

Air bubbles Take the Stage in Flow Cytometric Drug Screening

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ABSTRACT

Keywords: Drug Discovery; Biosensing; Crohn's Disease; Hyperchromacity; Squeezed Light; Plasmonics

Abbreviations: FRET: Förster Resonance Energy Transfer; PBFRET: Photobleaching Resonance Energy Transfer; LSC: Laser Scanning Cytometry

Introduction

Those who ever used a stream-in-air flow cytometer and when running their most interesting samples they just got an air bubble trapped in the nozzle never dreamed of a time when this awful situation just turned back showing its good face in helping to find the best drug for curing a disease! The following story is somewhat reminiscent of that of photobleaching, which was originally has been discovered as a pure nuisance reducing visibility of fluorescent samples, but with time it started to be used as a „kinetic ruler” for probing phenomena mediating relaxation of the excited states of dyes, leading to elaboration of methods like „photobleaching fluorescence resonance energy transfer” or „pbFRET” [1]. The central task of simultaneously probing thousands-millions of protein-protein, protein-DNA interactions, posed by a demand for solving biological complexity and problems of drug discovery has been tackled by introducing a highly versatile family of bisensing technologies of outstandingly high speed, sensitivity and degree of multiplexation, collectively termed as „high throughput-high content (or capacity) screening assays, HT-HCS-A” [2]. Driven largely by the development of microscopy and imaging techniques (mainly confocal microscopy) different imaging solutions have been introduced such as the different plate-readers in biochemistry.

In the field of cell biology, the analogue candidates are the different types of cytometers (microscopes with moving objects), basically slide-based or flow-based platforms, which inherently have the demanded high-throughput-high capacity attributes, originating from the large number of different (mainly optical) signal channels (up to ~17 different colour fluorescence and light scattering signals) and the high speed of cell detection (~103-104/sec). While slide based cytometry (laser scanning cytometry, LSC) is an excellent tool for monitoring adherent cells in their close to-native environments, for non-adherent cells in suspension, flow cytometry is the method of choice. Now, the problem at hand is how to convert a flow cytometer traditionally used in a „single sample-single run mode” into a device with which different samples can be measured at a 40-100 samples/minute rate, i.e. to find the appropriate interface between an array of a huge number of different cell samples and the flow cytometer! The answer to this problem is given by the group of L. A. Sklar in New Mexico, in their works aiming at converting flow cytometry into a potent tool for drug discovery during the past decade. Experiencing initially with an 8-pole flow switch - „plug cytometry” [3] -, their work culminated in inventing a very ingenious sample „aspirating probe and transfer” arrangement called HyperCyt platform [4-6].

The goal of the arrangement is a sliding probe capable of high speed motion in the x and y directions above a sample holder plate of wells, and alternatively sucking sample from the well or air between two subsequent wells, in succession, which are further transported towards the flow cytometer by a peristaltic pump. The end result is a train of samples from the respective wells with air bubbles separating them, each having relative lengths determined by the time periods spent by the probe in the well and in the air. Although this idea at first looks rather simple, its practical application demanded a lot of optimisation procedures regarding fitting of tubings of proper material and inner-diameter, peristaltic pump frequency (sample-air bubble frequency) to minimize particle carry-over between successive samples (if the tubings are not washed in between) and operating pressure of flow cytometers to avoid skewness of histograms belonging to the different samples (wells) occurring at such high sample speeds (~2 µl/s), proper shaping of wells, and sample mixing by rotation to ensure sample homogeneity [6,7].

In addition to that it can be conveniently fit to any flow cytometer, another remarkable feature of the HyperCyt platform is that it can be parallelized. In their work, B.S. Edwards, et al. [8] demonstrate the usage of HyperCyt when 4 probes connected to their respective cytometers are operated in tandem, allowing simultaneous sampling from four parallel 384-well sections of a 1536-well plate taking only 11 minutes! By applying multiplexed bead and cell based assays, the authors demonstrate that also at these high sampling rates good quality data - as assessed by the „Z' scores" significantly larger than 0.5 - can be obtained. After setting the stage for the realization of drug screening experiments in flow conditions, for what kind of assays can the new approach be used? Practically any cell constituent of physiological parameter - even dynamical parameters such as rotational motion (via fluorescence anisotropy) and proximity (via Förster transfer), i.e. molecular conformation - can be assayed depending on the availability of appropriate labels. Moreover signal-modulators (agonists and antagonists) can be screened in competition type assays like the nice illustrations of the authors: a G protein-coupled receptor-related, Formylpeptide Receptor (FPR) ligand binding inhibition assay, a protease inhibition assay by which anthrax lethal factor can also be detected amongst others, and a G protein-coupled receptor-kinase 2 (GRK2) enzyme inhibition assay. It should be noted here that, besides the high statistical power, another strength of flow cytometry is its high sensitivity, originating from its low background signal detection.

A promising approach to drug screening is when the physical screening process is carried out on a preselected collection of drugs and receptors structurally fitting into a classification scheme constructed in-silico based on previous experience on the behaviour (whether agonist or antagonist) and chemical structure of drugs and receptors („virtual screening"). After prefiltering by a „virtual

screening" scheme, the „hit-rate" of finding drugs of the appropriate properties can be substantially increased, by >10-fold, as reported by the authors [9]. As opposed to measurement of ligand binding in equilibrium („primary" or „endpoint" assays), time-kinetical recordings of ligand binding („secondary" or „timed" assays) are also made possible by the HyperCyt system, during which cells are continually mixed („incubated") with preformed cocktail of reagents during the aspirating probe-cytometer sample delivery time. More detailed binding characteristics of drugs and their inhibitors, even the time course of the elicited cell-signaling events - e.g. dose response curves by realising preformed concentration gradients in the wells of the plate [5,7] - can be recorded this way with a time resolution determined by sample delivery and working frequency of the aspirating probe.

A proposed future application of this method would be e.g. when pair-wise proximity mapping of a set of receptors is made by measuring Förster Type of Resonance Energy Transfer (FRET) on preselected cell populations (different phenotypes, e.g. CD4+ or CD8+ T-cells, or CD4+CD25+ double-positive helper T, or Treg cells) of peripheral blood or surgical samples of persons suffering in different illnesses (e.g. Crohn's disease, colorectal cancer). In this measuring scheme at least 3-4 different colours are needed: two colours for FRET donor and acceptor, and another one or two colours for gating out the necessary cell population(s) [10]. In this case the number of samples is „exponentially growing" with the number of receptors involved in the proximity mapping, due to the need for the donor-only and acceptor-only samples in addition to the double-labeled ones, a typical system amenable for multiplexing. This sampling technology is also amenable for a larger degree of multiplexing by applying new types of light emitters with sharper emission spectra - the „hyperchromacity principle" [11]. A future marriage with the emerging nanocrystal, nanolaser, and plasmonics technologies seem to be promising in this respect [12]. For slide and imaging based screening platforms the optical resolution is also decisive. New types of light sources - e.g. time-ordered squeezed light from a nonlinear crystal, with a reduced Poisson-noise [13] - offering larger spatial resolution are good candidates for developing more sensitive biosensors.

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