TECHNOLOGY NOTE

The SemaCyte® Multiplexing Platform: Accelerating Image-Based Cell Panel Screening

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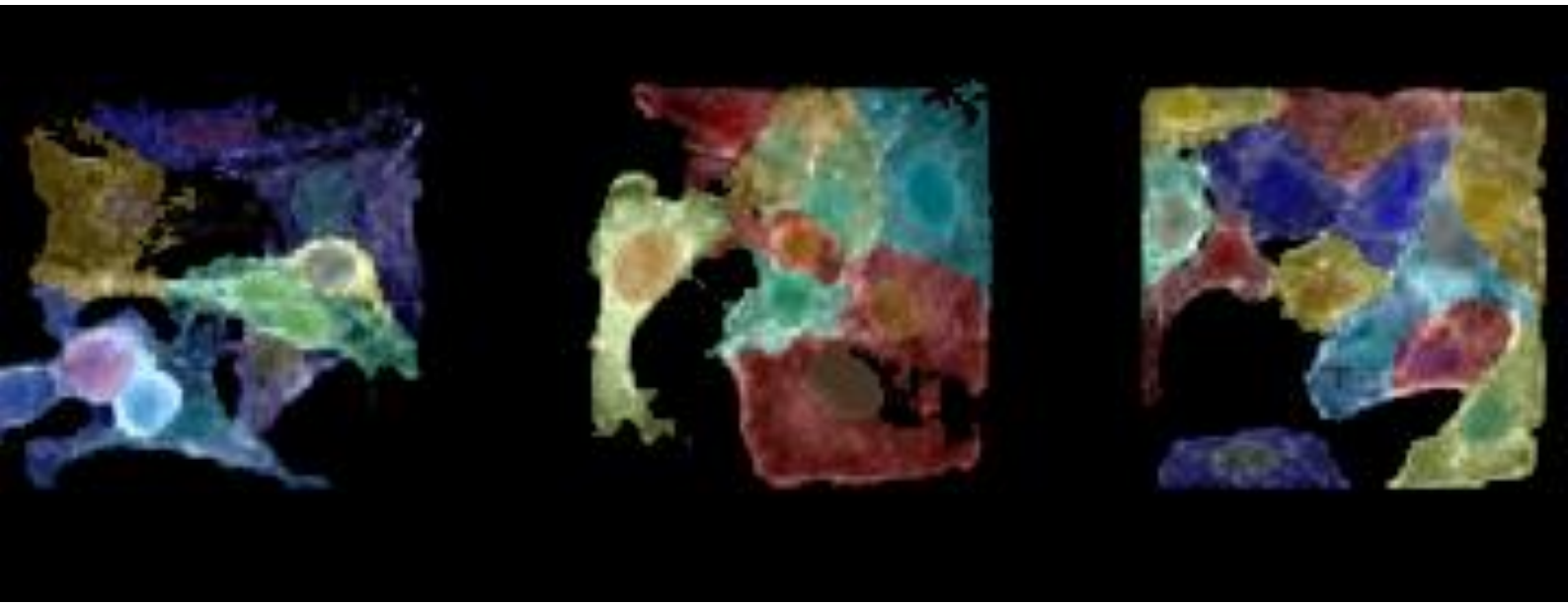
Abstract

