

Center for Human Health and the Environment

The vision of the Center for Human Health and the Environment (CHHE) is to become a global leader in environmental health sciences (EHS) along the continuum from genes to populations by building on NC State's unique research strengths and resources in quantitative biology, -omics and analytical technologies, and diverse model organisms as well as its emerging strength in human population science. CHHE provides consultation and affordable access to analytical and -omics technologies, bioinformatics support, and pathologic assessment of the effects of exposures on model organisms as well as access to the Integrative Health Sciences Facility Core (IHSFC) translational partnership.

Systems Technologies Core - The **overall objective** of the Systems Technologies Core (STC) is to provide expertise, state-of-the-art instrumentation, and novel analytical approaches to address a broad range of scientific questions presented across the Center for Human Health and the Environment (CHHE) at NC State University. Expertise in analytical techniques is essential to support CHHE's mission to understand how human health is impacted by environmental factors and to implement this knowledge to reduce the adverse impacts. The STC has partnered with the Molecular Education, Technology and Research Innovation Center (METRIC) (described below) and the Genomic Sciences Lab (described below) to provide CHHE investigators with analytical expertise and state-of-art methodologies to create an integrative, mechanistic picture of the underlying effects of environmental exposures on human health. These methodologies include: 1) genomics, epigenomics, and transcriptomics; 2) discovery and targeted proteomics; 3) discovery and targeted metabolomics including environmental toxicants; and 4) metallomics including metal speciation

Comparative Pathology Core- Vertebrate animal model use is essential to answer fundamental questions and elucidate the mechanisms in vivo through which environmental exposures may influence human disease and produce adverse human health outcomes. Diverse animal models, such as zebrafish, medaka fish, mouse, rat, rabbit, guinea pig, vole, xenopus, domestic animal (dogs, pigs, sheep, etc.) and others, along with their genetically engineered counterparts, represent powerful in vivo approaches to link molecular mechanisms to pathologic lesions (disrupted organ/tissue function) and toxic effects. Animal models remain the gold standard for studying these effects of environmental toxicants on development, cancer, reproduction, organ toxicity, and neurological and endocrine systems. These approaches are powerful because in vivo model organisms operate in a systems biology framework integrating all levels of biological organization - biomolecule, pathway, cell, tissue, organ, and organism - and inform us of potential adverse human health outcomes. Understanding these adverse effects requires expert pathologic evaluation of the affected tissue/organ/cells. To fill this need, the **mission** of the Comparative Pathology Core (CPC) is to provide CHHE member scientists dedicated access to cost-free pathology expertise to assess the effects of environmental factors in a diverse range of animal models.

Integrative Health Sciences Facility Core - The Integrative Health Sciences Facility Core (IHSFC) of the Center for Human Health and the Environment (CHHE) is the central interface between basic scientists, data scientists, epidemiologists, clinical researchers, and public health practitioners. Its mission is to catalyze multidirectional translation of research between diverse model systems, population-based studies, and community partners to advance understanding about the effects of environmental exposures on human health and facilitate use of this information by affected communities to improve public health. The intentional disciplinary

diversity of the IHSFC leaders has developed the Core into a highly effective resource for CHHE's internationally renowned scientists who leverage diverse models for environmental health science (EHS) research, bioinformaticists who integrate data streams from across the health sciences, and epidemiologists and community engagement specialists who are substantively involved in local communities and translating CHHE research to improve public health.

Molecular Education, Technology and Research Innovation Center (METRIC)

Beginning in late 2016, a convergence of institutional planning identified molecular characterization of simple and complex chemical and biological systems as requiring strategic investments by NC State. With strong support from the Chancellor, the Provost, the Vice Chancellor for Finance, and the Vice Chancellor for Research and Innovation, detailed planning for the development of METRIC commenced and was announced formally in November 2017. METRIC encompasses three key platform technologies: mass spectrometry, magnetic resonance (NMR and EPR), and X-Ray crystallography (small molecules and proteins). METRIC resources are housed in Dabney Hall, Polk Hall, Partners III, and the Toxicology Building and total more than 12,500 square feet, including offices for 15 scientists, instrumentation rooms with UPS and back-up power, bio-specimen and general-purpose storage (-86°C and -20°C freezers) with back-up power, and wet laboratories for sample preparation. Space renovations (~\$2M+) to accommodate METRIC produced state-of-the-art laboratories with independent climate control, galleys to isolate noise and vibration, chilled water, DI water, and appropriate power. Laboratories are equipped with sample storage, analysis, and preparation instrumentation, including Bio-Rad ChemiDoc XRS Gel Imager, Bio-Rad Pharos FX Plus Fluorescent Imager with three wavelengths, Bio-Rad iMark Microplate Reader, Stereomicroscopes, column packing stations, data analysis hardware and software, a state-of-the-art LMD system with live-cell imaging capability, Leica CM 1950 cryostat, BSL2 laminar flow hoods, 1D Electrophoresis, Eppendorf benchtop centrifuges, vortexers, Thermo Fisher Sorvall ST 16R centrifuges, and Nanopure water purification systems. All personnel in METRIC have personal PCs with access to networked printers, a color copy machine, and several 20 TB RAID data storage systems.

