New Graduate Course Proposal by Katya Ravid
Evans Center for Interdisciplinary Biomedical Research
Course Outlined and directed by Sarah Haigh and Katya Ravid
(4.8.10)

Biological Core Technologies (MM730)

Location: Boston University School of Medicine
Course Directors: Drs. Sarah Haigh Molina and Katya Ravid
Course Instructors: Course and Core Directors

Major course goals, structure and learning objectives:
The major goal of the course "Biological Core Technologies" is to provide an overview of the principles and applications of modern techniques, which are regularly employed in academia and industry as tools for biomolecular and biomedical investigation. This course will focus on technologies, which are available at BUSM. One of the missions of the course is to also present clinical researchers and clinicians with the opportunity to learn more about basic core technologies and common biological tools of research.

General Course structure: This is a 15-session course (one per week), comprised of 10 didactic lectures and 5 observational practicals. Each session will be 2 hours in duration, and will be taught by faculty who are experienced with BU Institutional Core facilities. During the first part of the course, instruction will focus on image-based and *in vitro* applications [sessions 1-5, including Principles of Light, Fluorescence and Detection, Optical Sectioning-Confocal Microscopy, and Flow Cytometry]. The second part of the course will focus on Analytical techniques [sessions 6-10, including real time PCR, Immunohistochemistry, microarry, proteomics and high throughput screening]. The third part of the course will start with a description of mouse genetic manipulations, and will continue with *in vivo* analyses of such mice [sessions 11-15, including mouse imaging, ultrasound and metabolic phenotyping].

Structure of each lecture: Each instructed session will consist of 4 components:
1. General outline of the theories, which guide the technology;
2. Scope of the core technology – advantages and limitations;
3. Example(s) of application to a biomedical problem (i.e., case studies);
4. Data processing and analysis pertinent to this technology.

Structure of each practical observation: Practical observations will take place in the relevant core facility. Students will have the opportunity to see the technologies in action, but may not have personal hands-on time (specifics are outlined in each observation). During observation, the students will be presented with a follow up of an analysis and an overview of how to use the core. Of note, for lectures that do not include observation, typically owing to limitations based on institutional regulations (e.g., IACUC approval when mice are involved), may include a video demonstration (with institutional approval, as needed).

Target students**:** Graduate students from both the Charles River and Medical campuses; senior undergraduates with permission of the instructor; clinical researchers. This course will count towards elective courses in all departments at the medical school.
Course Location*:* This course will be held on the BU Medical Campus.

Course prerequisites: One of the following courses or a college course equivalent with consent of the course director:

Cell Biology (GMS 753)
General Biochemistry (BI755)
Molecular Biology (BI782)

Course evaluation*:* A mid-term quiz (take-home, open-book) and a Final Project guided by the Course Director

General Web Resources:
http://www.microscopyu.com
http://www.olympusmicro.com/primer/
http://www.invitrogen.com/site/us/en/home/brands/Molecular-Probes.html
http://www.chroma.com/resources/filter-handbook

Suggested Course book:
None

Sessions (each lecture is 2 hours in duration):

Sessions 1-5: Image-based and *In Vitro* techniques

1. Lecture: Principles of Light, Fluorescence and Detection
Understanding the properties of light is fundamental to understanding the principles and theories which underlie many core technologies including microscopy, flow cytometry, immunohistochemistry and fluorescence molecular tomography.

We will begin the course with a review of the properties of light. Light exhibits both wave-like and particle-like properties (wave-particle duality), thus we will discuss light as both a wave (form, length and spectra) and as a particle (photon).

We will focus on the properties of photons as we cover the principles of luminescence, specifically fluorescence. This class will review the physics of electronic states and transitions, absorption and emission efficiency and Stokes Shift.

We will review different classes of fluorescent molecules and proteins: fluorescent dyes; probes for living cells; fluorescent proteins; nanoparticles.

What is a micrscope: history and application. How have fluorescence technologies revolutionized microscopy? Applications of fluorescence micrscopy: tracking; co-

localization; sensors (e.g. Ca^{2+}, pH). We will discuss the advantages (sensitivity, non-invasive, real-time, multiplexing, control) and disadvantages (phototoxicity, photobleaching, autofluoresence, artifacts) of utilizing fluorescence for biomolecular techniques.

We will learn how to select appropratiate dyes based on excitation/ emmission; mechanism of action; subcellular localization and suitablility for live cell imaging.

