

MAJOR EQUIPMENT BIOMARKER CORE

GENERAL EQUIPMENT: The Biomarker Core is located in the Haughey Lab and has full access to the laboratory equipment that includes a fully equipped tissue culture facility with 2 laminar and 1 vertical flow hoods, two double stack copper lined CO₂ incubators, water baths, and dissecting microscopes. All equipment necessary for various types of electrophoresis, Western, Northern and Southern blot analyses are present. A Syngene G:Box, and dark room are available for chemiluminescent and fluorescent image analyses of protein and DNA blots. A Speedvac evaporator/concentrator for processing DNA and RNA samples is present. A Spectra Max M2^e fluorescence plate reader, a visible plate reader, a spectrophotometer are present for fluorescent based 96-well assays. A full range of equipment for immunohistochemical and immunofluorescent studies are available including a Leica cryostat, a sliding microtome, and staining facilities. All equipment necessary for blood and fluid sample processing including organic and solid phase extraction platforms, OA-SYS heated nitrogen dryer, and Isotemp vacuum oven are present for sample processing. Two applied biosystems PCR cyclers and one 7300 RTPCR systems are present for RNA analyses. Three Zeiss Axiovert inverted microscopes are present. One microscope is equipped with a Quant EM 512sc digital camera, a perfusion and atmosphere control system for rapid fluorescent imaging of calcium and other fluoroprobes in live cells at high resolution (up to 20 images/second). The second microscope is equipped with an Apotome, and a motorized stage for optical sectioning by structured illumination. The third microscope is equipped with a motorized stage with auto-focus controlled by Neurolucida software/hardware for semi-automated high field strength imaging of dendrites and dendritic spines. One additional Zeiss upright microscope is present to image histological sections from brain slices. A Seahorse cellular bioenergetics analyzer is available in the Hoke laboratory (Dr. Haughey was Co-I on the equipment grant to purchase this instrument). Five large 80°C freezers, 6 refrigerators, and 5 -20° C freezers, and a liquid nitrogen storage tank are also in the laboratory for sample and reagent storage.

CORE SPECIFIC EQUIPMENT

MASS SPECTROMETRY: Mass spectrometers dedicated to lipid and metabolite analyses supported by the NIMH CAHN Center include an AB/Sciex API 3000, AB/Sciex API 4000 QTRAP electrospray ionization tandem mass spectrometers (ESI/MS/MS), and an AB/Sciex 5600 triple TOF. Each of these instruments is equipped with HPLC or UPLC and auto-samplers. AB/Sciex Multiquant and LipidView software and high performance computing are available for data analysis.

Mass spectrometers dedicated to proteomic analyses (located in Dr. Cole's laboratory) include Agilent 6500 Q-TOF and Chip Cube Chromatography System, ABI 4800 TOF/TOF bioanalyser, ABI Tempo Maldi Spotting System. Equipment available on shared equipment grants includes: LTQ Orbitrap MS (Thermo), 1D and 2DE gel equipment, one GE IPG apparatus, Ettan Gel electrophoresis apparatus and power packs, 5 x 2nd dimension vertical slab gel apparatus, Typhoon 9210, multiple Western blot systems (Invitrogen); Langendorff Apparatus. 1 PF2D (2DLC system, Beckman). Agilent OffGel System.

The Applied Imaging Mass Spectrometry (AIMS) core facility (directed by Kristine Glunde) offers targeted MALDI imaging of drugs, and drug metabolites, and discovery MALDI imaging of metabolites, lipids, peptides, intact proteins, tryptic peptides, and glycans. The AIMS core is equipped with a variety of sample handling and automated sample preparation tools that allow the usage of controlled and reproducible protocols. AIMS core staff closely collaborates with users to perform MALDI-imaging compatible cryo-sectioning in gelatin and other MALDI-compatible cryo-media in the facility. Protocols for MALDI imaging sample preparation of formalin-fixed, paraffin-embedded (FFPE) tissues are also well established, and are performed in the AIMS core facility. Technical staff in the AIMS core performs standard histology and IHC protocols in close collaboration with users, followed by slide scanning for co-registered overlay with MALDI imaging data. In contrast to other mass spectrometry techniques, MALDI imaging allows for the precise anatomical localization of metabolites, lipids, proteins and other agents in tissues. The instrumentation in this core was obtained through a large equipment grant in which Dr. Haughey is a Co-I.

