

PSU College of Medicine Core Facilities (Summer, 2007)  
Section of Research Resources H093  
(More information available at [www.hmc.psu.edu/core](http://www.hmc.psu.edu/core)):

•**Macromolecular Core Facility** (C1732) —Current services include:

*Protein/peptide sequencing:* An Applied Biosystems Procise 491 protein sequencer using Edman degradation chemistry is available for sequence analysis. We have the capability to sequence from blots and can routinely obtain sequence from 10-20 pmol of non-blocked peptide. Costs are **\$15/residue** with a **5 residue (\$75) minimum charge**.

*Peptide synthesis:* One Milligen 9050 and one Perseptive Biosystems 9050 Fmoc peptide synthesizer are available for peptide synthesis. A standard synthesis delivers between 50-250 mg of peptide. A standard synthesis on either of these machines delivers between 50-80 mg of peptide, at a cost of **\$20 per residue (0.1 mmol scale)**, or **\$30 per residue (0.2 mmol scale)**. Custom syntheses are also possible (e.g., phosphopeptides, biotinylated peptides), with the additional cost being determined by the reagents used. **Mass Spec** of the synthesized peptide is available for an **additional \$15**.

*Oligonucleotide synthesis:* One Expedite 8909, and a PolyGen DNA synthesizer are used to generate oligonucleotides. Depending on the scale of the reaction, **costs range from \$0.33 - \$1 per base, with no setup fee**. **Purification** is routinely performed using Nensorb columns at a cost of **\$10 per oligo**. Custom syntheses such as modified bases (e.g., methylated bases, non-standard nucleotides) and phosphorothioate chemistries are also available, with the price depending on the cost of the specialized reagent necessary.

*Mass Spectrometry:* A state-of-the-art Applied Biosystems 4800 MALDI TOF TOF and a Proteomics 4700 Analyzer (tandem ms/ms MALDI TOF-TOF, capable of high-throughput, automated data-dependent tandem MS/MS CID analyses and database searching/protein identification), with complete 2D-LC nanoflow separation systems, are available (See). A self-service (after training) Perseptive Biosystems Voyager DE-PRO reflectron MALDI-TOF is also available for protein identification, protein modification, and protein expression studies, as well as for oligonucleotide and carbohydrate analyses. For small molecule, metabolite, and post-translational modification analyses, an MDS/Sciex 4000 QTrap (hybrid Ion Trap) is available. The DE-PRO and 4000 QTrap instruments are available on a signup basis, at a rate of **\$25/hr**, or non-complex samples can be analyzed by facility personnel at a fee of \$15 for a nominal mass, or \$25 for an accurate mass. For more complex analyses, see Mass Spec/Proteomics Facility below

*Image analysis:* A Biorad FX Pro Plus imager coupled with a BioRad GS 800 densitometer and Quantity One and PDQuest software is available for acquisition and quantitation/analysis of radioactive, fluorescent, and film images. Three separate lasers (488, 535, and 633 nm) allow fluorescent image acquisition from a large variety of fluorescent probes, including multicolor applications like DIGE analysis from 2D gels. A Typhoon fluorometer is also available for DIGE gel image acquisition, with spot analysis through DeCyder software). After image acquisition and analysis, spot cut lists can then be exported to our BioMachines/LEAP Technology 2DiD spot cutter, or to an Ettan Spot Picker, for subsequent proteomic analyses (See *Mass Spec & Proteomics Facility* section below). A Molecular Dynamics 425E Phosphorimager is also available for analysis of radiometric images. These imaging instruments are available on a signup basis, at a **rate of \$5/hr**.

*The purpose of the Macromolecular Core Facility is to provide state-of-the-art methods for analysis and synthesis of protein and nucleic acid structures. The Macromolecular Core Facility was established in 1987-1988, with primary grant support from the National Science Foundation (Macromolecular Instrumentation for Cell and Molecular Biology; NSF DR-8804758, 11/1/88 - 4/30/91; \$258,222 direct costs; Drs. S. Goodman, A. Hopper, M. Billingsley, Co-PIs). The facility has also received grant support from the NIH (Two-Dimensional Image Analyzer, NIH-RR04811, 5/1/89 - 4/30/90; \$72,000 direct costs, Dr. M. Billingsley, PI; Instrumentation for Peptide Analysis, NIH RR14851, 4/1/2001-3/31/2002, Dr. B. Stanley, PI), the State of Pennsylvania via the Ben Franklin Partnership (Macromolecular Synthesis Facility; 9/89 - 8/91; \$100,000 direct costs; M. Billingsley, PI), and matching funds from the College of Medicine (approximately \$500,000 in salary support, fringe benefits, supplies, new equipment, and equipment upgrades.*