We will compare different detectors including the Photo multiplier tube (PMT), the charge-couple device (CCD), and the electron multiplying charge-couple device (EM CCD). The advantages and disadvantages of each detector will be discussed, from which we will generate a list of suitable applications for each.

Reading Assignment (1-2 sources, to be finalized by the instructor):
• "Fluorescent probes for living cells" Iain Johnson Histochemical journal 30, 123-140 (1998)
• Fluorescent probes for super-resolution imaging in living cells. Fernández-Suárez M, Ting AY. Nat Rev Mol Cell Biol. 2008 Dec;9(12):929-43
• Intracellular ion measurement with fluorescent indicators. MARK B. CANNELL and MARTIN V. THOMAS.
http://www.utdallas.edu/~tres/microelectrode/microelectrodes_ch12.pdf
• Intracellular Ion Indicators. R.P. Haugland and I. D. Johnson. Fluorescent and luminescent probes (book).
• The Carl Zeiss Lecture: The Cell and the Microscope, Peter Evennett

Non Mandatory Web resources:
• http://probes.invitrogen.com/resources/education/tutorials/2Spectra/player.html
• http://probes.invitrogen.com/resources/education/tutorials/3Light_Sources_Filters/player.html
• http://probes.invitrogen.com/resources/education/tutorials/1Intro/player.html
• http://probes.invitrogen.com/resources/education/tutorials/2Spectra/player.html

Instructor: Dr. Anthony Molina

2. Lecture: Optical Sectioning; Practical observation: Confocal Microscopy

During the first hour, we will compare optical sectioning, mathemical sectioning, and physical sectioning. We will discuss the advantages and limitations of each method.

What is a microscope: design and function. We will review the advantages and disadvantages of upright vs inverted miscorscopes. We will review widefield microscopy in preparation for understanding the advantages and limitations of confocal microscopy.

We will learn the principles of confocal microscopy and the advantages and limitations of different confocal modes (point scanning, line scanning, nipkow disc, 2-photon). The biological application of each mode will be illustrated by an example.

In preparation for the practical observation, we will learn how to understand the information on the objective (Description, magnification, N.A., tube length), and how to choose the optimal objective for our experiments. We will learn how to choose optical filter sets, and we will briefly review source illumination (xenon/ mercury lamps, LEDs, monochromators and lasers).

In the second hour, the class will be divided into two groups, both of which will observe the confocal microscope. One group will work with Dr. Vickery Trankaus-Randall, the other with Dr. Mike Kirber in the Cellular Imaging facility. This observational class is intended to familiarize the students with the equipment, and how the equipment can be used for cellular imaging.

Instructors: Dr. Mike Kirber and Dr. Vickery Trankaus-Randall
Reading Assignment:
http://micro.magnet.fsu.edu/primer/techniques/fluorescence/fluorhome.html

Potential follow on course on Confocal Microscopy: GMS CM 766

3. Practical Observation: Methods in Image Processing and Analysis
During this session we will learn how to extract data from images and how to prepare images for publication. The fundamentals of computational image processing and analysis will be explored including deconvolution, resolution, the Nyquist/ Shannon theorem as it relates to pixel size, and the two most important parameters for photodetection which are Quantum Efficiency and noise.
Students will have the opportunity to develop practical experience of digital filtering (low pass averaging, median, Gaussian, edge enhancement), deconvolution and co-localization through projects using the freeware program Image J.

Instructors: Dr. Mike Kirber
Reading Assignment:
http://micro.magnet.fsu.edu/primer/techniques/fluorescence/fluorhome.html

4. Lecture: Flow cytometry and FACS
This section of the course will treat basic principles of how cells in a laminar flow medium interact with laser light, including measurement of forward and side scatter properties and fluorescence. The specific properties of emitted fluorescent light offer a powerful source of information about the state of cellular DNA synthesis and cell cycle, surface markers, signal transduction and apoptosis. A distinct advantage of flow cytometry techniques over immunoblot is the assignment of biological function to individual cells, rather than to a population average that drastically reduces information; an advantage over fluorescence microscopy is the ability to measure a large number of events in a few minutes; modern high-speed instruments are able to process 15+

fluorescent parameters at 20,000 events per second. The lecture will cover sorting strategies and fluorescence compensation in the FACS analyzer; multiparameter data analysis and computational methods of gating and handling large numbers of events/rare events; and advanced techniques such as staining of intracellular antigens for signaling and sorting of different types of stem cells.

