instrument is capable of performing both acquisition/analysis of samples, in addition to its main cell sorting function. The BD FACS Aria-IIu is equipped with BD Accudrop and Sweet Spot technologies that increase sort accuracy and stability. The sorter is capable of high-speed sorting through over 50x106 cells per hour with up to four populations simultaneously and ability to sort for single- cell applications. An Automated Cell Deposition Unit, ACDU, is integrated to allow for sorting cells into microtiter plates up through 96-well layouts. The sorter is fitted with 70, 85, and 100 μm nozzles to allow for sorting a wide variety of cell types. The instrument is installed in a Baker BioProtect IV Class II Biosafety Cabinet to allow for appropriate sorting of BSL-2+ samples. The Biosafety cabinet provides a means to evacuate the sort collection chamber and traps aerosols that may be generated during the sorting process. The BD FACS Aria IIu is located in the MSUFCCF South Campus Hub (IQ Room 2522).

**10x Chromium Controller:** The 10x Chromium controller is capable of single-cell portioning and barcoding to create single-cell cDNA libraries for downstream next-gen sequencing. Pairing with barcoded antibodies also allows for concurrent single-cell surface protein analysis. This instrument is also paired with a Bio-Rad C1000 Touch Thermal Cycler. The 10x Chromium controller is located in the MSUFCCF South Campus Hub (IQ Room 2521).

**Multiplex Systems**

**Luminex-200:** The Luminex 200 System is a diagnostic instrument that performs bead-based multiplex (96-well format) analysis of up to 100 analytes simultaneously in a single small volume samples. This system utilizes fluorescently labeled microbeads that are conjugated with a specific reagent (eg, antibody, antigen, enzyme substrate, etc.) to enable capture and detection of analytes of interest. The Luminex 200 is equipped with 2 lasers: a 635nm classification laser for excitation of fluorescently-labeled microbeads and a 532nm reporter laser for excitation of the analyte signal. The Luminex 200 has a dynamic range typically ≥ 3.5 logs. A handheld magnetic plate washer, microtiter plate shaker, bath sonicator, and vortexer are available in the Core Facilities to enable multiplex assay performance. Operation of the system and data analysis are performed using the Luminex XPonent Software. Commercially available and custom multiplex kits can be utilized to quantify mouse, rat, and human protein expression (e.g., cytokines, growth factors, phosphoproteins, etc.) in a variety of sample types (e.g., cell culture media, plasma, serum, BALF, cell lysates, etc.). In addition to immunoassays, the platform can be used for enzyme assays, genotyping, gene expression analysis (*Quantigene, ThermoFisher*), or assessment of protein-protein interactions. The Luminex 200 is located in the MSUFCCF South Campus Hub (IQ Room 2521).

**High-Content Imaging**

**Yokogawa CQ1:** The Yokogawa CQ1 is a compact high-throughput spinning disk confocal microscope equipped with automated focus, acquisition, and data analysis. The CQ1 provides rich feature extractions, facilitating sophisticated cellular image analysis, and due to its proprietary microlens array disk, facilitates high speed scanning and reduces phototoxicity and photobleaching. The CQ1 is equipped with 4 lasers (violet 405nm, blue 488 nm, yellow-green 561 nm, and red 640nm) and has the capability of detecting up to 16 fluorescence channels with additional brightfield/phase contrast detection. The CQ1 is equipped with the following objectives to image a wide variety of sample types: 2x (NA-0.08), 4x (NA=0.16) , 10x (NA=0.4), 20x (NA=0.8), 20x LWD (NA=0.45), 40x (NA=0.95), and 60x (NA=0.9). The CQ1 is additionally equipped with environmental controls capable of regulating temperature, CO2, and O2. Operation of the CQ1 is performed using Yokogawa Cell Pathfinder software. The Yokogawa CQ1 is located in MSUFCCF North Hub (BPS Room 4120).