Director: Dr. Bruce Stanley ([bstanley@psu.edu](mailto:bstanley@psu.edu))

Senior Research Support Associate and Lab Manager: Anne Stanley ([aes7@psu.edu](mailto:aes7@psu.edu))

Facility Staff: Suja Maddukuri ([srm25@psu.edu](mailto:srm25@psu.edu))

**•Transgenic Mouse Facility (ARF 173, 174)** — This facility provides injection and breeding services for development of knockout or transgenic mouse lines. To create such transgenic or knockout lines, the core lab performs microinjection of purified DNA into the pronucleus of fertilized mouse eggs on two separate injection days. Eggs are then returned to pseudopregnant females, and founder lines established. The core lab harvests eggs, injects them on two separate injection days, reimplants eggs, and coordinates litter stocks up to the establishment of a founder line. Procedures for targeted gene disruption using embryonic stem cells have also been developed, and over 80 transgenic and knockout mouse lines have been created.

*Costs for Transgenic Mouse Production* depend on the strain of mouse used as follows:

Mouse Strain	Set-up Fee (non-refundable)	Injection Fee	Total Price
B6D2F1	\$500.00	\$2120.00	\$2620.00
C57BL/6	\$700.00	\$2490.00	\$3190.00
FVB	\$500.00	\$2560.00	\$3060.00
Investigator Provided*	\$200.00	\$1530.00	\$1730.00

\*Mouse strain provided by the investigator: Male and female mice needed for production of fertilized embryos for injection are supplied by the investigator. These mice must be kept under the investigator's mouse protocol and be proven pathogen free before being moved into the Transgenic mouse room. No guarantee applies to these mice.

**Knock-out Mice:**

# of Injections	Set-up Fee (non-refundable)	Injection Fee	Total Price
1 Injection Day	\$500.00	\$980.00	\$1480.00
3 Injection Days	\$1500.00	\$2640.00	\$4140.00
5 Injection Days	\$2500.00	\$4250.00	\$6750.00

**Rederivations:**

Mice	Set-up Fee (non-refundable)	Rederivation Fee	Total Price
1 Line	\$300.00	\$2170.00	\$2470.00

*Investigator Responsibilities:* The individual investigator is responsible for providing ultrapure DNA fragments or Embryonic Stem Cells for ES work, and for performing appropriate Southern blot or other validated analyses on founders and their progeny to establish integration of the transgene.

*The purpose of the Transgenic Core Lab is to develop transgenic and targeted gene disruption mouse models for use in biomedical research, thus providing powerful approaches for the study of gene function in relation to normal health and disease. As with other cores, centralizing the basic microinjection and stem cell technologies in a common core lab avoids unnecessary and expensive duplication of efforts, and more importantly, experience with similar facilities at other institutions strongly indicates that the efficiency and success rates of transgenic experiments are greatly improved in a core facility where the procedures are conducted routinely. The Transgenic Core Facility is housed in the Animal Research Farm in dedicated core space proximate to the animal care facilities. Establishment of this facility was funded by an NIH grant (Drs. Billingsley, Levenson, and Pegg) and by a commitment of institutional resources, which provided funds for salary and fringe benefits for a core lab technician and equipment for microinjections, a Leica microscope with dual micromanipulators, a Sutter programmable pipette puller, a Narashige microforge, an Olympus dissecting stereoscope, a laminar flow hood, and two incubators.*

Co-Directors of the facility are Dr. Sarah Bronson ([sbronson@psu.edu](mailto:sbronson@psu.edu)) and Dr. Bruce Stanley ([bas12@PSU.EDU](mailto:bas12@PSU.EDU))

Lab Manager: Alane Seidel ([aks2@psu.edu](mailto:aks2@psu.edu))

(Faculty Advisory Committee: Dr. Ron Wilson, Dr. Rob Bonneau)

•**Molecular Genetics Core Facility (C1727)** — This lab provides DNA sequencing, standard primer sets, and PCR amplification for sequencing from plasmids, BACs, and PCR products, as well as sequencing-based SNP and Genotyping analyses (SNPlex assays).