METRIC provides NC State and CHHE researchers with access to a world-class measurement science facility that includes mass spectrometry, nuclear magnetic resonance spectroscopy, electron paramagnetic spectroscopy, and X-ray crystallography of small molecules and macromolecules. NC State has committed significant resources (>\$11M in capital investments, including service contracts on all instrumentation to reduce downtime) and state-funded, highly-qualified personnel. The latter includes a Director (**Dr. David Muddiman**, Jacob and Betty Belin Distinguished Professor of Chemistry and Director of CHHE's Systems Technologies Core), Associate Director (Joseph Barycki, Professor of Biochemistry), seven PhD scientists (plus an additional hire in mass spectrometry with search ongoing), one MS-level scientist, one BS-level scientist, and a laboratory manager to serve all three platform technologies. METRIC is a University-wide Shared Core Research Facility administered by NC State's Office of Research and Innovation and is governed by an active and engaged Executive Committee, Faculty Advisory Committee, External Advisory Committee, and three User Committees.

Each Platform Technology in METRIC is listed below. Instrument scheduling and/or training, as well as billing for services, is managed by a cloud-based Lab Management Software platform (MENDIX).

Mass Spectrometry Platform Technologies and Software

- 6560 Agilent ion mobility spectrometry-QTOF mass spectrometer with a Rapidfire 365 SPE platform and Agilent 1290 UPLC System
- Thermo Fisher Scientific ID-X Tribrid MS with EQUAN and Vanquish UPLC System with PAL Autosampler
- QE HF with Easy nanoLC 1200 UPLC System with Autosampler
- QE HF X with Vanquish Horizon UPLC System and ZipChip™ CZE System with Autosamplers
- QE HF X with Nano Proflow 2D UPLC System with Autosampler
- TSQ Altis Triple Quadrupole Mass Spectrometry with Horizon Vanquish and Eksigent nanoflow UPLC Systems with Autosamplers
- QE Plus with Custom IR-MALDESI Source for Direct Analysis and Mass Spectrometry Imaging
- ICAP RQ ICP-MS with Ultimate LC System and Autosampler
- 5800 MALDI TOF/TOF
- 5975 GC/MS with NIST and Wiley Libraries
- Exactive Plus with UltiMate 3000 UPLC System for Elemental Composition Determinations
- Orbitrap Elite with nanoUPLC and ETD
- Off-Line SPE System for sample preparation (Autotrace for Thermo Fisher)

Software for mass spectrometry experimental design and data analysis includes: SAS JmP Pro v11.0, Mascot, Proteome Discoverer 2.2, Scaffold v1.4, Biopharma 2.0, LipidSearch 4.1, Compound Discover 2.1W, MaxQuant, X-Caliber, and MSiReader v1.0 for MSI Experiments. Each software suite operates on an independent, high-performance computer with four processors.

Magnetic Resonance Spectroscopy

- Avance NEO 400 MHz NMR with TopSpin 4 Acquisition and Processing, RT BBO iProbe, VT, and SampleXpress Automatic Sample Changer
- Avance NEO 500 MHz NMR with TopSpin 4 Acquisition and Processing, BBO PRODIGY LN₂-Cryoprobe, VT, and SampleCASE automatic Sample Changer
- Avance NEO 600 MHz NMR with TopSpin 4 Acquisition and Processing, RT BBO Smart Probe and TXI 1H-13C/15N-2H Probe, VT, and SampleXpress Automatic Sample Changer
- Avance NEO 700 MHz NMR with TopSpin 4 Acquisition and Processing, TCI 1H/19F-13C/15N-2H LHe Cryoprobe, TXI 1H-13C/15N-2H Probe, VT, and SampleXpress Automatic Sample Changer
- Avance III 700 MHz NMR with TopSpin 3.5 Acquisition and Processing, TCI 1H-13C/15N-2H LHe CryoProbe, TXI 1H-13C/15N-2H Probe, VT, and SampleXpress Automatic Sample Changer
- Varian Mercury 400MHz NMR with VNMRJ4.3 Acquisition and Processing, VT, 5mm ASW 4-nuclei 1H/19F/13C/31P Probe
- Varian Inova 400 MHz NMR with VNMRJ4.3 Acquisition and Processing, VT, 5 mm PFG gradient 4-nuclei probe (1H/19F/13C/31P)
- Varian Mercury 300MHz NMR with VNMRJ4.3 Acquisition and Processing, 5 mm ID Probe (1H/13C)
- Varian Mercury Plus 300MHz NMR with VNMRJ4.3 Acquisition and Processing, 5mm PFG 4-nuclei Probe