Instructor: Dr. Gerald Dennis
Reading Assignment:
• "Isolation and Functional Properties of Murine Hematopoietic Stem Cells that are Replicating In Vivo" Margaret A. Goodell, Katja Brose, Glenn Paradis, A. Stewart Conner, and Richard C. Mulligan

Web resources (1-2 sources as finalized by the instructor):
• http://www.bu.edu/cores/flow-cytometry/LosAlamos2009.html
• http://probes.invitrogen.com/resources/education/tutorials/4Intro_Flow/player.htm
l
• http://probes.invitrogen.com/resources/education/tutorials/5Data_Analysis/player.
html

5. Practical observation: Flow cytometry and FACS

Sessions 6-10: Analytical Techniques

Lecture 6A: Immunohistochemistry
Instructor: Dr. Xuemei Zhong

A. During the first hour, we will review the principles which underpin Immunohistochemistry (IHC). IHC utilizes specific antibody and antigen binding to visualize protein expression and cellular structures in tissue. The detection is achieved by directly or indirectly labeling antibody with a reporter enzyme such as alkaline phosphatase (AP) or horseradish peroxidase (HRP). Colors will be developed upon brief incubation with different chemical substrates (chromogens). Immunofluorescence (IF) utilizes the same principle as IHC except that the antibodies are labeled directly or indirectly with fluorescent dyes and florescence microscope or confocal microscope is required.

We review the scope of the equipment which is available in the IHC core facility, and how this technology can be used to probe biological systems. Such applications include:
✓ Understand the distribution and localization of biomarkers
✓ Determine differential expression of proteins in different parts of a tissue
✓ Diagnosis of abnormal cells (i.e. cancer cells vs. normal cells in a tissue)
✓ Analysis of antibody deposition on inflamed tissue
✓ Study cell proliferation, activation, apoptosis in a tissue using specific markers
✓ Visualize different cell types in blood smear
✓ Study cultured cells using cytospin or cover slips
If time allows, data analysis modes will be only briefly explored including Microscope

and image processing software, which is covered in detail in lectures 1-3.

Reading Assignment (one of the sources below as finalized by the instructor):
• “Histotechnology: A Self-Instructional Text” by Frieda L Carson and Christa Hladik
• “Triple immunofluorescence labeling of atherosclerotic plaque components in apoE/LDLR -/- mice” Mariusz Gajda, Jacek Jawieñ, £ukasz Mateuszuk, Grzegorz J. Lis, Andrzej Radziszewski, Stefan Chopicki, Jan A. Litwin
• "Immunohistochemistry: Methods Express Series" By Simon Renshaw
Published January 19, 2007

Optional (non mandatory) Web resources:
B. Biocare Medical intelliPATH FLX Automated Slide Stainer (for IHC and IF staining)
http://slidestainer.com/features
C. Decloaking Chamber (for antigen retrieval)
http://www.biocare.net/decloaking-chamber.html

Lecture 6B: quantitative PCR (qPCR/ RT-PCR)
Instructor: Dr. Lynn Lingyi Deng,

B. During the second hour, we will review the principles and applications of quantitative PCR (qPCR).
We will briefly review the general concept and the history of Polymerase Chain Reaction (PCR). We will also point out the disadvantage of general PCR and then start to introduce the purpose and the principles of qPCR or Real-Time PCR. We will emphasize the importance of studying gene expression and it’s quantification, followed by an introduction to the instrument, ABI StepOnePlus. Two major methods will be introduced in this class, including using fluorescent reporter probes and using double-stranded DNA dyes. Methods of data analysis will be briefly introduced, and finally, the applications of Real-Time PCR in medicine and biological research will be discussed.

