

High-content analysis of DNA damage markers in multiplexed cell lines using the SemaCyte® microcarrier platform

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Abstract

High-content imaging (HCI) offers a considerable depth of analysis options when considering the requirements for developing a new cellular assay. The information-rich image data collected during an HCI assay allows access to measures of spatial, quantitative, temporal and morphological markers of target engagement, often requiring monolayers of adherent cells seeded to assay plates. The SemaCyte microcarrier system developed by Semarion enables powerful new strategies to manage cell monolayers in drug discovery workflows. In collaboration with Semarion, o2h Discovery have updated an established HCI approach to DNA damage to explore SemaCyte-enabled enhancements in cellular assays; miniaturisation of seeding, assay-ready adherent cells, multiplexed cell lines and subpopulation barcoding are investigated here.

Objectives

Perform a first-pass test using the SemaCyte system to obtain and analyse cellular HCI data, making use of cell multiplexing capabilities and same-day set up of assays from frozen cell SemaCyte stocks

Assay details

- **96-well plate, 2hr, 11-point etoposide (EP) DRC**
- Cells presented as **pooled SemaCytes** in each well
- Using **frozen SemaCytes** on same day as recovery
- Fixed endpoint - probes for **DNA damage markers**
- **3 multiplexed cell lines per well** – U2OS, A549, U87MG
- 4 SemaCyte **barcodes used for deconvolution** by HCI
- **Plate reference control:** U2OS cell line re-used for 4th barcode providing fixed 50µM set, spiked to every well

Key findings & next steps

Multiplexing - Early analyses with this data set yield sufficient information to characterise and differentiate variability in DNA damage dose responses between cell lines grown in parallel using SemaCytes

Thawed monolayers – recovery of frozen assay-ready monolayers worked well (>= 75% viable) and offers a new flexibility to scheduling and maintaining cells for deployment to assays

Data handling - The ability to deconvolute mixed populations using image processing to sort SemaCytes by barcodes proved accessible and opens further options for how to organize and control HCI assays

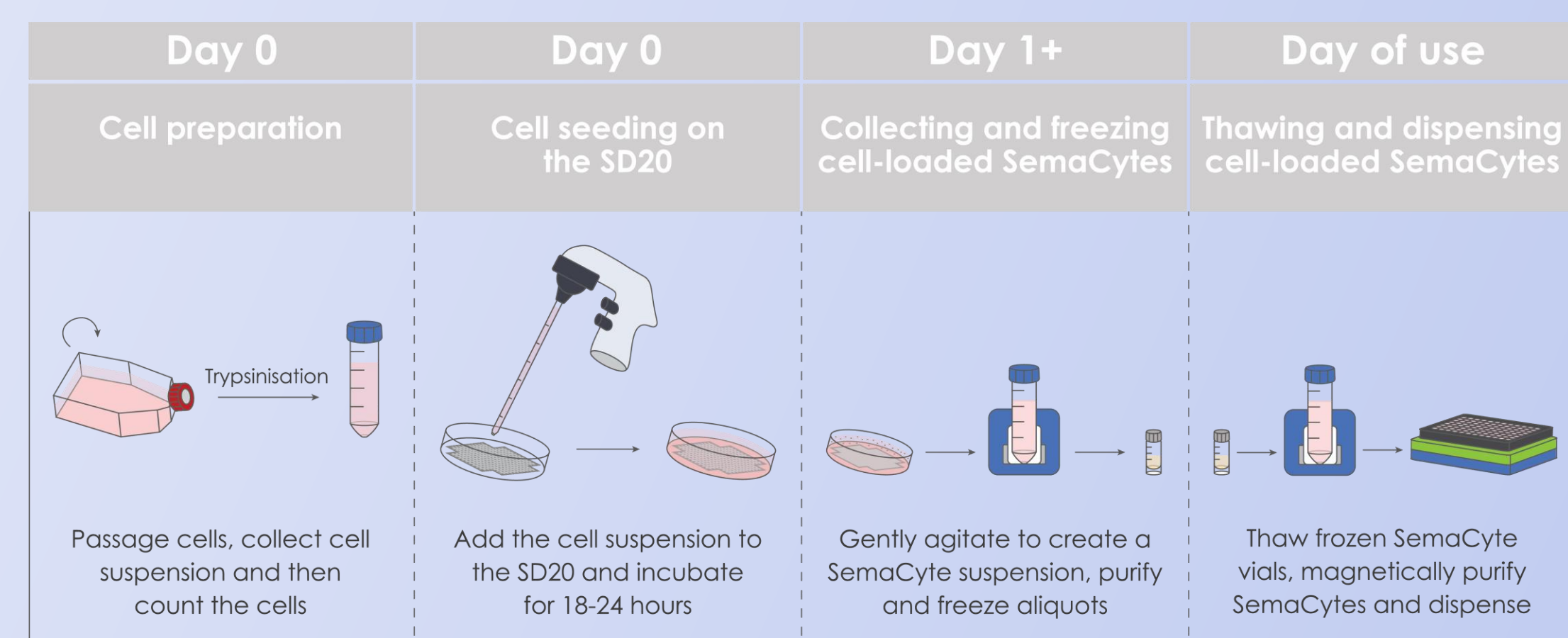
Next steps – A deeper dive into the current data set is planned, including exploration of RAD51 and implementation of dynamic image acquisition strategies such as the CellInsight EurekaScan