MASS SPECTROMETERS

Mass Spectrometers (Haughey)	Ionization	Detector	Vendor
AB Sciex 4000 QTRAP ABSciex 5600	Electrospray Electrospray	Triple quadrupole Triple-TOF	AB Sciex AB Sciex

Mass Spectrometers (Cole)	Ionization	Detector	Vendor
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Q-Exactive LTQ Orbitrap Velos Voyager DE-STR MALDI-TOF	Electrospray Electrospray MALDI	Orbitrap Orbitrap or Ion Trap TOF	ThermoFisher ThermoFisher AB Sciex
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Mass Spectrometers (Glunde)	Ionization	Detector	Vendor
Bruker Rapiflex Bruker timsTOF fleX	MALDI MALDI-2	TOF-TOF TOF	Bruker Bruker

NUCLEAR MAGNETIC RESONANCE

NMR (Xu)	Field Strength
Bruker NMR Spectrometer	400 MHz
Bruker NMR Spectrometer	500 MHz
Bruker NMR Spectrometer	750 MHz

CHROMATOGRAPY EQUIPMENT

Chromatography (Haughey)	Vendor	Interfaced with
Shimatzu UFLC Autosampler Degasser	Shimadzu Shimadzu Shimadzu	AB Sciex 4000 QTRAP
Shimatzu UFLC XR Autosampler Nexera XR Degasser Nexera pumps	Shimadzu Shimadzu Shimadzu Shimadzu	AB Sciex 5600

Chromatography (Cole)	Vendor	Interfaced with
Easy-nLC 1000 Dual Column Nanospray Source NanoAquity UPLC 2D NanoLC system (3 units) 1100 series Autosampler (3 units) Nanospray source (3 units) 1100 series CapillaryLC-variable wavelength 1200 series CapillaryLC-multi-wavelength 1200 series Micro-Fraction Collector (2 units) Probot Fraction Collector Peristaltic pumps (2 units)	ThermoFisher (Proxeon) Phoenix Waters Eksigent Agilent ThermoFisher Agilent Agilent Agilent Dionex Fisher Scientific	Q-Exactive LTQ Orbitrap Velos Offline fractionation applications Offline fractionation applications Offline fractionation applications Offline fractionation applications Mini-Prep Cell protein purification

GEL ELECTROPHORESIS AND ACCESSORIES

Gel Electrophoresis and Other (Cole)	Function	Vendor
Mini-Prep Cell (2 units) IPGphor Isoelectric Focusing system (3 units) Ettan DALTsix System (4 units) Typhoon 9400	Protein purification by gel electrophoresis IEF 1 st dimension separations. SDS-PAGE 2 nd dimension separations. Red/green/blue laser scanner, fluorescence and phospho-imaging.	Bio-Rad GE Healthcare GE Healthcare GE Healthcare
Perfection V750 Pro	Light scanner for silver and Coomassie blue stain imaging.	Epson
Ettan Spot Picker robotic Laminar Flow Hood AlphaSpec µL Spectrophotometer	Excise spots from gels. For manually excising gel spots. For measuring peptide/protein concentrations in small volumes without sample loss.	GE Healthcare Fisher Scientific Alpha Innotech
Positive Pressure 96 well manifold	For using 96 well flow through plates containing affinity resins to remove interfering reagents.	Waters

BIOINFORMATIC SOFTWARE

Software (Haughey)	Function	Vendor
LipidView	Molecular characterization and quantification of lipid species.	AB Sciex
Peakview	Interpretation of mass spectral data, processing accurate mass, structural interpretation, and batch analysis.	AB Sciex

MarkerView	Metabolomics, lipidomics, protein/peptide biomarker profiling, and statistical analysis.	AB Sciex
MATLAB	Generation of random forest models.	Mathworks
SIMCA	Partial Least Squares Regression Analyses	Unimatrix
Ingenuity Pathway Analysis	Pathway and functional downstream analyses	Ingenuity sys.
Sciex OS-Q	Omics data quantification and qualitative processing.	AB Sciex
National Institute of Standards and Technology (NIST-2017)	ESI-MS/MS tandem mass spectral library.	NIST
Sciex Accurate Spectral library 2.0	ESI-MS/MS accurate tandem mass spectral library.	AB Sciex