Students should understand:
• The principle of PCR and Real-Time PCR
• The purpose of the experiment
• The concept of gene expression
• The “players” in the reaction
• The interpretation of the quantification results of gene expression
• Precautions for such an experiment

Web resource (1-2 sources as finalized by the instructor):
Real-Time PCR System by Applied Biosystems
https://products.appliedbiosystems.com/ab/en/US/adirect/ab?cmd=catNavigate2&catID=604109
Primer Express Software

https://products.appliedbiosystems.com:443/ab/en/US/adirect/ab?cmd=catNavigate2&catID=605537

Reading Assignment (1-2 sources as finalized by the instructor):
• VanGuilder HD, Vrana KE, Freeman WM (2008). "Twenty-five years of quantitative PCR for gene expression analysis". *Biotechniques* **44**: 619–626. doi:10.2144/000112776. PMID 18474036.
• Udvardi MK, Czechowski T, Scheible WR (2008). "Eleven Golden Rules of Quantitative RT-PCR". *Plant Cell* **20**: 1736–1737. doi:10.1105/tpc.108.061143. PMID 18664613.
• Nolan T, Hands RE, Bustin SA (2006). "Quantification of mRNA using real-time RT-PCR.". *Nat. Protoc.* **1**: 1559–1582. doi:10.1038/nprot.2006.236. PMID 17406449.

7A. **Practical observation: Immunohistochemistry; qPCR**
During this session, the class will be divided into two groups. For the first hour, one group will observe the Immunohistochemistry Core while the other will observe qPCR facility. The groups will swap for the second hour.

40 min video presentation in the facility of the following steps as well as 15 min introduction to the actual equipment.
1. Deparaffinization
2. Antigen retrieval
3. Computer programming
4. Bar-coding slides and reagents
5. Sample and reagent preparation and loading
6. Counter staining
7. Mounting
8. Observation under microscope

7B. Practical observation :qPCR
40 min video presentation for Real-Time PCR System, 5 min introduction of primer design and sample preparation, and final 10 min for Q and A.

1. Installation,
2. Basic principles of quantification,
3. Practical demonstration.
4. Primer design
5. Sample preparation
6. Ordering sources for reagent

8. Lecture: Genomics technologies: from microarrays to next generation sequencing
During this session we will first discuss different aspects of microarray technology: what are the microarrays in principle, what types of the microarrays are used and how are they fabricated, what kind of studies can benefit from using the microarrays and how to design a microarray study. We will discuss how to collect and process the samples for a

microarray study and how to analyze the microarray data. The services available through the BU Microarray Resource Core will be explained in detail. We will discuss successful examples of using microarrays in biology and medicine and currently available microarray-based diagnostics.

The second part of the session will be devoted to the next generation sequencing technology. First we will discuss the currently commercially available technologies, which were developed to replace the traditional Sanger sequencing. These technologies will be compared from the standpoint of the basic principles, throughput, types and quality of the sequencing data, costs, labor-intensity, etc. We will also look at the potential advantages of the newly emerging "next next generation" sequencing technologies that are being developed or becoming available on the market.
Finally, based on the successful published studies we will discuss the major applications of these new genomics technologies and how these technologies are revolutionizing science and medicine and discuss different aspects of the progress towards the personalized medicine.

Instructor: Dr. Yuriy Alekseyev
Reading Assignment:
• "Sequencing technologies - the next generation". Metzker ML. Nat Rev Genet. 2009 Dec 8.

9. Lecture: Proteomics
Mass spectrometry has become a cornerstone in the field of proteomics and protein chemistry research. This achievement has been made possible by revolutionary technical advances in MS, separation science and computational data analyses over the last decade. Thus a new age of research has been ushered in which allows for the direct study of the molecular and cellular biology of an organism's complete complement of proteins using this potent technology. With MS based proteomics it is readily possible to rapidly and directly document differential protein expression, protein sequence variants and positions and abundances of post-translational modifications (PTMs) and correlate these results with genome data and the clinical manifestations of an expressed phenotype.

In this lecture a very brief and non-technical overview on mass spectrometry based proteomics will be presented. Emphasis will be placed on more practical subject areas in order to facilitate the design and interpretation of a proteomics experiment one may initiate through collaboration with our Center for Biomedical Mass Spectrometry or another proteomics center. Topics of discussion will include a brief overview of instrumentation, interpretation of mass spectra, tandem mass spectra (MS/MS), protein identification via a database search using both peptide mass fingerprint (PMF) and MS/MS data, assignment of PTMs, use of isotope tags, sample purification, fractionation and separation strategies, design of a differential proteomics experiment and meta-analyses of protein data. Examples from ongoing clinically relevant biological projects will be provided. An assignment consisting of analyzing database search results from several projects will further assist course participants with the understanding of the material. Course notes will be provided in the form of lecture slides and supplemental A-

page review articles.