**Instrumentation:**

ABI 3130XL Capillary Sequencer

ABI/Perkin Elmer 377A DNA Sequencer (gel-based)

associated PCR machines

SUN BLADE 1000 workstation running the GCG/Wisconsin package with SeqWeb web-based access for more sophisticated analysis of DNA sequences, mapping alignments, and communication with other public sequence databases.

NanoDrop ND-1000 Spectrophotometer: Full-spectrum spectrophotometer needs only 1 µl of sample (free usage)

**Sequencing costs are \$9.50 per sequence**, with the individual investigator providing pure DNA template; the Sequence Analysis services in the lab are currently provided to our researchers without charge; however, a fee structure for use of these analytic packages may be established in Fall, 2007. There is no charge for the use of the NanoDrop ND-1000 Spectrophotometer.

This facility greatly enhances the infrastructure for molecular biology and genetics at the University.

*At the heart of this facility is the institutional need for automated DNA sequencing, genotyping, and sequence analysis. This core laboratory for DNA sequencing and DNA sequence analysis was established using institutional and NSF funds in 1994.*

Director: Dr. Bruce Stanley ([bstanley@psu.edu](mailto:bstanley@psu.edu))

Lab Manager: Joe Bednarczyk ([jbednarczyk@psu.edu](mailto:jbednarczyk@psu.edu)), with additional facility support from Dr. David Stanford ([drs19@psu.edu](mailto:drs19@psu.edu))

•**Microscopy Imaging Facilities (C3706, C3707, C7643)** – these facilities, which are co-supported between the Section of Research Resources and the Juvenile Diabetes Diabetic Retinopathy program project grant, provide **Confocal, Deconvolution, Multichannel Fluorescence, and Electron Microscopy** instrumentation and services as well as **Histology** services.

*Confocal Microscopy (C3706):* The facility is equipped for traditional and live cell confocal microscopy, including FRAP, , and FRET experiments, , etc. Instrumentation is a Leica TCS SP2 AOBS confocal system with a Leica DM/IRB/BE inverted microscope, including 20X, 40X, 63X objectives, in a combination of oil, water, and multi-immersion refractive indices appropriate to our combination of tissue, live mammalian and yeast cell, and fixed cell/tissue work; DIC/Nomarski optics necessary for detailed transmission imaging; an integrated environmental control system for live cell (heated stage and perfusion system). Four separate lasers provide 8 well-spaced excitation wavelengths (405, 458, 476, 488, 496, 514, 543, and 633 nm); acousto-optical tunable filters allow both rapid switching between these eight laser lines and attenuation of each individual line to optimize excitation while avoiding photo-bleaching. Individual investigators (faculty, staff, students and post-docs) may use the system on their own after being certified on the instrument following a training session (given each Friday by appointment) by the facility director, Dr. Alistair Barber ([abarber@psu.edu](mailto:abarber@psu.edu)). **Usage fees** are \$27 per hour.

*Deconvolution Microscopy (C3706):* a separate image capture and deconvolution software package fitted to an upright Leica fluorescence microscope using a Retiga EXi cooled CCD camera for image acquisition using QED Software followed by the Huygens algorithm for deconvolution. The system is mounted on a Leica DM RXA2 upright microscope with 10X, 20X and 63X UV-corrected objectives. Individual investigators (faculty, staff, students and post-docs) may use the system on their own after being certified on the instrument following a training session (by appointment) with Roland Myers ([rlm9@psu.edu](mailto:rlm9@psu.edu)). **Usage Fees** are \$5 per hour.

*Image analysis (C3706):* an additional computer is available for image analysis with several software packages including: Leica confocal software, Huygens deconvolution, Adobe Photoshop, and Image J.