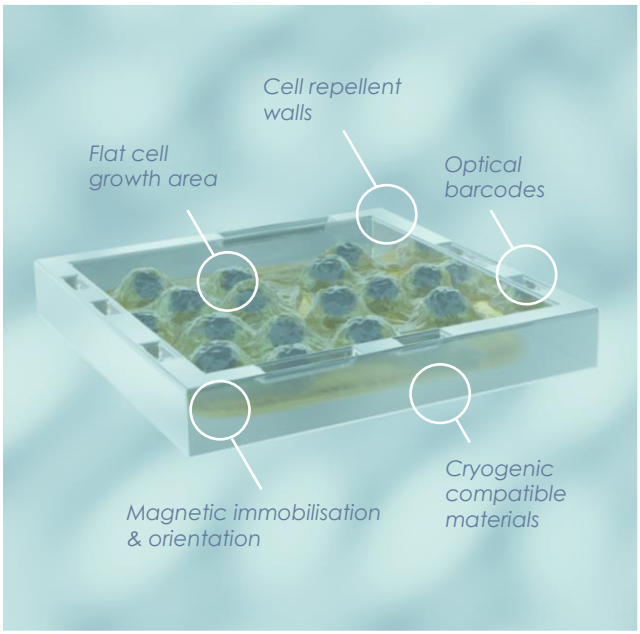
Multiplexing technology holds immense promise for enhancing the efficiency of cell panel screens. SemaCyte® microcarriers facilitate the movement and cryopreservation of adherent cells. By optically barcoding the walls of these cell microcarriers, we now enable true high-order cell multiplexing capabilities for image-based assays. Importantly, this innovative method seamlessly integrates with standard microplate workflows and high-content imaging systems. Our approach involves pooling diverse cell types within a single well, resulting in a remarkable 6-fold reduction in screening campaign time while simultaneously reducing reagent costs by up to 10-fold. In this white paper, we provide a comprehensive overview of the SemaCyte® Multiplexing Platform, including its protocol and potential applications.



Introduction

Cell multiplexing, the simultaneous analysis of multiple cell types within a single well or screening environment, holds immense promise for enhancing data robustness while minimizing time and reagent usage. In recent years, genetic barcoding techniques have successfully enabled high-order multiplexing for viability and omic assays. However, these approaches fall short in the realm of image-based screens, where only low-order multiplexing is currently feasible, often requiring the intricate engineering of fluorescently labelled cell lines.

The SemaCyte® Multiplex platform specifically tackles the multiplexing limitation of image-based assays. Building upon our established SemaCyte® microcarriers, which allow for the movement and cryopreservation of adherent cells, this platform introduces a transformative feature: optical barcodes embedded into the walls of the carriers. These barcodes, with over a million theoretical identifiers, enable high-order multiplexing within a single microwell. Imagine the potential — image-based analysis of tens or even hundreds of distinct cell lines or populations, all within the confines of a single well.



Optically barcoded microcarriers

The SemaCyte® Multiplex platform consists of barcoded microcarriers which are arrayed at the bottom of a petri dish, called a Seeding Dish 20 (SD20). Each dish has a diameter of 60 mm, a total area of 21.5 cm^2, and contains 50,000 immobilised microcarriers (**Figure 1**). Each standard SemaCyte® microcarrier has a cell attachment and growth area of 100 µm x 100 µm, or 0.01 mm^2. Standardly coated with a fibronectin mimetic polymer, they offer compatibility with various cell types, though other coating options are also available. Each SemaCyte® microcarrier features a flat cell growth surface and a cell-repellent wall, accommodating approximately 4 to 20 cells, depending on the specific cell type. Notably, barcoded SemaCytes incorporate unique, optically visible barcodes into their walls, enabling precise identification of distinct cell populations in pooled assays.

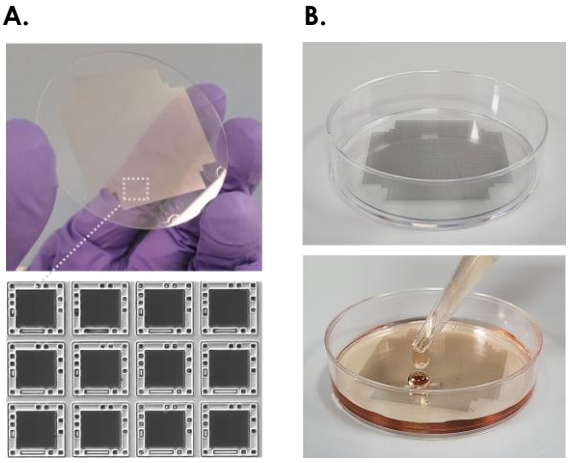


Figure 1. Optically barcoded SemaCyte® microcarriers. **A.** Macro (top) and microscopic (bottom) images of microfabricated arrays of barcoded SemaCyte® microcarriers. **B.** Cells are seeded into a Seeding Dish 20 (SD20) with immobilised microcarrier arrays.

Workflow

The SemaCyte® multiplexing protocol can be broken down into 6 steps as shown (**Figure 2, Table 1**). Importantly, this workflow integrates seamlessly with routine cell culturing and assaying methods and protocols.