Software (Cole)	Function	Vendor
DataExplorer	Analysis of MALDI data.	AB Sciex
DeCyder (5 licenses)	DIGE gel image analysis.	GE Healthcare
Mascot (3 licenses)	MS database search engine.	Matrix Science
Peaks	De novo sequencing and MS database search engine.	Bioinformatics Solutions Inc
Progenesis (5 editor licenses)	Gel image analysis.	Non-Linear Dynamics
ProSight PC	Top Down Analysis of Proteins.	Keller, Northwestern U.
Protein Deconvolution	Deconvolutes multi-charged envelopes of intact proteins.	ThermoFisher
Proteome Discoverer (3 licenses)	Analysis of ion trap, orbitrap and iTRAQ data.	Genologics
Proteus	Laboratory Information Management System.	Proteome Software, Inc
Scaffold (2 licenses)	Parse Mascot and Sequest Search Results	ThermoFisher
Sequest	MS database search engine	MacCoss Lab Software
Skyline	Targeted Quantification Method/Analysis	QIAGEN-Ingenuity Inc.
XCalibur	Collect/Analysis of ion trap data	Partek Inc.
Partek Genomics Suite 6.6	Normalization, statistical analyses and graphical Representation.	
Spotfire Genomics Suite	Statistical analyses and graphical representation	TIBCO Spotfire Inc.

Software publicly available	Function	Vendor
MetaboAnalyst 5.0	Omics data analysis, pathway and network analysis.	Open source
KEGG: (Kyoto Encyclopedia of Genes and Genomes)	Database resource for understanding high-level functions and utilities of the biological system.	Open source
Cytoscape	Visualizing molecular interaction networks and biological pathways.	Open source
METLIN	Mass spectral characterization and compound identification.	Open source
XCMS	Processing mass spectrometry data for metabolite profiling.	Open source
LipidMaps: Lipid Database	Lipidomics data characterization.	Open source
HMDB: Human Metabolome Database	Metabolomics data interpretation and biomarker discovery.	Open Source
SMPDB: Small Molecule Pathway Database	Pathway elucidation and pathway discovery in metabolomics, transcriptomics, proteomics and systems biology.	Open Source

ADVANCED COMPUTING: All data acquisition computers store raw spectral data from mass spectrometry onto a dedicated RAID 1 storage so that data is mirrored onto two hard drives in real time to prevent data loss in the case of a hard drive failure. For off-line analysis, spectral data is transferred to one of two separate dedicated analysis computers for subsequent processing, data analysis and mining. Each of these computers contains a Intel Xeon Gold 6128 3.4GHz, 3.7GHz Turbo, 6C, 10.4GT/s 2UPI, 19.25MB Cache processor with 48GB 6x8GB DDR4 2666MHz RDIMM ECC for multithread processing and a RAID 5 disk system for backup of processed data. An additional 3 Windows 10 PRO based PCs equipped with identical processors are present in the laboratory for statistical and mathematical modeling. Computers are networked to the Johns Hopkins High Throughput Biology Center (HIT) for access to Ingenuity Pathway Analysis software covered through a multiuser agreement.

The Biomarker Core has access to the Maryland Advanced Research Computing Center (MARCC) that is a shared computing facility located on the Bayview Campus of Johns Hopkins University that is funded by a State

of Maryland grant to Johns Hopkins University through IDIES. MARCC is jointly managed by Johns Hopkins University and the University of Maryland College Park. MARCC offices are located in the Bloomberg Building (Homewood campus). The mission of the MARCC is to enable research, creative undertakings, and learning that involve and rely on the use and development of advanced computing. MARCC manages high performance computing, highly reliable data storage, and provides outstanding collaborative scientific support to empower computational research, scholarship, and innovation.

Blue Crab is the main cluster at MARCC with over 23,000 cores and a combined theoretical performance of over 1.4 PFLOPs. The compute nodes are a combination of Intel Ivy Bridge (large memory nodes), Haswell, Broadwell and Skylake processors and several Nvidia K80/P100 GPUS linked via FDR-14 InfiniBand interconnects. It also features two types of storage: 2 PB Lustre (IEEL) and 14 PB ZFS on Linux.