- E500-10/12 EPR System with Digital High-Resolution Hall Field Controller and Dual Channel Signal Processing Unit

A workstation with nmrPipe, nmrView, Topspin, and Sparky is available to users for data processing and analysis.

X-Ray Crystallography

- Rigaku MicroMax007 X-ray generator with Eiger 4M Detector, VariMax-VHF ArcSec Optics, Oxford Cryosystem, and associated peripherals
- Art Robbins Instruments Crystal Phoenix
- Art Robbins Instruments CrysCam Digital Microscope
- Walk-in crystal growth chambers with vibration damping cradles for crystal tray storage (4°C/18°C)
- Bruker-Nonius X8 Kappa APEXII (CCD) instrument using MoK α radiation with an Oxford Cryostream 700 cold stream
- Leica M60 stereomicroscope, equipped with polarizing filters, rotating stage and video camera

Workstations with Cambridge Structural Database, Reciprocal Net, Phenix, COOT, CCP4, PyMol, and Chimera, are available to users for data processing and analysis.

Genomic Sciences Laboratory

NC State's Genomic Sciences Laboratory (GSL) was established in 2000 and is the primary genomics core facility for the University. The GSL currently provides sequencing and genomics services to over 147 laboratories across 5 colleges and 22 departments, as well as a large external user base that extends to other North Carolina Universities (Duke, UNC Charlotte, UNC Chapel Hill, NCCU, and ECU), multiple federal research agencies (NIH-NIAID, NIH-NIEHS, USDA-ARS, and NOAA), and CROs and other Biotech companies within the NC Research Triangle area. Operating under a net-neutral funding model, the GSL extends internal pricing rates for Next-Generation Sequencing (NGS) services to all federally-funded agencies, as well as to collaborators of NC State faculty.

The GSL occupies 2,500 square feet square feet of laboratory and office space in Thomas Hall, located on NC State's Main Campus. The GSL is led by its Director, **Dr. David Baltzegar** (CHHE's Genomics Navigator in its Systems Technologies core), and is staffed by four full-time technicians who have over 50 years of combined molecular biology experience. Capabilities available within this core facility include:

Sanger Sequencing & Microsatellite Genotyping. The GSL currently houses an Applied Biosystems 3730xl capillary sequencer, a Biomek FX liquid handling robot for dye-terminator cleanup, and multiple thermal cyclers.

Next Generation Sequencing. The GSL facility operates two Illumina MiSeq sequencers for small genome sequencing, phage sequencing, and metagenomics/microbial diversity analysis. Illumina NextSeq 500 and NovaSeq 6000 DNA sequencers are used for transcriptomics (RNAseq), whole genome shotgun sequencing, and Genotype By Sequencing (GBS) approaches. In addition, the GSL recently acquired a Pacific Biosciences Sequel, capable of long-read (> 5kb) NGS sequencing, for sequencing of large or complex genomes. The GSL also

utilizes an Agilent Bioanalyzer and TapeStation for sample Q/C analysis, and a Sage Pippen Prep fragment size collector.

Shared Use. The GSL maintains a suite of laboratory instruments for general use by trained NC State faculty, staff, or students. These include a Qiagen TissueLyzer for extraction of nucleic acids, a Covaris S220 instrument for DNA fragmentation, BioRAD CFX 384 qPCR and BioRAD QX200 digital PCR instruments for gene expression analysis and rare variant detection, and a Biomek Synergy H1 plate reader for colorimetric and fluorescent assays.

Cellular and Molecular Imaging Facility

CMIF is NC State's primary shared research and teaching resource for advanced optical microscopy and supports NC State faculty research programs spanning 6 colleges and over 20 departments. The facility is also open to other universities, government agencies, and industries. CMIF is currently located in Gardner Hall (1,300 square feet) with satellite labs in David Clark Labs (85 square feet) and Riddick Hall (185 square feet).

