APPLICATION NOTE

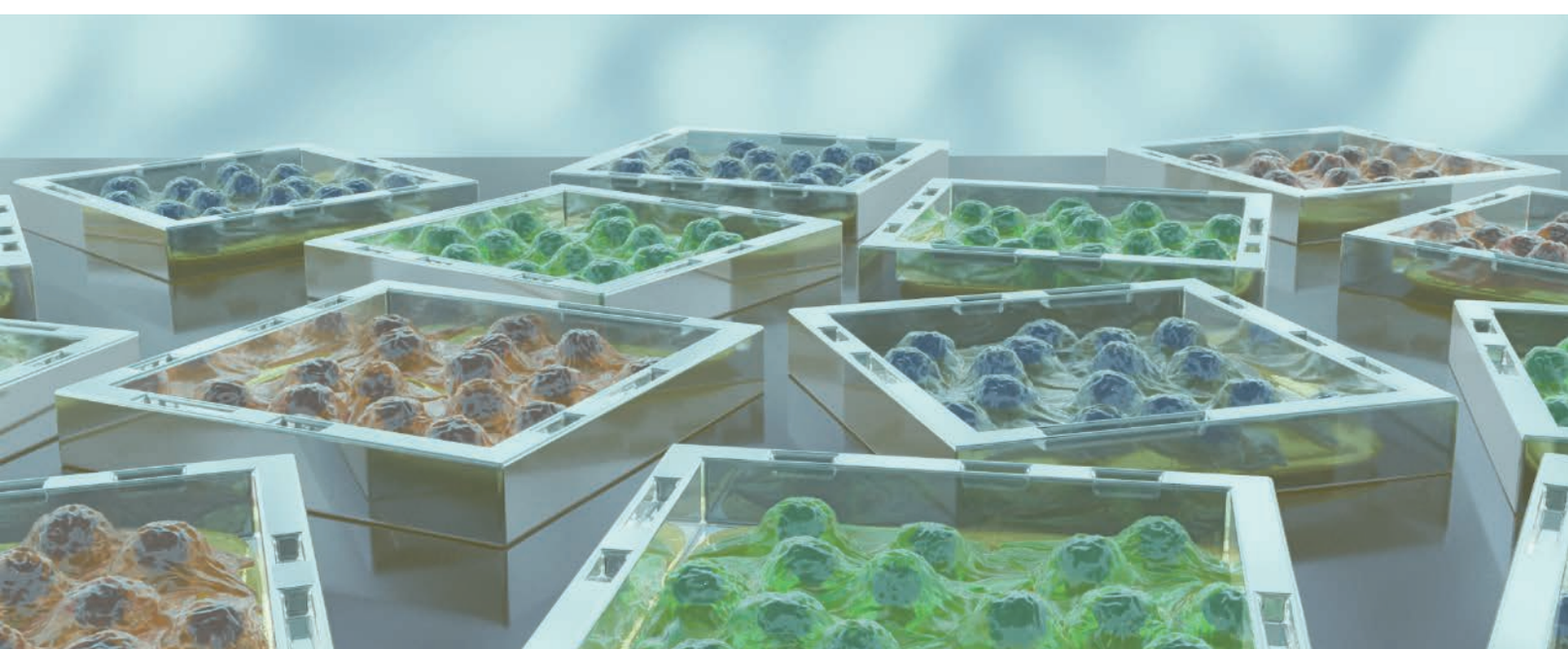
semarion

Scalable & Reproducible DNA Damage Assays

Barcode and pool diverse cell models with SemaCyte® microcarriers for reproducible, image-based DDR screening

