276/B155

Determining RNA Expression at the Single Cell Level in Live Cells Using Flow Cytometry

Don Weldon[1], Alex Ko[2], Yuko Williams[2], Laura White[2], Luke Armstrong[2], Victor Koong[2]
[1]R&D, EMD Millipore, Temecula, CA, United States, [2]EMD Millipore, Temecula, CA, United States

The ability to measure the level of an RNA target in a single cell provides researchers the ability to understand how individual cells may differ from the rest of the population. This is of increasing importance when studying the effects of stimulations or treatments on cells in culture as individual cells may respond differently to the experiment. Current methods to analyze cellular RNA require lysis and amplification of the entire population to understand the effect of the treatment as in the case of RT-PCR. The data is then a mere average of the expression within the population and small numbers of responding cells could easily be missed.

Here we present a novel live cell RNA detection technology which allows for single cell RNA detection without the use of any transfection methods. It is comprised of a gold nano-particle core conjugated to duplexed oligonucleotides on the surface. A fluorophore is linked to one of the strands and is quenched by the gold core until it is displaced by its target RNA in the cell and leaves the proximity of the gold allowing it to fluoresce. These RNA detection probes are non-toxic, do not alter gene expression, and utilize the cells own endocytosis mechanism.

Flow Cytometry can be used to detect the fluorescence which is relative to the amount of target RNA present in each cell. This provides a more complete understanding of the RNA expression for each cell in the experiment as opposed to the average trends seen in RT-PCR data. Furthermore, since the cells are unharmed and unchanged after interrogation with the probes researchers now have the ability to sort cells based on the expression level of their target of interest and use the sorted products for downstream experimentation.

277/B156

Simultaneous Tracking of Cell Type, Viability, Proliferation and Expression of Intracellular Proteins In Co-cultures of Leukemia and HS-5 Stroma Cells Using Multiparameter Flow Cytometry

Katarzyna Piwocka, Paulina Podszywalow-Bartnicka, Marta Brewinska-Olchowik, Lukasz Bugajski, Monika Kusio-Kobialka
Laboratory of Cytometry, Nencki Institute of Experimental Biology, Warsaw, Poland

Introduction: It is known that leukemia cells communicate with the stroma and vice versa by the mean of secreted soluble proteins. Previously we have discovered a novel, pro-survival pathway in chronic myeloid leukemia cells, which is based on activation of the PERK-eIF2alpha phosphorylation pathway (1). This signaling pathway leads to the rearrangement of the protein translation process what strongly influences the composition of expressed and secreted proteins. We aim to study different parameters of the cell-cell interactions detected in vivo in the co-culture conditions.

Methods: To this end we developed protocols to distinguish different types of co-cultured cells and simultaneously analyze their survival and proliferation rate together with detection of the level of intracellular proteins using multicolor flow cytometry. We used CellProliferation tracking compounds and Fixable Viability Dye reagent to monitor cell division and apoptosis in combination with dyes labeling each cell type. For a long term tracking of epithelial cells we used a CellTracker Blue CMAC dye, whereas leukemia K562 cells stably expressed proteins tagged with the green GFP protein. Intracellular proteins playing a role in the prosurvival pathways were detected by the antibodies previously stained with the fluorochrome using the Zenon Labeling technology.

Results: We were able to show that each type of cells, which were cultured together, responded in a different way to the imatinib treatment and modification of the secretome. Moreover, cells stained with both, CellTracker and Fixable Viability Dye, unlike 7-AAD and propidium iodide, can be washed, fixed, permabilized, and stained for intracellular antigens without any loss of staining intensity of the dead cells. We found that the Zenon labeling technology can be used together with the tracking dyes to specifically stain intracellular proteins.

Conclusions: This novel combination of cell tracking and the intracellular protein staining method allows for in vivo studies of cells properties as well as cell signaling upon the co-culture conditions. Thus it can produce a valuable information about the influence of cell-cell interactions for biology of cancer and stroma cells. Importantly, this methodology can be further improved and modified dependently on needs, as the number and type of tracking dyes available for different flow cytometry applications constantly increases.

