

What Analytical Ultracentrifugation Reveals about Macromolecular Interactions. **Borries Demeler**, Associate Professor of Biochemistry.

Analytical Ultracentrifugation (AUC) is a powerful and essential characterization method to study macromolecular mixtures in solution. AUC is suitable to study molecules such as DNA, proteins, nanoparticles, carbohydrates and synthetic polymers. Studies in the solution environment are of particular interest, since they can most accurately describe the behavior of molecules in a physiological environment. Such an environment most closely resembles the natural conditions occurring in cells, and therefore permit the study of dynamic processes which are inaccessible in other high-resolution techniques such as X-ray crystallography or transmission electron microscopy. In AUC, the molecules are able to freely interact with other molecules. These dynamic interactions can then be quantified. AUC can be used to study mixtures of molecules covering a very large size range ( $10^2$ - $10^8$  Dalton), and under a wide variety of solution conditions where pH, ionic strength, oxidation state, temperature and concentration of solutes, ligands and cofactors can be easily modulated. In this talk I will provide an overview of the technique and its applications.

Assuring Accurate qPCR Data: the MIQE Guidelines for qPCR. **Greg Shipley**, MIQE Co-Author and former Director of the Quantitative Genomics Core, UTHSC-Houston

The MIQE guidelines paper (Bustin, *et al.*, *Clinical Chemistry*, 55(4):611-622, 2009) was published in response to the large amount of inaccurate data being published from qPCR experiments. The MIQE guidelines provide a checklist for investigators to follow to ensure their experimental data are reliable. At first glance, the checklist can seem daunting, especially to those new to the technique. The purpose of my seminar is to provide an introduction to the MIQE guidelines and to go through each section of the checklist, provide clarification for many items and for others to show how some of the requirements can be easily accomplished with the least amount of time and effort. Generating data from a qPCR experiment is trivial. Generating data that accurately reflects the biology in question requires a concerted effort on the part of the investigator.

Levitating Cells for Developing In vivo-Like 3D Cell Culturing Tools and Assays. **Glauco R. Souza, Ph.D**, Director of Genomic Research, Nano 3D

*In vitro* cell culturing is an essential process in emerging areas of biotechnology, such as drug discovery, toxicity testing, cancer research, and regenerative medicine. Traditional cell culturing is carried out in Petri dishes or media-filled flasks where cells usually attach onto a flat glass or plastic surface in a two-dimensional (2D) cell monolayer. Cells grown in monolayers provide a poor representation of *in vivo* conditions and are widely acknowledged to be insufficient for demanding *in vitro* drug discovery needs. Many schemes for three-dimensional (3D) culturing are being developed or marketed to

address these challenges, such as bio-reactors or protein-based gel environments, but they typically suffer from high cost, low-throughput, poor scalability, complexity, or the presence of non-human biological factors that can alter cellular response and preclude therapeutic use. Here, we will present tools and assays based on 3D cell culturing by magnetic levitation using the Bio-Assembler where cells can be initially cultured in 3D at the air-liquid interface<sup>1</sup>. This technique is based on the magnetization of cells using poly-L-lysine based nanoparticle assemblies (Nanoshuttle) and levitation of cells by spatially varying magnetic fields. Our results culturing primary human pulmonary cells (fibroblast, smooth muscle, endothelial, and epithelial cells) by magnetic levitation show the rapid and reproducible formation of levitated 3D cell cultures at the air-liquid interface. Furthermore, the efficiency and spatial control when generating 3D cultures enables the application of this method towards *in vitro* and label-free cell based assays. In this presentation, we will also introduce n3D's BiO-Assay, where ring- or O-like 3D multicellular structures are generated by culturing/levitating human fibroblasts cells, primary human smooth muscle, adenocarcinoma cell line (A549), human embryonic kidney cells (HEK293) and mixture of these cell types in co-culture format. Our results show that rate of O closure can be used to quantitatively evaluate a dose-dependent response to doxorubicin (chemotherapy drug) and ibuprofen (nephrotoxic agent). These data estimate the point of zero growth for the various systems, which is in agreement with IC50 values reported in the literature<sup>2,3</sup>. We believe these assays have broad application in lung research, cancer research, cell-based drug discovery, toxicity testing, and tissue engineering.