Instructor: Dr. Mark McComb (Proteomic Center)
Reading Assignment (an article derived from the following source):
• "Mass spectrometry-based proteomics" Ruedi Aebersold & Matthias Mann
• "The biological impact of mass-spectrometry-based proteomics" Benjamin F. Cravatt, Gabriel M. Simon & John R. Yates III

10. Lecture: High Throughput Screening (HTS)
This session is designed to provide students with an overview of the High Throughput Screening process. We will discuss in detail how HTS can be used for both pathway- and target-validation, and for early stage drug discovery. We will focus our discussions on small molecule HTS, and will talk about different classes of small molecule libraries. We will discuss the advantages and limitations of two prevailing screening methods: target-based and phenotype-based. We will focus on the microplate reader and the HCA reader as major instrument technologies that underpin, enable and ultimately catalyze successful HTS campaigns. We will use examples of current screens to illustrate the differences between target-based and phenotype-based screens. We will talk about the volume of data produced in an HTS screen, and how this data can be stored, mined, and analyzed.

Instructor: Dr. Sarah Haigh Molina
Reading Assignment:
• Drug Discovery: A historical perspective. Jurgen Drews
• The potential of high-content high-throughput microscopy in drug discovery. V Starkuviene and R Pepperkok Br J Pharmacol. 2007 September; 152(1): 62–71.

Sessions 11-15: In Vivo-based technologies

11. Lecture: In vivo systems and the *Xenograph*
A. First hour- In this session we will review the general principles guiding the **generation of transgenic and knockout models**. We will then describe how different fluorescent model systems are generated and used, including the generation of chimeric proteins with a TAG, or Luciferin used for tracing of cellular processes in vivo**.** Application of such models in various research inquiries will be discussed. Analysis of expression of the tracing marker will be discussed in the context of lectures on immunohistochemistry and fluorescent microscopy.
B. Second hour- **Fluorescence molecular tomography applied in vivo: The *Xenograph*.**
During this hour, we will talk about the principles and applications of the Xenogen IVIS *in Vivo* imaging system. The IVIS Imaging System is a highly sensitive and large field of view imaging system that offers users the flexibility to image fluorescent and/orbioluminescent reporters both in vivo and in vitro. It relies on patented optical imaging technologies to facilitate non-invasive longitudinal monitoring of disease progression, cell trafficking and gene expression patterns in living animals. We will learn the basic principles of the technology including: the basis of the technology, required

labelling modalities including advantages and disadvantages for different systems and assays. Apllications include
• Tracking cells populations in marrow reconstitution experiments
• Tracking stem cells
• Tracking gene activity
• Tracking enzyme or biological activity with fluorescent or bioluminescent substrates
• Tracking bacteria infections
• Tracking cancer cell metastasis and cancer cell growth
We willc onclude the hour with a discussion of image processing and analysis using the Living Image 3.1 software. Using this proprietary software, we are able to automatically draw ROIs and compute photon flux, create a composite image to evaluate dual reporters in a single experiment, measure distances (cm or pixels) and view pixel data in histogram or line graph formats, quantify the depth, point location, and brightness of a biophotonic source using Planar Spectral Imaging, quantify the depth, geometry, and brightness of a biophotonic source in 3-dimensional space using DLIT or FLIT tomography, co-register organs from the Digital Mouse Atlas on a 3D image, import and co-register CT or MRI radiographs on a 3D image and export 2D image to DICOM-compliant format

Instructors: Drs. Katya Ravid and Louis Gerstenfeld

Reading Assignment (one of the sources below as finalized by the instructor):
• Mwakingwe A, Ting LM, Hochman S, Chen J, Sinnis P, Kim K. Noninvasive real-time monitoring of liverstage development of bioluminescent Plasmodium parasites. J Infect Dis. 2009 Nov 1;200(9):1470-8
• Zabala M, Alzuguren P, Benavides C, Crettaz J, Gonzalez-Aseguinolaza G, Ortiz de Solorzano C, Gonzalez-Aparicio M, Kramer MG, Prieto J, Hernandez-Alcoceba REvaluation of bioluminescent imaging for noninvasive monitoring of colorectal cancer progression in the liver and its response to immunogene therapy . Mol Cancer. 2009 Jan 7;8:
• Klerk CP, Overmeer RM, Niers TM, Versteeg HH, Richel DJ, Buckle T, Van Noorden CJ, van Tellingen O. Validity of bioluminescence measurements for noninvasive in vivo imaging of tumor load in small animals. Biotechniques. 2007 Jul;43(1 Suppl):7-13, 30.
• Sato A, Klaunberg B, Tolwani R. In vivo bioluminescence imaging. Comp Med. 2004 Dec;54(6):631-4
• Sadikot RT, Blackwell TS. Bioluminescence: imaging modality for in vitro and in vivo gene expression Methods Mol Biol. 2008;477:383-94 (Excellent set of links in refercne list to many key papers to show uses of technique)