*Electron Microscopy (C3707):* Electron Microscopy sample preparation (sectioning, osmium

coating, etc.) and EM imaging services are available using a Philips TEM 400 transmission electron microscope. Digitized images can be produced on demand from the higher resolution film images that this system produces. Individual investigators (faculty, staff, students and post-docs) may use the system on their own after being certified on the instrument following training sessions (by appointment) with the facility manager, Roland Myers ([rlm9@psu.edu](mailto:rlm9@psu.edu)).

**Fees: Facility-provided services are \$35 per hour. Use of the Electron Microscope by trained users is \$70.00 per hour**

*Multichannel Fluorescence Microscopy (C3706, C5742):* A Nikon TE-2000U inverted microscope with 4-channel epifluorescence and DIC modules, CoolSnap ES CCD camera, and NIS-Elements imaging software is available in room C5742 (Grigoryev lab). A fluorescent spinning filter disk system developed by a faculty member (Dr. Russ Scaduto) allows realtime acquisition and integration of fluorescence signals from up to 8 separate excitation-emission pairs using an advanced Fluorescence microscope from C & L Instruments in room C3706.

**Fees:** currently (Summer, 2007) there are **no fees** for the use of these instruments.

**Please contact Dr. Sergei Grigoryev** ([sgrigoryev@psu.edu](mailto:sgrigoryev@psu.edu)) to arrange use of the Nikon TE-2000U microscope

*Histology (C7643):* Services provided include Cryostat sectioning, Tissue processing and paraffin embedding, Paraffin sectioning, Routine hematoxylin and eosin staining, specialized staining (glycogen, neuron, connective tissue staining, etc.), Immunohistochemistry, and In situ hybridization. In addition to these procedures performed routinely in the lab, specialized staining procedures can be implemented in collaboration with individual investigators. **Fees** depend on exact work done, but tissue slicing and slide mounts are \$1 per slide.

Service	Fee
Tissue Processing & Paraffin Embedding	\$3.00 per block
Paraffin Sectioning	\$1.00 per slide
Cryostat Sectioning	\$1.00 per slide
Staining (H&E, Glycogen, Neuron, Connective Tissue, etc.)	\$1.00 per slide
Decalcification	\$1.00 per specimen
Immunohistochemistry	Variable
Training	\$20.00 per hour
Supplies	\$0.81 per slide

For histology services, please contact Dr. Kang Li ([kx130@psu.edu](mailto:kx130@psu.edu))

*The confocal microscope and QED/deconvolution microscopes were established in March, 2003 using funds from the NIH (Instrumentation for Confocal Microscopy, (PHS grant number 1 S10 RR 16861-01), 7/1/2002-6/30/2003, Dr. B. Stanley, PI), funds from a JDRF Program Project Grant in Diabetic Retinopathy (Dr. T. Gardner, P.I.) as well as institutional funds. The Histology and Electron Microscopy Facilities were previously established as local departmental facilities within the Department of Neuroscience and Anatomy, and became part of the institutional Shared Facilities in July, 2003). These services allow researchers to perform state-of-the-art imaging experiments, thus greatly enhancing the Cell Biology capacities at the institution. As with all other core facilities, fees are recalculated each year to cover maintenance, consumables, and salary costs only.*

Director: Dr. Alistair Barber ([abarber@psu.edu](mailto:abarber@psu.edu))

Lab Managers: Confocal Microscopy – Rhona Ellis ([rwe11@psu.edu](mailto:rwe11@psu.edu)); Electron Microscopy – Roland Myers ([rlm9@psu.edu](mailto:rlm9@psu.edu)); Histology – Dr. Kang Li ([kx130@psu.edu](mailto:kx130@psu.edu)).

•**Flow Cytometry Core Facility (C1733)** (Cell Identification/Cell Kinetics Laboratory) — Both Clinical samples and Research samples are routinely analyzed in this clinically-certified facility. Two 3-color Becton Dickinson FACScan instruments, a 4-color Becton Dickinson FACScalibur, a 6-color Becton Dickinson FACSCanto, and a Cytomation MoFlo high-speed six-color sorter are available for use by investigators at the College of Medicine.