Acknowledgements

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Workflow

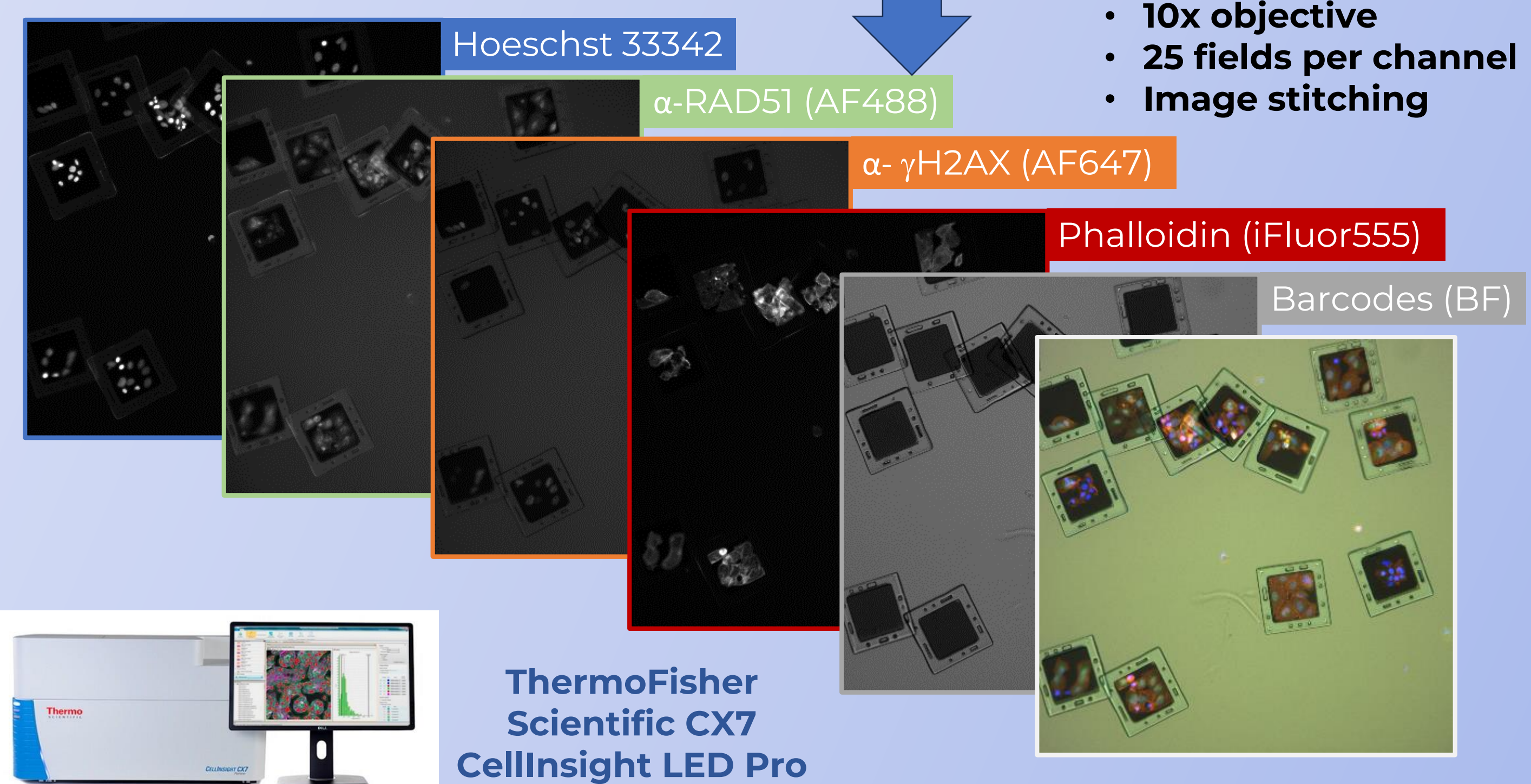
1. Cell handling



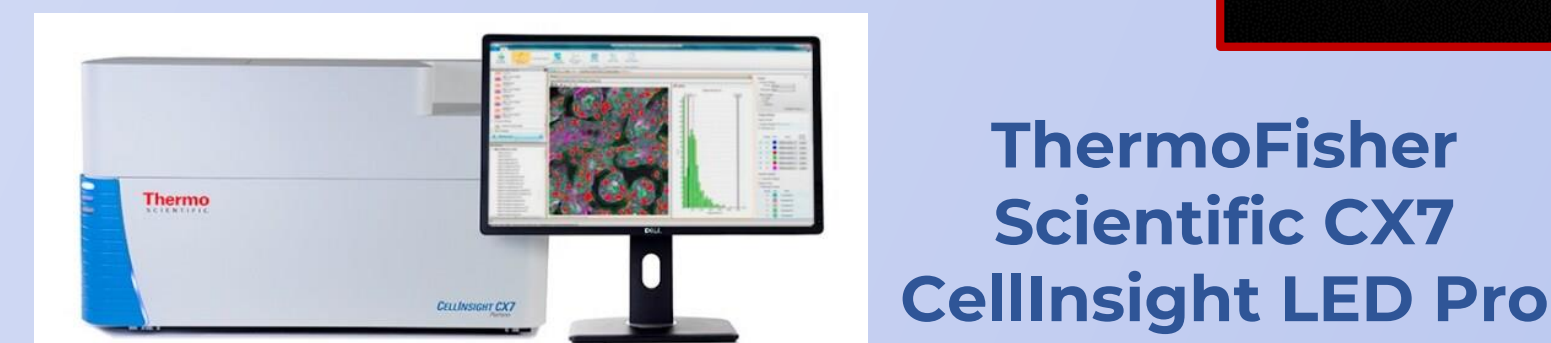
- **3 cell lines:**
 - U2OS
 - U87MG
 - A549
- Each assigned **SemaCyte barcodes**
- **Revived following frozen storage**
- **Pooled for assay**
- **Drugged 2hr with EP DRC same day**
- **Fixed and stained for subsequent HCI**