Follow on course: GMS BI 776 Gene Targeting in Transgenic Mice

12.Lecture: Ultrasound-based *in vivo* micro-imaging, molecular imaging and DNA-delivery

A. First hour: We will cover the principles that guide *in vivo* ultrasound-based micro-

imaging, specialized Doppler analysis, molecular imaging, and sonoporation-based DNA/drug transfer. Secondly, we will cover the scope of applications for these ultrasound-based emerging technologies for *in vivo* analysis of animal models of disease. More specifically, rather than standard ultrasonography, we will focus on high-resolution ultrasound imaging of anatomy, function and molecular changes in *in vivo* rodent models of human diseases.

a. Micro-imaging to 30 to 50 micron-resolution
b. Specialized Doppler-based analysis: power Doppler, tissue Doppler methods
c. Contrast-enhanced non-specific microvascular imaging (2-3 micron-resolution), and target-specific imaging of vascular molecular changes
d. In vivo sonoporation for regional and molecular target-specific DNA/drug delivery

B. Second hour: We will gain a perspective on applications of ultrasound micro-imaging, contrast-enhanced imaging, molecular imaging and sonoporation to biomedical problems in different organ systems as it applies to different disease paradigms (cardiovascular pathology, tumor angiogenesis, and inflammation). We will also gain a perspective on experimental design and analysis guidelines to maximally utilize the strengths of ultrasound-based technologies, while concurrently attaining an understanding of the limitations of said technologies.

Instructor: Dr. Victoria Herrera
Reading Assignment (one of the sources below as finalized by the instructor):
:
• "Molecular imaging of cardiovascular disease with contrast-enhanced ultrasonography". Jonathan R. Lindner
• "Microbubbles in ultrasound-triggered drug and gene delivery". Sophie Hernot, Alexander L. Klibanov

13. Practical observation and/or "how-to" discussion: Fluorescence molecular tomography and Ultrasound micro-imaging of anatomy, function and molecular biology

During this session, the class will be divided into two groups. For the first hour, one group will observe Fluorescence molecular tomography while the other will observe High-resolution Ultrasound technologies. The groups will swap for the second hour.

A. Ultrasound-based microimaging of anatomy, function and molecular biology: Dr. Victoria Herrera
We will view a video-based demonstration of research applications of ultrasound micro-imaging capabilities, contrast-enhanced non-specific and targeted molecular imaging, and sonoporation-based DNA transfer in *in vivo* research models.
We will also discuss experimental design questions specific to each student's research interest – to explore potential grant applications and research applications.

B. Fluorescence molecular tomography: Dr. Anna Studwell

During this session, students will learn how to use the IVIS technology to track bioluminescent of fluorescently tagged cells to monitor growth and metastasis. Next, we will demonstrate how to use the IVIS to monitor all forms of cell transplant experiments to quantify engraftment, cell growth or cell differentiation (i.e. for stem cell transplants, reconstitution of hematological and immune systems after irradiation). We will also show how in vivo gene activity can be monitored in transgenic animals carrying appropriately tagged promoter indicator trangenes. We will monitor in vivo gene activities in cells that have been reconstituted to carry a bioluminescent or fluorescently tagged expressed gene. We will also describe how to monitor any form of biochemical activity that can be assayed using a bioluminescent or fluorescently tagged substrate. Finally, we will discuss how to quantify depth, geometry and intensity for both bioluminescent and fluorescent sources in three dimensional spatial resolutions.

Instructors: Drs. Victoria Herrera and Anna Studwell

14.Lecture: Metabolic phenotyping
Instructor: Dr. Ravi Jasuja
A. We will cover the fundamental principles of techniques (NMR and CT scanning) that allow for the non-invasive, longitudinal monitoring of body composition. Relative benefits, precision and accuracy of the techniques will be discussed.