**Fees:** Rates for Flow Cytometer Use

Standard Sample Acquisition (Researcher provides operator)	\$50.00 per hour
Standard Data Analysis on Computer (Researcher provides operator)	\$10.00 per hour
Assisted Sample Acquisition or Data Analysis (Core provides operator)	Additional \$25.00 per hour
Additional Set up Charge for <b>Sorting</b> on the MoFlo	\$50.00 per set up
MoFlo <b>Sorting</b> will be charged at a minimum of:	\$100.00 per use

*The goals of this core, originally established in 1983, are to provide sophisticated fluorescence-based analyses and fluorescence-activated cell sorting at reasonable hourly rates. Funding for this facility has come from institutional and departmental funds, from Pennsylvania Tobacco Settlement Funds, and from the NIH (Instrumentation for Flow Cytometry/Sorting, PHS RR14851, 4/1/2002-3/31/2003, Dr. B. Stanley, PI). The services of this core are indispensable for investigators in the field of immunology, and for running clinical samples. In addition, many cell biology projects use the flow cytometers for increasingly multiplexed experimental determinations, and the sorter is used for tetrad and rare-event cell-enrichment and analysis.*

Director: Dr. Bruce Stanley ([bas12@psu.edu](mailto:bas12@psu.edu))

Flow Cytometry Specialist and Lab Manager: Nate Sheaffer ([nas2@psu.edu](mailto:nas2@psu.edu)), with additional facility support from Dr. David Stanford ([drs19@psu.edu](mailto:drs19@psu.edu))

[Advisory Committee: Dr. Elaine Eyster and Wendy Hayden (Hematology/Oncology), Dr. Rob Bonneau and Dr. Richard Courtney (Microbiology and Immunology), and Joe Mierski (Bone Marrow Transplant unit)]

•**Functional Genomics Core Facility (HCAR 3200)** The Functional Genomics Core Facility offers microarray and quantitative PCR expertise, instrumentation, and services.

*Microarray Analysis:* Microarray experiments provide a means to **determine the changes between different experimental conditions in thousands of mRNA levels**, with candidate “changed level mRNAs” subsequently validated by Northern Blots or quantitative Real-time PCR, or at the protein level by western blots or mass spec/proteomic experiments. The standard Core microarray platforms are the Agilent and GE Healthcare CodeLink arrays, with arrays available for human, rat and mouse. Microarrays are available in 10,000 gene, 20,000 gene and whole genome formats. We also offer hybridization and analysis for gene chips for other species and other slide microarray formats, as well as analysis services for Affymetrix data acquired from other scanners. **Microarray analysis software packages (Agilent/Silicon Genetics**

**GeneSpring 6 and Celera Discovery System**) are available for use in the core facility, and allow high level analyses such as clustering of elevated genes into gene families or biological pathways, as well as giving complete well-organized information on the genes themselves.

**Fees:**

Agilent whole genome MicroArray in the 4x44k format, the **normal price per sample/array is \$450**. (Should we get discounts from Agilent, the price per array will be less.)  
**Codelink Microarrays Whole Genome \$657 per array**. As the supplier has recently changed (Summer 2007), this price may change soon.

*Quantitative Real-time PCR systems:* **qRT-PCR** provides an alternative to Northern Blots for **more accurate quantitation of the levels of different mRNAs**, for example in validating the levels of candidate mRNAs initially discovered in Microarray experiments. Applied Biosystems 7900HT and 7300 qRT-PCR analyzers are available for use by individual investigators (after training), using associated Primer Express and Sequence Detection Software. Alternately, full analysis of samples can be performed as a service by staff in the Core Facility. The Facility recommends using Applied Biosystem predesigned TaqMan Assays-on-Demand for qRT-PCR, although any validated assays will work fine with this system.

**Fees:**

Instrument use cost is **\$25/run for the 96-well ABI 7300** and **\$50/run for the 384-well ABI 7900HT**. (Price includes the optical plate and cover.)

Additional reagents (Master Mix and Assays on Demand) are available for additional charges.

*SNP detection:* SNP detection can be performed on the 7900HT and 7300 platforms, using validated TaqMan SNP assays (or investigator-designed and validated assays). If you are performing analyses for a few SNPs at a time on a limited number of samples, this is the most economical and accurate way to go. For larger numbers of SNPs (up to 48 multiplexed SNPs), the SNPlex platform may be a better choice (See Molecular Genetics Core Facility section above).

