Miniaturization of Cell Based Assays- Part 1: Problems and Solutions

The last decade has seen a year on year increase in the production and testing of new biologically active compounds against a rapidly growing portfolio of potential therapeutic targets. This elevated research and development activity has arisen as a direct result of the completion of the human genome-sequencing project accompanied with advances in chemical synthesis and drug design.

(Read also the second part of this story: Micro Array Study)

To reduce costs the pharmaceutical industry is attempting to improve efficiency by investing in automated technologies that utilise more physiologically relevant cell based models. An example of such a technological approach is the newly emerging High content imaging technologies. High Content Imaging technologies are becoming increasingly used within drug discovery as they permit a rapid and robust means of identification and validation of therapeutic as well as providing a means of assessing the efficacy and/or toxicity of drug candidates. Despite the obvious advantages this technology offers, the reagent costs associated with cell based screening can be prohibitive.

With the advent of micron resolution robotics and nano litre capable liquid handlers, large scale and automated assay miniaturization is now possible. The advantages of miniaturization are clear, when one considers the savings in reagents and experimental materials. However miniaturization of cell based assays can be technically difficult and costly to deploy.
Miniaturization in Cell Based Screening
The growing trend toward biological assay miniaturization has been driven by the need to reduced costs and has been facilitated by scientific and technological progress in automation and detection instrumentation. The best contemporary example of biological assay miniaturization is the DNA microarray [1] and protein microarrays [2]. These analytical tools provide a fast and inexpensive means of gathering highly detailed information on gene expression or protein interaction at the cell or tissue level.

Cell-based assays are commonly performed in micro-titre plates which are now available in a multitude of well densities ranging from 96-1536 wells/plate (with working reagent volumes from 5 to 100 μl per well depending on the plate format).

Although micro-titre plates are ubiquitously used in cell biology labs, the experimental costs of performing large scale experiments using these technologies even in the high density formats can be prohibitive. As such, many are now turning to cellular micro array technologies as an alternative to the more costly conventional micro plate technologies.

The use of cellular microarrays were first described by Ziauddin and Sabatini [3], who preprinted defined spots of plasmid and transfection reagent onto a glass slide before seeding with cells. It was demonstrated that seeded cells subsequently attached to the array substrate and absorbed the DNA and expressed the genes contained within the plasmid. The position of the spots and the in-situ transfected cells were identified by spatial correlation. Since then, the same principle has been shown to successfully work for RNA interference assays and drug discovery screens [4; 5].

High Content and Analysis Technologies
Since its inception in the mid 1990’s, High Content Screening and analysis (HCS/A)
technologies have become ever increasingly adopted within the fields of drug discovery and cell biology. One of the most attractive features of this technology is that it provides contextual information at the level of the intact cell or tissue and permits the quick, straight forward and reproducible measurement of the morphological and structural properties cells and organelles [6; 7]

High Content and Screening and Analysis (HCSA) technologies represent the convergence of several mature laboratory analysis technologies, namely fluorescent light microscopy coupled with the automation and ease of use of a plate reader and the ability to analyze cellular subpopulations functionality associated with flow cytometry. The key feature of this technology is the ability to perform highly detailed analysis on cellular images acquired by these platforms.

This combined functionality hence enables for the first time the study of the functional characteristics of genes and proteins in the context of the whole cell (A definition of the discipline cellomics).

**The High Content Screening Process**

The High Content Screening process can be divided into 5 discrete steps, 1. Assay preparation; 2. Image acquisition; 3. Image analysis; 4. Data storage and retrieval; 5. Data analysis and visualization. In this review we will focus on steps 1 to 3.

**1. Assay Preparation**

Involves setting up the cell based assay for the screen, this step is often preceded by an assay development step where the assay is optimized and assessed for use within the High Content Screen.

As an example a typical assay workflow for high content screening is performed in three discreet workflows namely (a) cell seeding, Compound and (b) ligand dosing and (c) Fixation/ Permeabilization, washing, Antibody and substrate addition.

**Cell Seeding**

This task is performed using bulk liquid handling technologies such as the Thermo Well- Mate with integrated stackers. In larger screening centres, such as those housed in the AstraZeneca Oncology Research Centre (Alderley Park, UK) Utilisation of upstream centralized cell culture automation and use of cryopreserved cells has increased efficiency in this workflow minimising the need for local cell culture in routine screening.

**Compound Dosing and Ligand Stimulation**

The efficiency of the assay preparation process has been significantly improved with the implementation of acoustic dispensing technologies such as the Echo 555
(Labcyte). As the name suggest acoustic dispensing technologies utilise sound waves to move liquids from one location to another. The key advantages of these types of system is that they are; capable of reliably and reproducibly and rapidly dispensing volumes as small as 2.5 nl and they non-contact indeed the commercially available technologies do not require tips at all. Collectively this ensures that minimal quantities of material can be dispensed (reducing waste) with no risk of cross contamination. To further improve efficiency these dispensing and liquid handling technologies are arrayed into integrated work cell configurations such as the PAA workcell shown in figure 1.

**Fixation/Permeabilization, Washing, Antibody and Substrate Addition**

This part of the process involves the chemical fixation of cells, which effectively stops the experiment, this is essential for any cell based assays where time dependant reactions or processes are being monitored also fixation will serve to preserve the cells allowing them to be stored until needed.

Typically the aldehyde cross linking reagent formaldehyde or para-formaldehyde would be used to perform this task (although others fixatives are used depending on experimental requirements).

At AstraZeneca Oncology Research Centre (Alderley Park, UK) these tasks are typically performed on PAA work cells (fig. 2) utilising both Biotek EL405 and Tecan plate washers and a Thermo Multidrop dispenser linked to Hamilton valves. This Multidrop/valve arrangement enables a single Multidrop to add multiple reagents without the requirement for manual intervention.

To improve process efficiency and to ensure that the automation work cell can be used to full effect, the use of integration an software has been employed. An example of this is Overlord integration software and batch manager. These types of software enable the user to automate multiple assay steps without the need for exhaustive machine programming. Each assay preparation work cell also incorporates tissue culture incubators set to the appropriate conditions to enable the automation of cellular incubation steps.

In figure 2 both phases b and c have been incorporated on the same robotic platform to further minimize manual handovers between systems.

**2. Image Acquisition**

Put very simply this image acquisition involves the capture of high resolution digital images using automated multi channel fluorescent microscopy. An example of such an instrument platform would be the INcell 1000 (fig. 3), or the Array Scan VTI. These systems are typically used in the conjunction
with multi-welled plates and are capable automatically focusing, filter wheel, sample alignment (moving stage) image capture (by means of an automated ccd camera or photomultiplier), and data storage.

3. Image Analysis

Image analysis initially begins with the selection and configuration of an image analysis algorithm which has been setup to selectively identify and segment labelled cellular and sub-cellular targets of interest (see table 1 below). The image analysis process may be subdivided into the following steps (a) object identification. For this step the images are automatically detected by the software.

(b) Image segmentation: Depending on the configuration of the algorithm i.e. whether cellular images meet pre-selected user defined criteria the software will segment the image into regions of interest. For example A simple nuclear / cytoplasmic segmentation is illustrated in figure 4.

(c) Feature extraction: This is where numerical information is extracted from the image. For example, in figure 5 segmented images of peripheral t-lymphocytes in this case the cells are selected on the basis of their gross morphology (i.e. the elongated cells are selected shaded in green and excluded cells non elongated cells shaded in red).

References

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