

Lecture 12: Assessing drug efficacy and nanotoxicity at the single cell level

- 12.1 Introduction to measures of efficacy for nanomedicine
 - 12.1.1 for evaluation purposes, does structure/size reveal function?
 - 12.1.2 nanomedical treatment at the single cell level requires evaluation at the single cell level
 - 12.1.3 the difficulty of anything but simple functional assays (e.g. phosphorylated “functional” proteins)
 - 12.1.4 the need for assays which at least show correlation to functional activity
- 12.2 Quantitative single cell measurements of one or more proteins per cell by flow and image/confocal cytometry
 - 12.2.1 cell surface measures of protein expression on live, single cells
 - 12.2.2 high-throughput flow cytometric screening of bioactive compounds
 - 12.2.3 challenges of measuring protein expression inside fixed, single cells
 - 12.2.4 when location is important 2D or 3D imaging is required to get spatial location of proteins inside cells (“locational proteomics” at the single-cell level)
- 12.3 Quantitative multiparameter phospho-specific flow/image cytometry as a single-cell, structural-functional measurement
 - 12.3.1 attempts to measure “functional proteins” by detecting phosphorylation
 - 12.3.2 example of phospho-specific, multiparameter flow cytometry
 - 12.3.3 example of measuring single cell gene silencing by phospho-specific flow cytometry
- 12.4 Quantitative measures of gene expression – the promises and the realities
 - 12.4.1 is gene expression at the single cell level really possible?
 - 12.4.2 is it even useful to measure a single gene's changes?
 - 12.4.3 gene arrays of purified cell subpopulations
 - 12.4.4 RNA amplification techniques to attempt to perform single cell gene arrays

References

- Chan, S.M., Olson, J.A., Utz, P.J. Single-Cell Analysis of siRNA-Mediated Gene Silencing Using Multiparameter Flow Cytometry. *Cytometry Part A* 69A:59–65 (2005).
- Krutzik, P.O., Irish, J.M., Nolan, G.P., Perez, O.D. Analysis of protein phosphorylation and cellular signaling events by flow cytometry: techniques and clinical applications. *Clinical Immunology* 110: 206– 221 (2004).
- Szaniszlo, 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).
- Szaniszlo, P. Gene Expression Microarray Analysis of Small, Purified Cell Subsets. University of Texas Medical Branch, Galveston, TX April, 2007 (mentor: Dr. Leary)