B. Complementary tools that quantify spontaneous activity, feeding and resting energy expenditure will be discussed. Exemplary data sets from research problems will be discussed.
C. In the last part of the lecture, the theory and practice of isotopic tracers (stable and radioactive) for the study of metabolism in man and animals using mass spectrometry and NMR. This section will summarize isotopomer analysis for metabolic flux rates and metabolic regulation.

Reading Assignment (one of the sources below as finalized by the instructor):
• Yoshinori Arai, Ayuta Yamada, Tadashi Ninomiya, Takafumi Kato, Yuji Masuda (June 2005) Micro-computed tomography newly developed for in vivo small animal imaging. /Oral Radiology/, Vol. 21, No. 1:14-18.
• Quantitative magnetic resonance (QMR) method for bone and whole-body-composition analysis. Taicher GZ, Tinsley FC, Reiderman A, Heiman ML. Anal Bioanal Chem. 2003 Nov;377(6):990-1002. Epub 2003 Sep 16.
• Myostatin modulates adipogenesis to generate adipocytes with favorable metabolic effects Brian J. Feldman, Ryan S. Streeper, Robert V. Farese, Jr., and Keith R. Yamamoto, PNAS, October 17, 2006, vol. 103, no. 42, 15675-15680

15. Practical observation: NMR, CT scanning and Comprehensive Laboratory Animal Monitoring System (CLAMs).
Instructor: Dr. Ravi Jasuja
During this session, the class will be divided into two groups. One section will observe the CT scanning demo and NMR measurements for body composition while

the other group will observe the setting up of the CLAMs instrumentation and data acquisition.

How to test:
- **Attendance and participation** in all classes and in observation classes will grant 10% of the total grade. Students are expected to attend every classand to have read the assigned material before class, and to make thoughtful contributions to class discussions.
-**Mid Term** Requirement (due after Lecture 10):
Take home, open book test to reinforce the principles learned during the lectures. This will constitute 30% of the grade.

-Final project: based on students proposing a research plan to investigate an application of at least two of the Biological Core Technologies in their own scientific research. The projects will have to be approved by the course directors. This will constitute 60% of the grade. University polices on plaugarism are supported in this class.

Example: Utilization of metabolic caging, NMR and CT to measure the body composition, distribution and metabolic activity of a transgenic mouse.

The final project should be 4-6 single-spaced pages (12 pt type) in length, not including references. Like a grant application, the goal of the proposal is to establish a testable hypothesis. Writing should be clear, concise, and to the point. The main components of the writing should be:

1. Hypothesis and Specific Aims. State the overall goal of the research proposal and the hypothesis to be tested. Present the individual specific aims (usually 1-2 aims) with a very brief description of approaches tobe used to address the aims. (Typically 2/3 of a page)

2. Background and Significance. Review the essential literature in order to provide the background to and the rationale for the proposed experiments. Identify sources of information including reviews and Medline. Summarize primary literature for a knowledgeable but not necessarily expert reader. State the importance of the research to broad, long-term scientific questions. (Typically 1.5 pages)

3. Experimental Design and Methods. Provide an outline of the design of the experiments to be used to address each of your specific aims. Treat each aim individually and describe the approaches to be used and the controls. Expected results should be delineated, potential experimental difficulties should be discussed, and alternative strategies to achieve the aims should be outlined. Figures that illustrate important Background information or provide models that will be tested may be included (and welcomed) in the body of the text or at the end. (2 - 3 pages)

4. References. Provide references at the end of the Research Proposal. Each citation must

be complete, and include the names of all authors, title of the article, journal or book, volume, page numbers, and year of publication. The reference list is not included in the page limit of the proposal.

GRADING: grades will be on a scale of 100/100. The final projects will be scored by course instructors (depending on the focus of the project), guided by specific instructions on how to evaluate the above project components (e.g., 25/35/40). Projects will be re-read by the course directors to ensure uniformity in evaluation.

List of Instructor CV's

- Dr. Anthony Molina
- Dr. Michael Kirber
- Dr. Vickery Trinkaus-Randall
- Dr. Gerald Dennis
- Dr. Lynn Deng
- Dr. Xuemei Zhong
- Dr. Yuriy Alekseyev
- Dr. Mark McComb
- Dr. Sarah Haigh Molina
- Dr. Katya Ravid
- Dr. Louis Gerstenfeld
- Dr. Victoria Herrera
- Dr. Anna Studwell
- Dr. Ravi Jasuja