*Quality Assessment of Samples:* “Lab-on-a-Chip” instrumentation is also available for **assessing the quality and quantity of DNA, RNA, and protein samples** (Agilent BioAnalyzer 2100), and is used in all cases before more extensive experimental analysis is attempted to assure that quality data can be obtained.

**Fees:**

DNA, RNA and protein chips are available at a cost of \$50 for a 12 sample chip.

Pico RNA chips hold a maximum of 11 samples.

*Laser Capture Microdissection:* A Zeiss P.A.L.M. **Laser Capture Microdissection System** can be used for collecting specific cellular populations from fixed tissue slices to use in microarray, qRT-PCR, and proteomic experiments. Note – this is a shared instrument that you can use after training, we do not currently provide this as a service (Summer, 2007)

**Fees:** Cost for trained users is **\$25 per hour**.

*NanoDrop ND-1000 Spectrophotometer:* Full-spectrum spectrophotometer needs only 1 µl of sample

**Fee:** \$5 per usage (as many samples as you wish)

[Molecular Devices SpectraMax M2 Plate Reader](#): provides plate-based absorbance and fluorescence readings for multiple samples

**Fee:** \$10 usage fee per plate, includes the price of the facility-supplied optical plate

*Custom Microarrays and Protein Arrays:* For **custom spotted microarrays**, the facility has used an Apogent Discoveries MicroGrid arrayer, and 10,000 gene human, mouse, and rat oligo sets are available for spotting at reduced prices compared to commercial whole genome chips. **Peptide** and **protein-protein interaction arrays** were also under development in the lab. However, these services are currently (Summer, 2007) not being offered.

*The Functional Genomics facility was started in the fall of 2001 entirely with institutional funds, and additional institutional funds (from Tobacco Settlement Funds) contributed to expansion of this facility in 2003 and 2004, adding the capability to make custom microarrays; adding the capacity to use and analyze GE-Amersham CodeLink arrays; and adding the capacity to make protein arrays. Funding through the Penn State Cancer Institute in 2005 allowed the replacement of older qRT-PCR instrumentation by adding newer instrumentation for high-throughput analyses.*

Director of the Functional Genomics Core Facility: Dr. Bill Freeman ([wfreeman@psu.edu](mailto:wfreeman@psu.edu))

Lab Managers: Rob Brucklacher, Research Support Associate ([rlb6@psu.edu](mailto:rlb6@psu.edu)), & Georgina Bixler, Research Support Associate ([gbixler2@psu.edu](mailto:gbixler2@psu.edu)).

•**Mass Spec & Proteomics Facility (C1734, C1735):** This facility provides multiple separation, digestion, chemical derivatization, mass spec, and database searching services for proteomic, carbohydrate, oligonucleotide, lipidomics, and small molecule analysis.

*Mass Spectrometers:* An Applied Biosystems 4800 MALDI TOF TOF and a 4700 Proteomics Analyzer (MALDI TOF-TOF); a reflectron, delayed extraction MALDI-TOF mass spectrometer (Voyager DE-PRO, Perseptive Biosystems/Applied Biosystems) and an MDS/Sciex 4000 QTrap (Hybrid Ion Trap) are available for **analyses of proteins, peptides, carbohydrates, lipids, polymers, small molecules, and oligonucleotides.**

*Sample Preparation Services:* Multiple **sample preparation services** are available, including complete **1D and 2D LC separation services** (ABI's Tempo LC-MALDI and an Eksigent 2D Nanoflow LC system coupled to an LC Packings/Dionex ProBot MALDI plate spotter for peptide level separations, Agilent 1100 HPLC for small molecule separations, Shimadzu HPLC for general HPLC separations, and a Beckman-Coulter PF-2D system for **whole protein level separations and quantitation**).

*1D and 2D gels:* A 12-gel casting and running apparatus for **2D gels** (Ettan IPGPhor II and Dalt 12) can be used by any investigators needing such separations, and standard or DIGE 2D gels can be analyzed on our BioRad FX Pro Plus Fluorimager/ PhosphorImager/ Densitometer using Quantity One and PDQuest software, or with a GE Typhoon DIGE gel reader and DeCyder software. Subsequent **automated spot excision, proteolytic digestion, and MALDI plate spotting** can be done using our BioMachines/LEAP Technologies 2DiDx spot-cutting robot, or our Ettan Spot Picker. Digested samples can also be collected for further separation and injection using either an ESI (electrospray) or nanospray ionization source on our 4000 QTrap Hybrid Ion trap mass spectrometer.