1. Kusio-Kobialka et al. 2012, Cell Cycle Nov 1; 11(21):4069-78. doi: 10.4161/cc.22387.

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Microbiology and Aquatic Sciences (B157 – B159)

278/B157

Scanning Flow Cytometry for Static and Dynamic Characterization of *E. coli* cells

Anastasiya Konokhova[1,2], Maxim Yurkin[1,2], Valeri Maltsev[1,2]
[1]Laboratory of Cytometry and Biokinetics, Institute of Chemical Kinetics and Combustion SB RAS, Novosibirsk, Russia, [2]Department of Physics, Novosibirsk State University, Novosibirsk, Russia

Background: Light scattering by a particle is determined by its overall morphology, including shape and internal distribution of the refractive index. Therefore, light scattering is a powerful physical method for identification and characterization of bacteria. In addition to identifying or distinguishing microorganisms by its morphology, light-scattering can also potentially provide real-time monitoring of bacterial growth in order to study cell cycle kinetics or analyze growth rate for antibiotic sensitivity testing. This abstract describes a method for high-precision characterization of individual *E. coli* cells from light scattering measured with Scanning flow cytometer and its implementation for static and dynamical analysis of *E. coli* cells.

Methods: We used Scanning Flow Cytometer (SFC) – a technique capable of measuring angle-resolved intensity light scattering patterns (LSPs) of individual particles in flow (Novosibirsk, Russia, http://cyto.kinetics.nsc.ru/).Characterization of particles morphology from measured LSPs requires the solution of the inverse light-scattering (ILS) problem. This solution is based on fitting an experimental LSP by theoretical ones, calculated from the modeling *E. coli* cell as a cylinder capped with hemispheres of the same radius. To accelerate the fit we used a precalculated database of 300 000 LSPs in a wide range of model parameters (length, diameter, refractive index and orientation angle of cell in the flow) and performed the nearest-neighbor interpolation on it. This allowed us to determine length and diameter of individual bacteria including errors of these estimates.

Results: The developed method was tested by two strains of *E. coli*, showing 135 and 15 nm median precision in determination of length and diameter of single cells, respectively, which is very good for optical methods. Obtained length and diameter distributions showed a good agreement with microscopic measurements of the same samples. The method was also applied for monitoring morphological changes of *E. coli* cells during the exponential-growth phase. According to obtained distributions the decrease in cell volume was observed as bacteria cells approached stationary phase of growth.

Conclusions: The presented method allows characterization of a population of rod-shaped bacteria by their length and diameter distributions. It can be used for determination of dynamic characteristics of bacteria, monitoring morphological changes of bacteria cells over time. In particular, it can be used to study cell growth or cell cycle kinetics. Another advantage or this method is sensitive identification of bacteria cells in flow without fluorescent staining. It is important to note, that the SFC-based method is not specific to *E. coli* and can be directly applied to any rod-shaped bacteria. The only additional effort may be needed for extension of the database to larger or smaller bacteria sizes.

279/B158

Moflo Astrios™ Forward Scatter: Cell Sorting of Nano and Large Phytoplankton Simultaneously with High Purity

Carley Ross, Alan Dean, Robin Morris
Research and Development for Cell Sorters, Beckman Coulter Life Sciences Division, Fort Collins, CO, United States

Background: Phytoplankton conversion of light on the upper limits of the ocean consists of half of the photosynthesis on the Earth. The population densities indicate the health of not only the phytoplankton, but the entire aquatic ecosystem. The isolation and sorting of aquatic samples using flow cytometry allows for quick and effective population analysis. The forward scatter on the MoFlo Astrios allows for differentiation of small and large particles from 0.2 to 30 µm on FSC. This design provides researchers greater flexibility to isolate and sort specific phytoplankton of different sizes while utilizing the 7 laser, 42 parameter MoFlo Astrios.