Expert personalized training and support for all available instrumentation and software is provided by CMIF Director **Dr. Eva Johannes** and Research Associate **Dr. Marius Zareba**, each of whom has over 15 years of experience. CMIF is administered by NC State's Office of Research and Innovation (ORI) and governed by a Faculty Advisory Committee. Scheduling for equipment access and training, as well as billing for services, is managed by a cloud-based Lab Management Software (built on the MENDIX platform by NC State's Office for Information Technology). Currently available instrumentation and software in CMIF includes:

Microscopy workstations

- Zeiss LSM 880 with Airyscan: laser scanning confocal microscope
- Zeiss LSM 710: laser scanning confocal microscope
- Zeiss Lightsheet Z.1
- Zeiss Axioimager fluorescence microscope with DIC, 2 cameras
- Andor Dragonfly 302 spinning disk confocal with TIRF and ablation laser
- 3i Marianas spinning disk confocal with ablation laser
- Olympus MVX10 (fluorescence Macroscope with 2 cameras)
- Leica MZ III (stereo microscope with color camera)

Image Analysis Software

- Zeiss Zen Black and Zen Blue – several licenses
- Arivis 4D 2
- Imaris 7.0
- Metamorph 7.7
- Slidebook 6.0
- Matlab
- FIJI

Histology Laboratory

The Histology Laboratory at NC State's College of Veterinary Medicine is a biomedical service facility that resides in the same department as the Comparative Pathology Core. Histology support services include all aspects of processing, embedding, and sectioning of frozen and paraffinized tissues for routine hematoxylin and eosin staining, many special histochemical

tissue stains, and numerous immunohistochemical stains. The laboratory also supports research protocol development for investigators needing new services.

The Histology Laboratory staff includes five certified histotechnologists with extensive experience in biomedical histology. The Histology Laboratory is equipped with modern processing equipment that includes:

- Sakura VIP and VIP5, and Shandon PathCentre automated paraffin tissue processors
- Surgipath Embedding Centers (2 units)
- Leica Rm2135 (3 units), Leica RM2235, Leitz 1512, and Reichert 820 paraffin microtomes
- Leica CM1850 cryostat freezing stage microtome
- Sakura automated hematoxylin and eosin stainer
- Biocare Intellipath automated stainer for immunohistochemistry
- Dako ArtisanLink automated stainer for special stains and a Pascal Target Retrieval unit
- Leica 2065 Supercut Plastics Microtome
- Sorvall Triangular glass knife breaker

The Histology Laboratory facility occupies 2,500 square feet and has four fume hoods.

Biological Resources Facility

The Biological Resources Facility (BRF) provides centralized sites for the humane care of research animals while accommodating the research and teaching needs of faculty members at NC State, as well as researchers from private industry and other governmental agencies under conditions required by local and federal regulatory agencies. The BRF houses a multitude of research animals including mice, rats, voles, frogs, and fish, whereas the Toxicology Animal Facility houses mice.

The BRF is located on NC State's North Campus in Raleigh, NC, and it contains approximately 20,000 square feet including office and administrative support spaces, a clinical laboratory, necropsy and preparation rooms, and behavioral space. The BRF also operates a satellite facility on Centennial Campus that supports research within CHHE, Toxicology, and Biomedical Engineering.

These facilities house laboratory animals under conditions required by local and federal regulatory agencies. The BRF is regulated locally by the NC State University Institutional Animal Care and Use Committee (IACUC) and is equipped with backup electrical and air-conditioning systems to ensure the welfare of the animals.

Both locations are served by a laboratory animal veterinarian who is based at the College of Veterinary Medicine's Laboratory Animal Resource unit. The Veterinarian provides weekly rounds and is available for animal health checks and research consults as requested.

Flow Cytometry and Cell Sorting Facility

The Flow Cytometry and Cell Sorting facility at NC State's College of Veterinary Medicine provides instrumentation and assistance with multi-parameter flow cytometry analysis and cell sorting. It is available to all NC State researchers, as well as users outside of the university community. The facility is equipped with two state-of-the-art analyzers (BD LSR II and a Beckman Coulter CytoFLEX), a MoFlo-XDP high-speed cell sorter, and an AutoMACS magnetic

cell sorter. The facility was also recently upgraded to support cell sorting of Biosafety level-2/2+ (BSL2/BSL2+) samples.

The flow core facility offers multiple software options free of charge for current users. Currently the facility has Cyt-Expert, FACSDiva, FCS express with multi-cycle analysis capabilities, and Kaluza. The facility also provides experimental design services as complimentary services to all users.

Users have the option of getting fully trained to operate the instrumentation independently or to pay for a facility-assisted run. The rates for the services are comparable to other flow cytometry core facilities in the area including, the UNC-Chapel Hill and Duke University flow cytometry core facilities.

This facility occupies 263 square feet in the CVM Main Building and is managed by **Javid Mohammed**.