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High-throughput flow cytometry for drug discovery: principles, applications, and case studies

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Flow cytometry is a technology providing multiparametric analysis of single cells or other suspension particles. High-throughput (HT) flow cytometry has become an attractive screening platform for drug discovery. In this review, we highlight the recent HT flow cytometry applications, and then focus on HT flow cytometry deployment at AstraZeneca (AZ). Practical considerations for successful HT flow cytometry assay development and screening are provided based on experience from four project case studies at AZ. We provide an overview of the scientific rationale, explain why HT flow cytometry was chosen and how HT flow cytometry assays deliver new ways to support the drug discovery process.

Introduction

Flow cytometry is a powerful technology that provides a high content analysis of single suspended cells or other particles. The ability of multiparameter analysis in combination with fluorescent dyes allows the analysis and identification of populations of cells in a heterogeneous sample [1,2].

In drug discovery, flow cytometry has been used in many phases [3]. However, until recently, flow cytometry was only amenable to small-scale experiment because of slow sampling technology. Recent development of HT flow cytometry sampling technology has transformed the technique into an attractive drug-screening platform [4,5]. Here, we review recent HT flow cytometry applications, HT flow cytometry deployment at AZ, and HT flow cytometry applications used to support high-throughput screening (HTS), phenotypic, and structure–activity relationship (SAR) screening, also at AZ.

Principles and applications of HT flow cytometry

All types of flow cytometer comprise fluidics, optics, and electronics. They all perform the same basic tasks: suspend particles (often cells) in a stream of fluid and then analyze the physical and chemical characters of particles as they pass through a laser beam [2]. Flow cytometers perform high-speed analysis of particles (up to thousands of single cells per second) and measure multiparameters on single cells simultaneously. Flow cytometry is a high content assay technology providing information-rich multiparametric analysis and, as such, is an important tool for drug discovery.

However, broader applications of flow cytometry as a screening tool for drug discovery have been hampered by the slow sampling speed and the unavailability of HT flow cytometry platforms. The development of fast, automated sampling technologies for use with flow cytometers has significantly increased the speed of this approach, rendering flow cytometry an attractive drug-screening platform [3–5]. An ideal HT flow cytometry system would be user-friendly with analysis software for multiparametric measurements and high-speed quantitative analysis of single cells and other particles. Researchers have reported using different HT flow cytometry platforms in pharma, biotech, and academia, such as an integrated HyperCyt platform on flow cytometers CyAn (Beckman Coulter), FacScan, and Accuri C6 (BD Bioscience), as well as the commercial iQue Screener (Intellicyt Corporation, Albuquerque, NM, USA) [3,6,7].

During the past decade, various HT flow cytometry screening applications have been reported, such as ligand-binding assays for screening novel G-protein-coupled receptor (GPCR) ligands [8], HT screening and signaling profiling using barcoded cells [9], phenotypic screen of cytotoxic T lymphocyte lytic granule

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exocytosis in a 1536-well format [7], and yeast-based screens [10]. In addition to cell-based applications, there has also been an increase in performing HT flow cytometry using bead-based immunoassays to screen for secreted cytokines and other proteins in a multiplexed format [11–13].

Compared with other assay technologies with simple readouts, both imaging- and flow cytometry-based screening enable multiparametric measurements in the more complex physiological environment of a cell, so-called 'high content screening'. HT imaging has been widely used as a screening platform in drug discovery [14]. Flow cytometry has several advantages over imaging-based screening. First, it can physically separate cells and analyze one cell at a time from a heterogeneous cell population without the need to develop complex segmentation algorithms for data analysis, as is required for imaging-based screening. Second, the technology is not only optimal for screening suspension cells, but can also be used for screening adherent cells after detaching cells from plates or cell culture flasks [15,16], while imaging assays are more suitable for screening adherent cells. Therefore, HT flow cytometry complements HT imaging and is rapidly becoming an attractive drug-screening platform.