Methods: Plankton species; *Chlorella, Phormidium inundatum, Phormidium persicinum, Cryptomonas, Rhodosorus, Synechococcus, Skeletonema, Fremyella,* were acquired from the UTEX: The Culture of Algae and grown in photobioreactors and cultured in specialized salt and fresh water media. Plankton species *Prochlorococcus marinus* and *Emiliana huxlei* were grown in sterile 2 L containers supplied with 0.2 µm filtered air in salt water with fertilizer. Instant Ocean, ½cup per gallon of deionized water, was added to the culture with Microalgae Grow Mass Pack with Silicate. Cells were harvested by gentle centrifugation, roughly 300 x g for 5-10 minutes. The supernatant was decanted/aspirated and the pellet resuspended in a sterile saline solution to achieve $1x10^6$ cells/mL. Plankton were then stained with SYTOX Green(Invitrogen, S7020), at a maximum concentration of 5 µM for 10 minutes after vortexing. Samples were filtered with a 70 µM Partec filter and kept on ice before flow cytometric analysis.

Flow Cytometry: The MoFlo Astrios was configured with 7 lasers and setup with a 100 µm tip to accommodate the larger phytoplankton (*Cryptomonas*). Cells were selected on their "live" status by being highly fluorescent in the red channels (chlorophyll) and low in the green channels (Sytox -). For small particle analysis, 1 µm beads were run simultaneously with the *P. marinus* and *Synechococcus* and analyzed on FSC-Log parameters. Populations were sorted based on fluorescence and size as a 6-way sort into 5 mL tubes. The plankton were sorted at 25K eps to collect at least 100,000 events per each population using sort mode Purify 1-2.

Results: Plankton populations were distinguishable using fluorescence and scatter patterns on both log and linear scales simultaneously. With the Astrios optical flexibility, the plankton fluorescence spectra were optimized for signal to noise. Isolation of the *P. marinus, Synechococcus* and other plankton species using cell sorting achieved 99% purity for all populations.

Conclusions: The forward scatter and optical collection design of the MoFlo Astrios provide flexibility to detect large and small populations and sort them with high purity.

280/B159

Development of a Plankton Cell Sorter Utilized with the Amnis ImageStream

Gordon Wiegand
Estuary Biophotonics, Baltimoe, MD, United States

Microscopic plankton form the foundation of the food web in aquatic ecosystem such as the Chesapeake bay. Data describing the abundance and distribution of these organisms indicates a direct response to specific forms of pollution. Phytoplankton are the primary producers and vary in size while zooplankton are the primary consumers and are generally larger. The goal of this study is to index size, shape, and morphology from images obtained with stage microscopy and AmnisImageStream then correlate this data with scatter and fluorescence profiles generated by 2 specialized cell sorters.

Drags of the upper Chesapeake by were made at three benthic levels of the shore and outer region of Swan Point located within the upper Chesapeake bay. Size spectra of plankton was compiled for species ranging from 60nm to 300um that included dinoflagellates , diatoms and larger particles. The diatom skeletonemacostatum is closely associated with oyster harvest. We compiled a 3 dimentionalmap of the waters off Swan Point that included a distribution of species considered important to this local ecosystem. Conditions vary with seasons: less bioproduction in winter and more eutrfication from over enriched water in late summer. This causes low oxygen and loss of habitat.

Nano-particles present unique challenges when analyzing and sorting by flow cytometers. A detection limit of 60nm was achieved with a specialized flow cytometer manufactured by PartecInc. by utilizing 90 degree light scatter.

The Partec Space provides very limited sorting capability so we developed an electro-optical system within a test bench that included a quartz flow cell, specialized beam shaping optics and an efficient scatter detection module with low noise PMT and preamps. This subsystem was transferred to a FACVantage cell sorter for sorting of nanometer sized phytoplankton.

An assortment plankton were characterized by combining image and pulse cytometry. Alterations of the FACSVantage optics and detection channel allowed measurement and sorting of plankton within the nanometer range. Large plankton were sorted with a macrosort 400nm nozzle and flow cell.