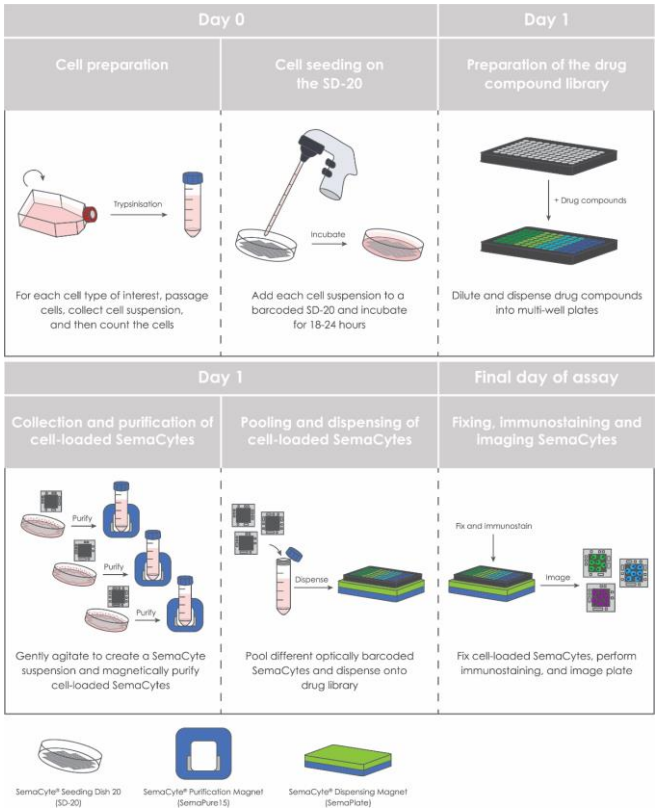


Figure 2. Schematic of SemaCyte® Multiplex assay workflow

At the start of the assay, a suspension of the cell types of interest are prepared and seeded onto SemaCyte® Seeding Dishes (**Day 0**). Each cell type to be assayed is seeded onto its respective Seeding Dish, with each dish containing microcarriers tagged with a unique barcode.

After a 16-24 hour incubation period, depending on the cell type, SemaCyte® microcarriers are collected, purified, pooled, and dispensed into microplates with libraries of drug compounds, CRISPR gRNAs, siRNAs, or other modalities (**Day 1**). Cells are then incubated for the desired amount of time. On the final day of the assay an appropriate imaging endpoint assay is performed. Examples of assays include live or fixed cell dyes, fluorescent protein analysis, and immunocytochemistry. Plates are then imaged in the appropriate fluorescent and brightfield channels **(Figure 3)**.

	Steps	Timing
Day 0	A. Cell preparation B. Cell seeding on the SD20	Dependent on cell type, approximately 15 min
Day 1	C. Preparation of the drug, RNAi or CRISPR library in multi-well plates	30 min
	D. Collection and purification of cell-loaded SemaCytes	45 min
	E. Pooling and dispensing of cell-loaded SemaCytes onto multi-well plate	30 min

Table 1. Example SemaCyte® Multiplex assay workflow.

Batches of cells attached to barcoded SemaCytes can be prepared upfront and cryogenically stored in cryovials for later use. When it is time to perform an assay, the cell types of interest are thawed, pooled, and dispensed for assaying within 1 hour of thawing.

The maximum plex-number is defined by the size of the microwells (**Table 2**). For instance, a 384-well plate can accommodate up to 100 SemaCyte® microcarriers, with a recommended multiplexing capacity of 5. This translates to approximately 20 microcarriers (or 100-200 cells) per cell type.

