

Lecture 16: Assessing drug efficacy at the single cell level

- I. Introduction and overview
 - A. nanomedical treatment at the single cell level requires evaluation at the single cell level
 - B. for evaluation purposes, does structure reveal function?
 - C. the difficulty of anything but simple functional assays
 - D. the need for assays which at least show correlation to functional activity
- II. Quantitative single cell measurements of one or more proteins per cell by flow and image/confocal cytometry
 - A. cell surface measures of protein expression on live, single cells
 - B. high-throughput flow cytometric screening of bioactive compounds
 - C. challenges of measuring protein expression inside fixed, single cells
 - D. when location is important 2D or 3D imaging is required to get spatial location of proteins inside cells
- III. Quantitative multiparameter phospho-specific flow cytometry
 - A. attempts to measure "functional proteins" by detecting phosphorylation
 - B. example of phospho-specific, multiparameter flow cytometry
 - C. example of measuring single cell gene silencing by phospho-specific flow cytometry
- IV. Quantitative measures of gene expression – the promises and the realities
 - A. is gene expression at the single cell level really possible?
 - B. is it even useful to measure a single gene's changes?
 - C. gene arrays of purified cell subpopulations
 - D. RNA amplification techniques to attempt to perform single cell gene arrays

References

Steven M. Chan, Janelle A. Olson, and Paul J. Utz. Single-Cell Analysis of siRNA-Mediated Gene Silencing Using Multiparameter Flow Cytometry. *Cytometry Part A* 69A:59–65 (2005).

Peter O. Krutzik, Jonathan M. Irish, Garry P. Nolan and Omar D. Perez. Analysis of protein phosphorylation and cellular signaling events by flow cytometry: techniques and clinical applications. *Clinical Immunology* 110: 206–221 (2004).

Susan M. Young, Mark S. Curry, John T. Ransom, Juan A. Ballesteros, Eric R. Prossnitz, Larry A. Sklar and Bruce S. Edwards. High-Throughput Microfluidic Mixing and Multiparametric Cell Sorting for Bioactive Compound Screening. *J Biomol Screening*; 9; 103 – 111 (2004).

Szanişzlo, P., Wang, N., Sinha, M., Reece, L.M., Van Hook, J.W., Luxon, B.A., Leary, J.F. "Getting the Right Cells to the Array: Gene Expression Microarray Analysis of Cell Mixtures and Sorted Cells" *Cytometry* 59A: 191-202 (2004).

Szanişzlo, P. Gene Expression Microarray Analysis of Small, Purified Cell Subsets. University of Texas Medical Branch, Galveston, TX April, 2007 (mentor: Dr. Leary)