#3030 Protein Array Analysis with Laser-Excited Solid-Phase Time-Resolved Fluorometry. Liu-Ying Luo and Eleftherios P. Diamandis. *Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada.*

The advantages of time-resolved fluorometry over conventional fluorometric analysis are well known. However, time-resolved fluorescence did not as yet find wide applications in protein microarray or other multiparametric methods of analysis. We here describe a general method which is suitable for multiparametric and microarray analysis, based on time-resolved fluorometry. A polystyrene surface is coated with different monoclonal antibodies, specific for certain analytes. The analyte mixtures are then universally biotinylated by using an active biotin ester. After removing excess biotin, the biotinylated samples are applied on the polystyrene surface, incubated and the excess is washed away. The bound moieties are then quantified by adding a universal detection reagent containing streptavidin, labelled with a fluorescent europium chelate. After washing and drying of the solid surface, the immobilized moieties are detected by using solid-phase, laser-excited time-resolved fluorometric analysis. In a preliminary examination of this principle, we have demonstrated that we can correctly identify up-regulation of three secreted proteins, prostate specific antigen (PSA), human glandular kallikrein 2 (hK2) and pS2 protein, following stimulation of the breast carcinoma cell line BT-474 with various steroids. PSA and hK2 were up-regulated by androgens and pS2 by estrogens. Our method should be suitable for high-density microarray analysis of proteins, captured by specific monoclonal antibodies or other binding reagents.