Microplate Wells	Well Growth Area (cm^2)	Number of SemaCytes per Well	Suggested Multiplexing Capacity
6	6.4	12,000	600
12	3.8	7,000	350
24	1.9	3,500	175
48	0.76	1,400	70
96	0.32	600	30
384	0.056	100	5

Table 2. Suggested multiplexing capacity for different microplates

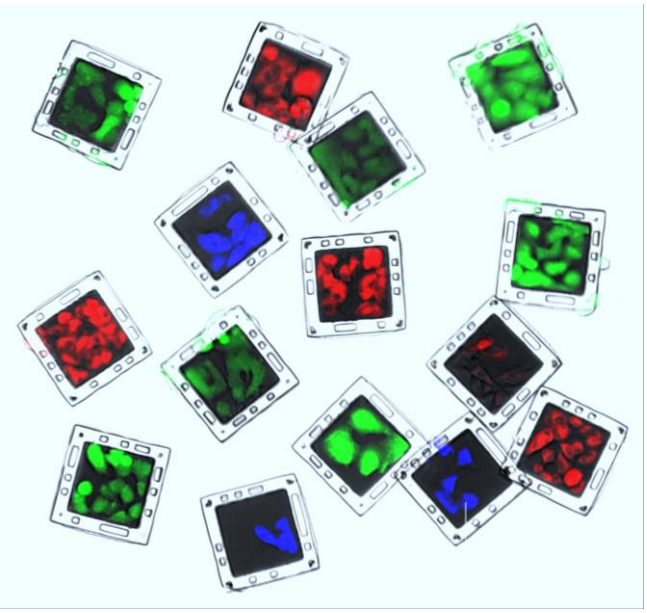


Figure 3. Pooling of cell types in microplates. Four different SemaCyte® SD20s, each with a different barcode, were seeded with A549 cells. 24 hours after attachment, microcarriers were released and purified. For each barcode, the A549 cells were stained with a different cell tracker dye before pooling them together and dispensing them into a 384-well plate at a density of 100 microcarriers per well.

The SemaCyte® microcarriers seamlessly integrate with automated liquid handling tools, including both peristaltic systems (e.g. the Multidrop™) and pipette-based systems (e.g. Integra Assist Plus). Additionally, these microcarriers are compatible with High Content Imaging systems, such as the Opera Phenix® and the ImageXpress®, allowing for efficient and precise imaging.

Read the full protocol here.

The Advantages

Augment microplate-based high-content imaging (HCI) to enhance both the quality and speed of data generation.

Increase screening throughput and reduce cost and time requirements by up to 6- and 10-fold, respectively.

Enhance target validation, compound screening, and compound profiling workflows.

Image processing and analysis.

Our Semalyse software streamlines multiplexed cell panel screening analysis process. In 5 steps (as outlined in **Table 3**), our image processing workflow enables data analysis from these SemaCyte® Multiplex experiments.

After capturing images of the microplates using both fluorescent and brightfield channels, the resulting image files are exported and loaded into Semalyse. This software performs two critical tasks: microcarrier identification (**Figure 4**) and barcode deconvolution (**Figure 5**).

Once Semalyse successfully identifies the microcarriers and their associated barcodes, it generates sets of tiled images. Each of these sets contain microcarriers from one specific barcode, corresponding to one of the multiplexed cell types. The sets hold the different colour channels which were initially imaged, now segregated by cell type.

These tiled images are readily exported for further analysis using standard image analysis software like CellProfiler and ImageJ (**Figures 6 and 7**). This approach enables seamless integration of the SemaCyte® Multiplexing solution with preferred imaging pipelines.

Step 1	Image microwell plate in fluorescent and brightfield channels
Step 2	Export images
Step 3	Import images to Semalyse to perform microcarrier recognition and barcode deconvolution
Step 4	Export images of microcarriers tiled by barcode from Semalyse
Step 5	Analyse images using standard image analysis software e.g. cell profiler and ImageJ

Table 3. Overview of image processing and analysis workflow.

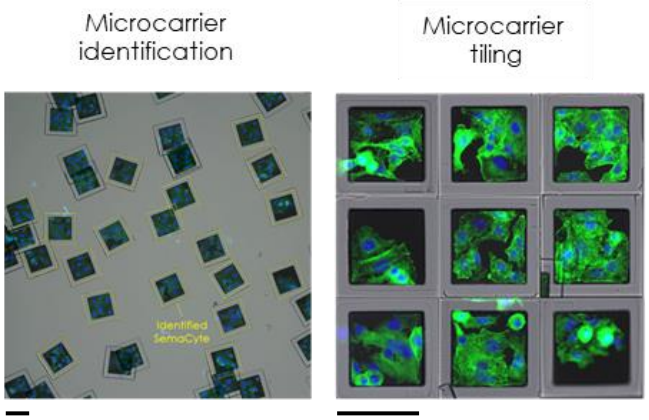


Figure 4. Microcarrier identification and tiling. Semalyse is able to identify SemaCyte® microcarriers from a brightfield image. By varying the recognition quality threshold, users can decide to include or exclude overlapping microcarriers. Here, we show an example using stringent values to exclude overlapping carriers. In this example, SemaCyte® microcarriers were loaded with A549 cells and stained with HCS nuclear mask (blue) and fluorescent phalloidin (green). Scale bar is 100 µm.