HT flow cytometry deployment at AstraZeneca and practical considerations for HT flow cytometry assay development and screening

In 2014, AZ deployed two iQue® Screener HD systems into its Global HTS center and one iQue® Screener Plus system in its assay development group to fill a technology gap for screening suspension cells and rare and/or primary cells in small sample volumes and to replace expensive and/or inflexible immunoassays for cytokine profiling. Now, the company has in place an array of HT flow cytometry applications ranging from HTS, multiplex profiling, and phenotypic to SAR screening. Selected project examples are detailed in the case study sections below to demonstrate the impact of HT flow cytometry on hit, lead, and novel target identification in the drug discovery process at AZ.

The critical questions to ask when we design an assay for a project include: What are the most physiologically relevant cells? Do these cells display a suspension phenotype? Is flow cytometry the most suitable technology for the project? What are the relevant biomarkers? Is there a need to monitor multiple parameters simultaneously? What is the assay cost? What are the assay throughput and quality requirements (e.g., is the assay for HTS or SAR screening)?

At AZ, HT flow cytometry assays are developed and utilized in screens according to several in-house-developed best practice guidelines. These guidelines are broadly similar to the published NIH guidelines [17] or those of the International Society for the Advancement of Cytometry [18]. During flow cytometry assay development, we optimize general parameters (e.g., cell density, compound treatment period, donor-to-donor variation if using human primary cells, and assay miniaturization) to improve the assay window, throughput, and assay quality. We also optimize parameters more specific to flow cytometry assays, as listed in Table 1.

Our current flow cytometry data analysis routine is to use the ForeCyt® software (Intellicyt Corporation) to perform Well Identification & data processing, and export parameters of interest as CSV files, which are then imported into Genedata Screener® (Genedata, Basel, Switzerland) to perform the analysis.

Similar to traditional flow cytometry, a good cleaning and maintenance routine is critical. The iQue Screener platform uses probes and/or tubing for sampling that have a recommended standard lifetime of 20 h. Fluidic stability can be disrupted by blockages because of cellular build-up, resulting in sampling issues. For this reason, data acquisition has to be monitored in real time to resolve potential clogging problems during a run. High shaking speed for the resuspension of beads in 1536-well plates can affect plate alignment calibration; thus, this parameter needs to be checked regularly during screening.

Case studies of HT flow cytometry applications

During the past few years, we have developed and performed various HT flow cytometry screens at AZ. We selected four recent examples as case studies, detailed below. These examples give an overview of the scientific rationale of the work, why HT flow cytometry was chosen, and how the HT flow cytometry assays have provided new ways to support the drug discovery process.

Case study 1: investigating compound toxicity

HTS is the primary hit-finding strategy within AZ. Key factors in generating successful HTS screens are the size, quality, and content of the compound library. Selecting poor starting points for lead generation and beyond leads to attrition post-HTS and can result in the best compounds either being missed or their progression being delayed. Safety-related attrition is a leading cause of project failure in AZ, where unacceptable toxicity profiles of compounds were the most important reason for failure between 2005 and 2010 and accounted for 82% of preclinical project closures [19].

To help address this problem, the AZ HTS group designed and ran an HTS to profile our compound library with respect to cell toxicity [20]. This assay was run against the THP-1 cell line using

TABLE 1

Q6 **Parameters that are more specific for flow cytometry assays**

Parameter	How
Fluorochrome panel	Design a fluorochrome panel based on antigen density, fluorochrome brightness, and settings of the flow cytometer
Compensation	Run a compensation test to identify a panel of fluorochrome combinations that has minimal spillover between channels and apply the compensation matrix to correct for it
Antibody titration	Perform antibody titration to find the optimal antibody concentrations to be used
Assay plates	Test the assay in different plate types: flat-, round-, v-bottom, etc.
Sample-acquiring protocol	Optimize sampling time, shake speed, interwell shake, mid-plate cleaning, and buffer composition (e.g., detergent) to maintain cells in suspension and reduce risk of forming cell clumps and clogging.

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