*Sensitivity and types of analyses available:* The mass specs can be used for **analyses of complex mixtures of peptides**, for example from proteolytic digests of proteins purified by column chromatography, by metal affinity chromatography, by SDS-PAGE or 2D gel electrophoresis, or from PVDF/nitrocellulose membranes, with a microliter of sample material at micromolar concentrations (equivalent to detection of sub-picomole amounts in

a single MALDI spot) sufficient for analysis in most cases – detection and identification of femtomole and sub-femtomole amounts is possible in many cases. These types of analysis can be used, for example, to **detect and analyze phosphoproteins and other post-translational modifications, for isozyme analysis, and for identification of unknown proteins**, as well as for quantitation of protein changes (see next section below).

*Quantitative Proteomics:* **Quantitative analyses** of differences in protein expression between different states can be performed at the **whole protein level** using (1) standard 2D gel analysis comparing spot-densities on two gels; (2) DIGE gel analysis using a single gel with proteins from different samples labeled with different fluorors; (3) whole protein 2D LC separations and comparing fraction densities between 2 samples using our Beckman-Coulter PF 2D system; (4) **Quantitation at the peptide level using differential heavy isotope ICAT or iTraq labeling** followed by gel and/or 2D LC separations, and finally differential relative quantitation on the MALDI TOF-TOF instrument itself using ProteinPilot 2.0 or GPS Explorer 3.5 software, or on the 4000 QTrap Hybrid Ion Trap instrument using ProteinPilot 2.0 software.

*Unknown proteins* can be further characterized by partial sequence determination of proteolytic fragments using tandem MS/MS ion fragment analysis. Additional analyses (e.g., partial sequence determination utilizing Collision-induced Dissociation (CID) fragmentation analysis with our 4000 QTrap or 4800 MALDI TOF-TOF are available in our facility

*Protein Sizing and aggregation state* can also be analyzed by the Wyatt DAWN Heleos Light Scattering Detection instrument located in John Flanagan's lab in C5747. This is a shared instrument purchased for general use, please contact Dr. Flanagan to arrange usage of this instrument ([jflanagan@psu.edu](mailto:jflanagan@psu.edu))

*A three year plan to create a full-service proteomics facility, using institutional funds made available through the Tobacco Settlement, was developed in the spring of 2002. The purchase and installation of the automated tandem MS/MS MALDI-TOF-TOF mass spectrometer (Applied Biosystems 4700 Proteomics Analyzer), in January 2003 was the first stage in this development plan. This was followed by the acquisition of a BioRad FX Pro Plus 2D gel analysis instrument, an Eksigent 2D Nanoflow LC separations system and LC Packings/Dionex ProBot automated spotter, an ABI BioCad separation system, and a Biomachines/Leap Technology 2DiD spot-picking/digestion/sample-spotting robot in 2003 and 2004. In 2005, in addition to 2D gel equipment, a Beckman Coulter PF-2D LC whole protein separation system was added. In 2006, an MDS/Sciex 4000 QTrap Hybrid Ion Trap Mass Spectrometer for additional small molecule and post-translational modification analysis capacity was added, as was a 2<sup>nd</sup> generation, more sensitive 4800 MALDI TOF TOF. Funds for this instrumentation and staffing of the facility have come from Pennsylvania Tobacco Settlement Funds, other institutional funds, and the Penn State Cancer Institute.*

Director: Dr. Bruce Stanley ([bas12@psu.edu](mailto:bas12@psu.edu))

Lab manager: Anne Stanley ([aes7@psu.edu](mailto:aes7@psu.edu))

•**MRI/MRS Facility** —The MRI/MRS Core Facilities are part of the Center for NMR Research, which occupies approximately 6,500 sq ft. in the MRI building behind the Biomedical Research Wing of the College of Medicine. The MRI Imaging Core provides both Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) services, for *in vivo* spectroscopy and imaging in animals and humans. These are particularly attractive techniques because they allow the viewing or measurement of closed internal structures or metabolism of

living animals or cells in a completely noninvasive and nondestructive manner. Magnetic Resonance offers a wide variety of fundamental measurements of anatomy and physiology. These include detailed anatomical imaging in soft tissues, quantitative measurements of blood flow or perfusion, fiber tracking of the nerve bundles in the brain, measurement of metabolism and kinetics in internal organs *in situ*, volume and staging of tumors, and functional MRI (fMRI) which can view the effects of specific stimuli on specific brain neurons or regions, allowing one a means to “see” the brain think.