2. High-content imaging

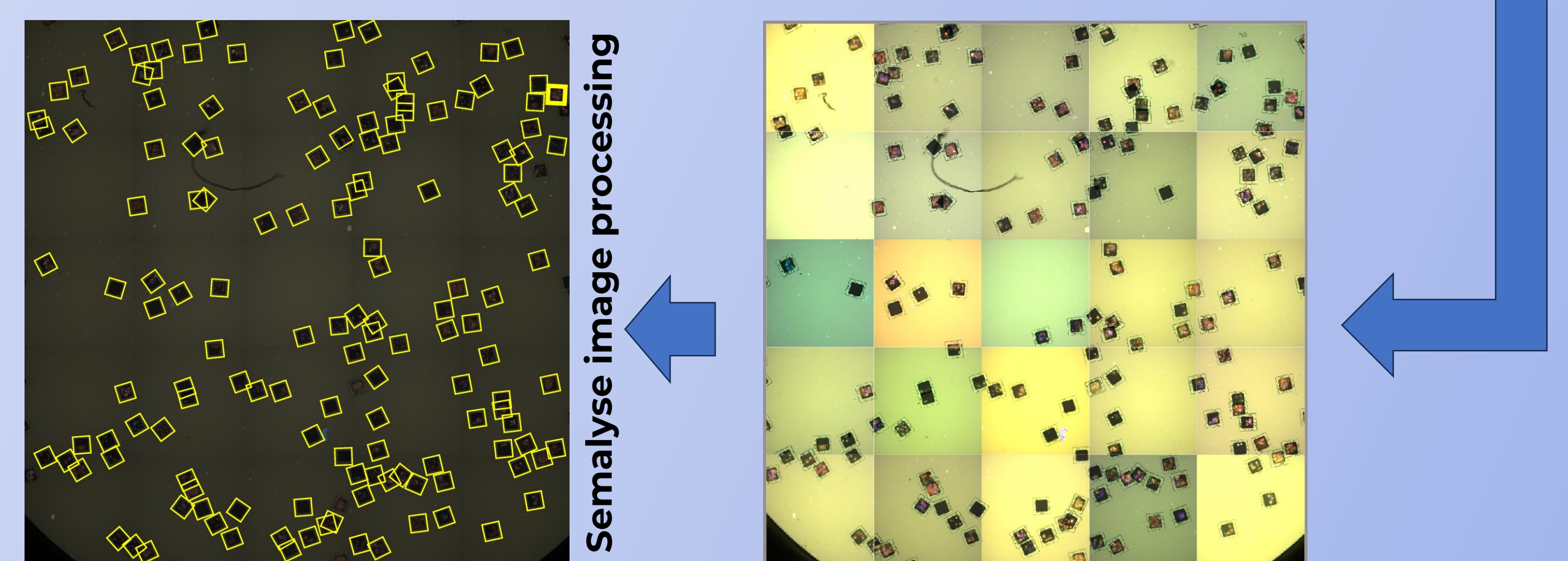


- **10x objective**
- **25 fields per channel**
- **Image stitching**



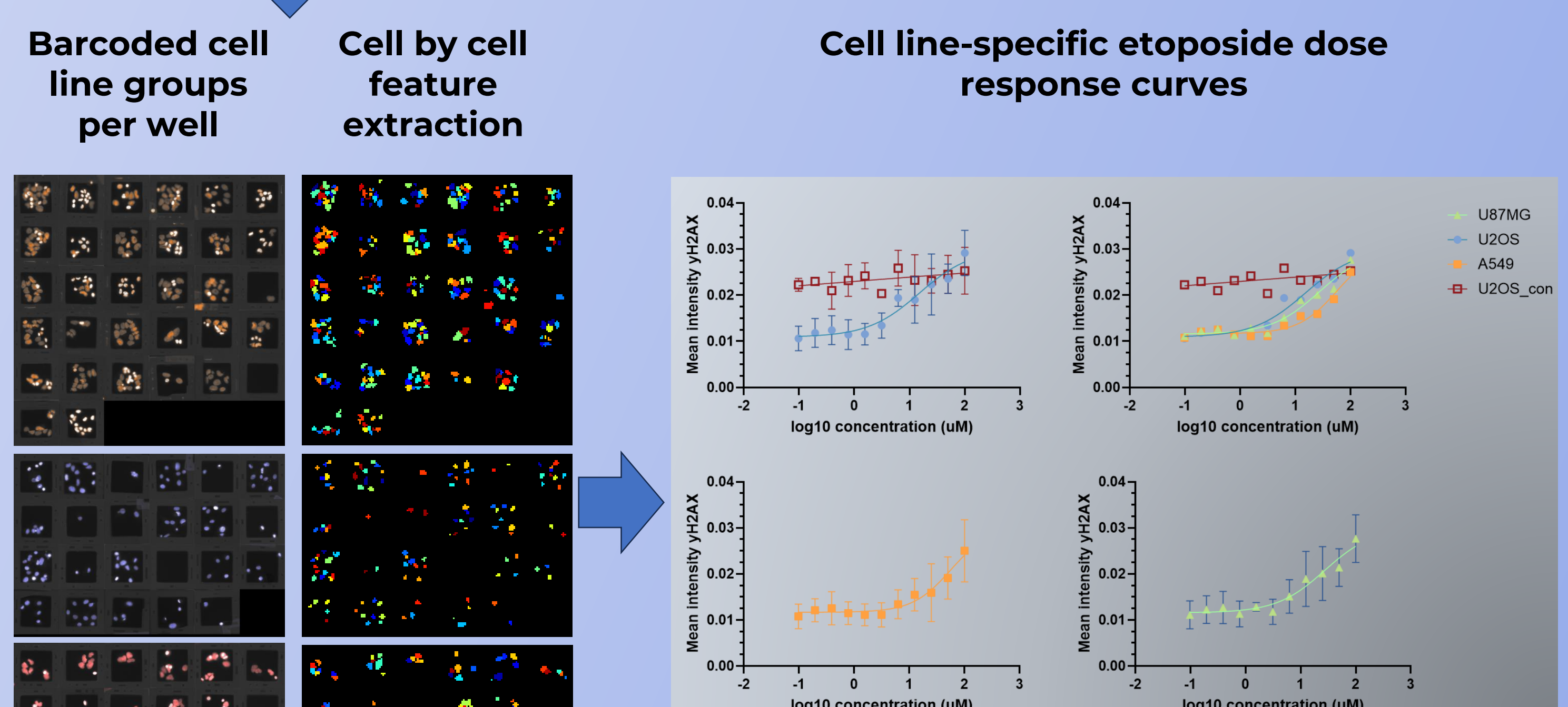
3. Image processing I – deconvolution

QC and extraction of SemaCytes according to cell line-specific barcodes



4. Image processing II – quantitation

Cell Profiler to detect and characterise cells within SemaCyte-defined image masks



- **First-pass analysis focused on γ H2AX marker of DNA damage**
- **Data resolves rank order cell line sensitivities to EP**
- **Separately barcoded technical control U2OS 50µM EP population correctly intersect main DRC**