High-Order Cell Multiplexing for Image-Based Assays with the SemaPlex Platform

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Multiplexing has the potential to drastically accelerate the efficiency of cell panel screens. SemaCyte® microcarriers enable the moving and freezing of adherent cells. With the SemaPlex platform, we can now optically barcode these cell microcarriers to enable true high-order cell multiplexing for image-based assays. Here, we present a proof-of-concept 4-plex oncology cell panel screen for p53 stabilisation. Cell multiplexing in this assay allowed for a 4-fold reduction in reagent use and a 60-fold reduction in cell use.

How a 10-Plex Cell Multiplexing Workflow Reduces Reagents & Time

Dispense multiplexed cells

directly onto drug library



Conventional Workflow

Adherent cells frozen on barcoded microcarriers

Thaw and pool 10 cell types



The SemaPlex platform enables the barcoding, freezing, and moving of adherent cells. Cells retain their adherent morphology as they are attached to micro-well shaped microcarriers. Here,



Culture 10 different cell types



10 flasks

Trypsinize, plate, wait until attached, and dispense drug library onto cells

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10 plates per 96/384 compounds

they are contained within a 100 x 100 μ m² growth area. The walls of these carriers contain optically visible barcodes able to generate millions of unique identifiers.

A cell panel screen using 10 adherent cell types typically requires lots of culturing and preparation. For each drug and cell combination, one well is necessary.

with unique SemaPlex microcarriers barcodes for each cell type can be thawed, pooled, and instantly dispensed onto a drug library. Here, each well would contain 10 cell types. For this 10-Plex setup, there would be a 10-fold reduction in the reagents needed. Depending on the size of the drug library, this approach could drastically shorten project timelines.

AM-Calceir

Preparation of Barcoded, Frozen, Adherent cells

Integration with Standard Image Analysis Workflows







Α.





Microcarrier Tiling







D.

A. The SemaPlex platform consists of microfabricated arrays of barcoded SemaCyte[®] microcarriers. **B.** Cells are seeded onto arrays of immobilised microcarriers which release over time into solution and can be used in standard cell assay workflows. C. Cells on SemaCyte[®] microcarriers can be frozen and thawed whilst maintaining their adherent morphology and cellular function. Top, calcein-AM and HCS nuclear mask blue labelled cells pre and post thaw. Bottom, A549 loaded SemaCyte[®] microcarriers were stained with a stain that preferentially binds to polarised mitochondria and reflects good mitochondrial health. Images were taken at 1-hour post-thaw. D. A549 cells were loaded onto 3 differently barcoded SemaCyte[®] microcarriers. The 3 populations were labelled with different cell tracked dyes and then mixed.



A. SemaCyte® microcarriers can be identified and tiled using the Semalyse software. Settings can be adjusted to allow or discard microcarriers with different degrees of overlap. This example uses high stringency to minimise microcarrier overlap. **B.** Tiled microcarrier images can be analysed using standard image analysis software such as cell profiler. C. Image based microcarrier barcode reading with the Semalyse software. Identified microcarriers are compared to reference codes and assigned to a matching barcode.

4-Plex Oncology Cell Panel Screen for p53 Stabilisation

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5-	PC3	A549	T98G	HEPG2		PC3	A549	T98G	HEPG2	A. F

Four different cell lines were loaded onto differently barcoded SemaCyte[®] microcarriers, pooled, and plated into wells of a 96-well plate. Wells were drugged in triplicate. Cells were fixed 16 hours post nutlin-3a treatment and p53 immunocytochemistry performed. Plates were imaged, barcode scanned, and quantified for p53 intensity. Data of mean p53 intensity from triplicate wells is shown. B. Representative images of untreated and 12.5 µM of nutlin-3a treated cells.





p53