**Major Equipment:**

3.0 Tesla 90 cm bore whole body MR spectrometer/imager (Bruker S-300)

7.0 Tesla 20 cm bore small animal imaging system (Bruker Biospec 70/20as).

These two systems are dedicated for research activities.

Additional 3.0 Tesla MRI Philips Intera Imagers with seven receiver channels for state-of-the-art SENSE technology are located in the main hospital complex. This system is reserved one day per week for research activities.

**MRI Methods (What MRI can do for your research)**

MRI allows for *in vivo* imaging studies for humans and animals. In addition to the conventional anatomical imaging methods, CNMRR provides the new MRI modalities for advanced research:

Functional MRI (fMRI)

Quantitative parametric mapping

Quantitative morphological measurement

Diffusion Tensor Imaging (DTI)

Magnetic Resonance Spectroscopy (MRS)

**Fees:** Rates for instrument use are:

7 Tesla Animal Imaging - \$150 per hour

3 Tesla Human Imaging - \$400 per hour.

**Director of the MRI/MRS Core Facility:** Dr. Qing Yang ([qyang@psu.edu](mailto:qyang@psu.edu))

**•Solution Phase NMR Facility (C2818A)** – the 500 MHz and 600 MHz Bruker NMRs in this facility provide high field strength NMR instruments necessary for a range of projects including macromolecular structure determination, organic chemistry and metabolomics. The 500 MHz instrument has several probes, including a broadband probe for direct detection of nuclei with frequencies ranging from Nitrogen to Phosphorous with proton decoupling, a dual fluorine and proton probe with detection and decoupling for either channel, a triple resonance cryoprobe (proton, carbon, nitrogen) with high sensitivity for proton and carbon, and a high-resolution magic angle spinning triple resonance (proton, carbon, phosphorous) probe for measurements involving solids and intact tissue samples. The 600 MHz instrument has a triple resonance cryoprobe (proton, carbon, nitrogen) with the greatest sensitivity for proton and carbon, and a 1 mm triple resonance (proton, carbon, nitrogen) microprobe for sample volumes as small as 5  $\mu$ l. Both instruments have cryofit accessories which use a robot to deliver multiple samples to the cryoprobe, allowing for a considerable degree of automation.

**Fees:** Currently (summer 2007) there are no fees for the use of this new instrumentation

**Director of the Solution Phase NMR Core Facility:** Dr. Ira Ropson ([iropson@psu.edu](mailto:iropson@psu.edu))

**•*Bioluminescent Imaging Facility*** — The Bioluminescent Imaging Core Facility provides a non-invasive way of tracking localized changes in the activities of particular gene promoters in cells or in whole animals, for example during normal development, after drug exposure or toxic insult, or during tumor growth. A transgene expressing luciferase under the control of a gene promoter of interest is incorporated into a transgenic animal or into cells in culture. After luciferin ingestion, areas where the transgene is being expressed can be clearly measured and differences in tissue or regional expression can be quantitated. Because the measurements do not involve sacrificing the animal, serial measurements or time courses can be obtained, and the response to multiple different treatments can be measured while minimizing inter-animal variability.

**Services offered:**

- Non-invasive whole animal imaging of gene promoter activity patterns in transgenic animals (developmental patterns, drug or treatment effect time courses, tumor staging, etc.)
- Imaging of bioluminescence from cells in culture

**Major Equipment:**

- Xenogen IVIS 50 Imaging System in the Animal Research Barrier Facility
- Remote analysis workstation for image processing

**Co-directors of the Bioluminescent Imaging Facility:** Dr. Jiyue Zhu ([joz1@psu.edu](mailto:joz1@psu.edu)) & Dr. Bruce A. Stanley ([bas12@psu.edu](mailto:bas12@psu.edu))