ABSTRACTS

CRG Core Facilities Technology Symposium: "Unsuspected Flow Cytometry applications on biological analysis"

28 November 2013
Marie Curie room, PRBB Building, Barcelona

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Optimization of flow cytometric analysis and sorting of nanosized particles and cell-derived membrane vesicles

Flow cytometry is widely used for the analysis and sorting of particles in suspension. Especially biological particles like cells and, to a lesser extent, organelles have been analyzed and purified using this technique for decades. Recently, extracellular vesicles(EV) attracted a lot of attention as vehicles for intercellular communication. These 50-200 nm sized vesicles consist of lipids, proteins and genetic material and were shown to play a role in several biological processes. Several methods are available to study EV's. Among the techniques available to study submicron particles, only flow cytometry allows to study single particles at high speed in a quantitative and qualitative manner. However, conventional flow cytometers do not allow visualization of EVs that have a size range between 50 and 300 nm. Here we present the results of our study to investigate the possibilities of flow cytometry to study particles in this size range. We modified a BD Influx to investigate, staining conditions, coincidence constraints and possibilities for sorting of subpopulations. Our results indicate that we are able to visualize and accurately quantify polystyrene particles as small as 100 nm. Similarly, EV and other biological particles of approximately comparable size can be analyzed. In addition, detection of fluorescently labeled antibodies on individual EV allows the identification of different EV subsets. We also demonstrate the possibilities to sort subpopulations of these particles for further analysis. This presentation will specifically address conditions by which EV and alike particles can be studied and sorted.

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Analysis of cells and chromosomes following in situ hybridization by flow cytometry

We developed flow FISH methods to analyze repetitive DNA in cells and chromosomes using FISH with directly labeled peptide nucleic acid (PNA) probes. Flow FISH is used to measure the content of telomere repeats in human and murine cells and the abundance of interstitial telomeric sequences in Chinese hamster chromosomes. The power and limitations of flow FISH will be illustrated and discussed.

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A systematic approach to evaluate instrument performance using Centre Stream Catch

Flow cytometers can be used to obtain highly homogenous populations from a heterogeneous particle mix. After the sort parameters are selected for, the instrument’s performance in this task is further evaluated with regard to Purity, Yield and Recovery of the sorted fraction. Purity is a check on the quality of the sort decisions made by the instrument. Yield establishes how well the instrument is at getting the target particles from the original sample. Recovery definitions vary with some authors regarding it as Yield while others use it to describe the accuracy of instrument sort counting. All of these performance parameters require counting of particles in the original and the sorted sample, which might not be feasible when dealing with rare populations in precious samples. Counting itself is open to large errors and sampling can equally increase the overall error. Here we describe a method to simplify evaluating instrument efficiency in sorting using knowledge from the percentage target population in starting mixed sample and the percentage of unsorted target population in the centre stream catch. This simple method can be performed with an ideal sample based only on beads avoiding wasting precious sample. Performance can be systematically compared for different sorting conditions (sort decision, drop drive frequency, nozzle size, etc), and used to identify and troubleshoot problems related to sorting based on deviations relative to the ideal recovery.