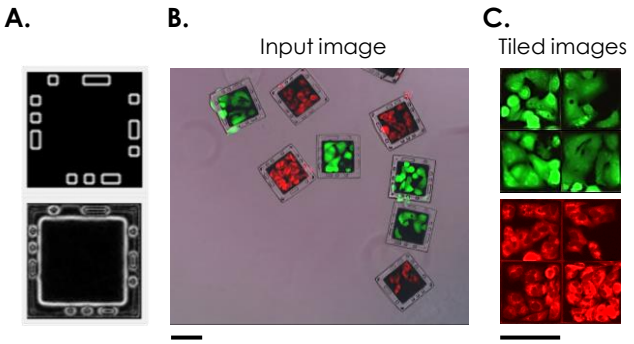


Figure 5. Barcode recognition. Semalyse generates reference images based on user inputted barcodes. These reference barcodes (**A, top**) are used to assign barcode values to identified microcarriers (**A, bottom**). Here, we show an example of Semalyse processing an input image (**B**) containing two different cell populations labelled with a red or green dye on differently barcoded SemaCyte® microcarriers to produce two separate tiled images (**C**). Scale bar is 100 µm.

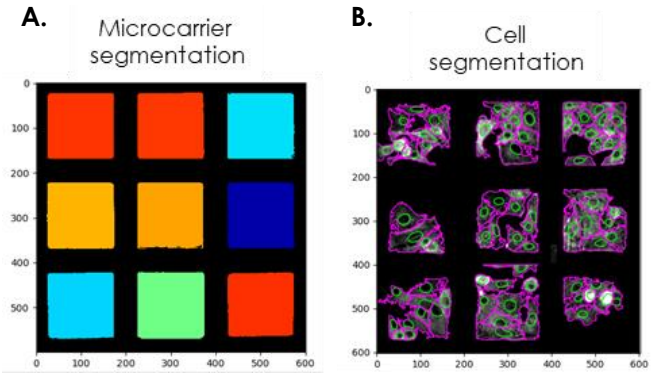


Figure 6. Microcarrier recognition and cellular feature segmentation using CellProfiler. Once exported from Semalyse, tiled microcarrier images can be analysed using CellProfiler. CellProfiler is able to both segment microcarriers for analysis of individual carriers (**A**) and segment cellular features such as nuclei and cytoplasm within these individual microcarriers (**B**). In this example, SemaCyte® microcarriers loaded with A549 cells and stained with HCS nuclear mask and fluorescent phalloidin were used.

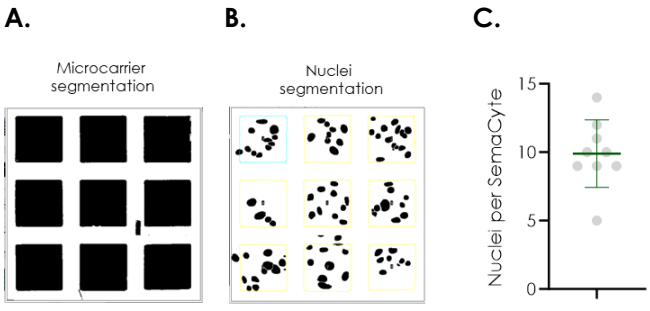


Figure 7. Microcarrier recognition and cellular feature segmentation using ImageJ. Once exported from Semalyse, tiled microcarrier images can be analysed using ImageJ. ImageJ is able to both segment microcarriers for analysis of individual carriers (**A**) and segment cellular features such as nuclei within these individual microcarriers (**B**). Nuclear count per microcarrier can be analysed (**C**). In this example, SemaCyte® microcarriers loaded with A549 cells and stained HCS nuclear mask and fluorescent phalloidin were used.