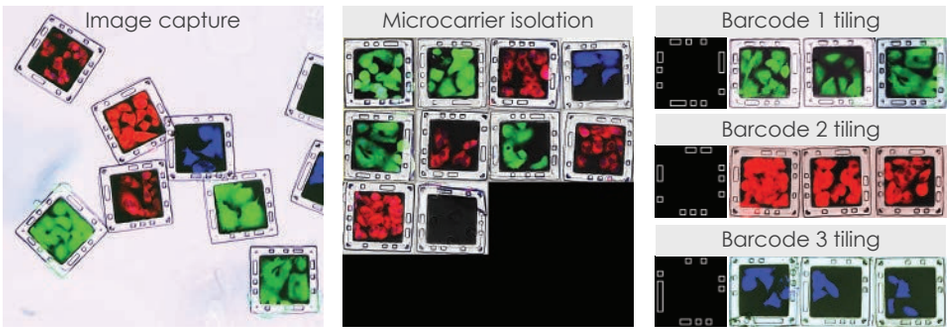
DNA damage response (DDR) pathways are key targets in oncology and neurobiology, but their dynamic, context-dependent readouts often require multiple cell models for robust interpretation. SemaCyte® microcarriers enable multiplexed screening of various adherent cells within a single well with optical barcodes to distinguish and profile each model. Here we present a high-content imaging assay using γH2AX as a marker for DDR in three cell lines (A549, U2OS, U87MG). Results demonstrate reproducible EC50 values from multiplexed assays using only ~80 cells per condition. Assay-ready frozen cells and embedded in-well controls further improved flexibility, comparability, and throughput in DDR screening.

Throughput: Multiplex 10 cells models to screen 10x faster
Efficiency: Assay-ready adherent cells to reduce time and resource use
Reproducibility: In-well prefixed contros for cross-study consistency



Cell Multiplexing with SemaCyte® Microcarriers

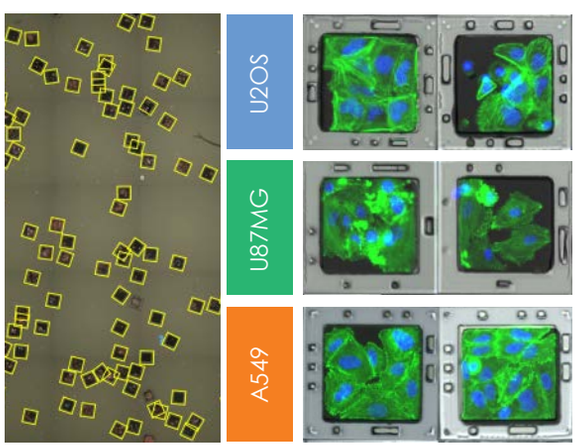
Each cell type is seeded onto a different SemaCyte® Seeding Dish, each containing SemaCyte® microcarriers with a unique optical barcode. Once the cells reach the desired confluency, the microcarriers are released, optionally frozen, and later pooled by mixing. This pooled mixture is dispensed directly onto a microplate containing an compound library for incubated. The optical barcodes, functioning like QR codes visible in the brightfield channel, enable precise identification. After imaging, software deconvolutes the barcodess, generating image sets for each cell type, which can be processed using standard image analysis workflows for further image analysis.



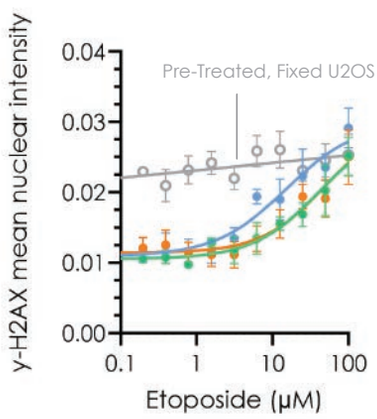
Barcode, freeze, pool, assay
Streamline profiling with mutliplexed adherent cells

3-Plex Cell Panel: DNA Damage Analysis with γH2AX Profiling

A



B



EC50 Values (μM)	
SemaCyte Mutliplex	Well Plate Control
15	16
43	49
48	57

(A) Three adherent cell lines (A549, U2OS, U87MG) were seeded onto optically barcoded SemaCyte® microcarriers, cryopreserved, then thawed, pooled, and dispensed into 96-well plates. A fourth barcoded population of pre-treated, fixed U2OS cells served as an in-well positive control. Cells were treated with etoposide, stained for γH2AX and RAD51, and imaged on a ThermoFisher CX7. SemaCytes were identified in brightfield and decoded by barcode; representative actin images for each cell line are shown post-deconvolution.

(B) Dose-response curves based on γH2AX mean nuclear intensity reveal cell line-specific EC50s. Multiplexed values closely aligned with historical single-line controls, despite using ~6× fewer cells per condition (i.e. 84±42). This approach enables faster DDR phenotyping with embedded controls and frozen assay-ready cells.