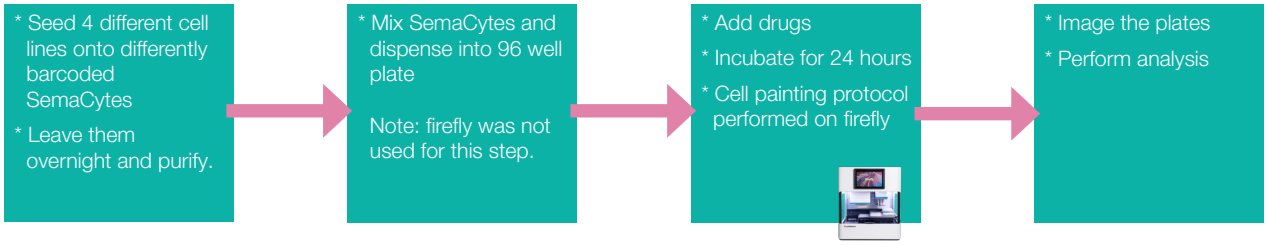
Automating and Multiplexing Cell Painting Assays

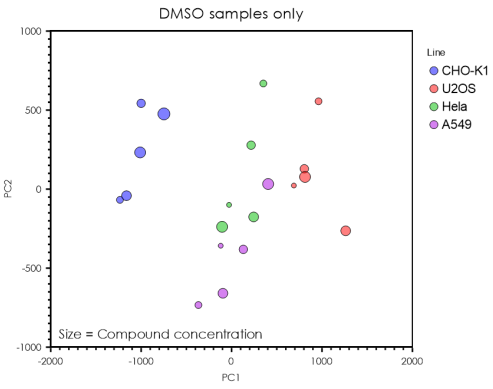
- Cell Painting is a high-content imaging assay for producing unbiased morphological profiling of cells, commonly used for mechanism-of-action (MoA) inference, off-target identification, and phenotypic screening in early drug discovery.
- Current limitation is that it is rarely applied to cell panels, limiting biological resolution and translational relevance. High well-to-well and plate-to-plate variability hinders combining data from different cell lines or screens.
- Also multiple liquid handling and staining steps in workflow can lead to inter and itra experiment variation. The use of automation can increase consistency of staining across experiments whilst supporting scalability. Whereas, use semaCyte platform enables cell multiplexing, allowing multiple cell lines (a panel) to be screened within each well. Making it possible to generate pan–cell line morphological fingerprints from a single screen.

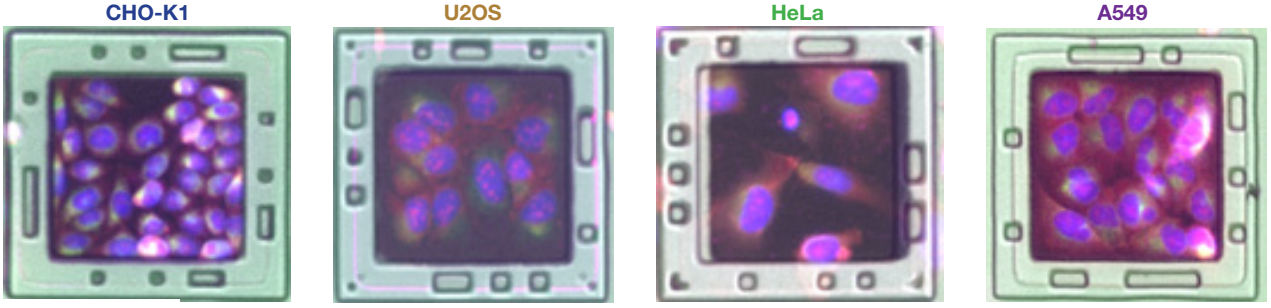
Automating and Multiplexing Cell Painting Assays - Workflow Overview

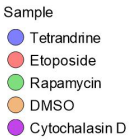


Cell Painting Protocol on firefly

1. Supplement 75ul PhenoVue 641 mitochondrial stain per well, for a working concentration of 500nM
2. Incubate for 30 minutes at 37°C
3. Add 50ul of 16% PFA per well, incubate at room temperature, in the dark for 20 minutes
4. Place plate on SP, remove 100ul of working volume per well
5. Add 100ul HBSS, incubate for 1 minute, remove 100ul working volume per well.
6. Repeat step seven for a total of four times
   - Here the original protocol adds 0.1% Triton x-100, incubates at RT for 10-20 minutes in the dark, repeat HBSS wash.
7. Remove 50ul of working volume per well
8. Add 50ul of Staining Mix as prepared in PhenoVue protocol, incubate at RT for 30 minutes in the dark.
9. Repeat step seven for a total of four times. Leave a working volume of ~70-100ul per plate to ensure cells remain in a carrier.







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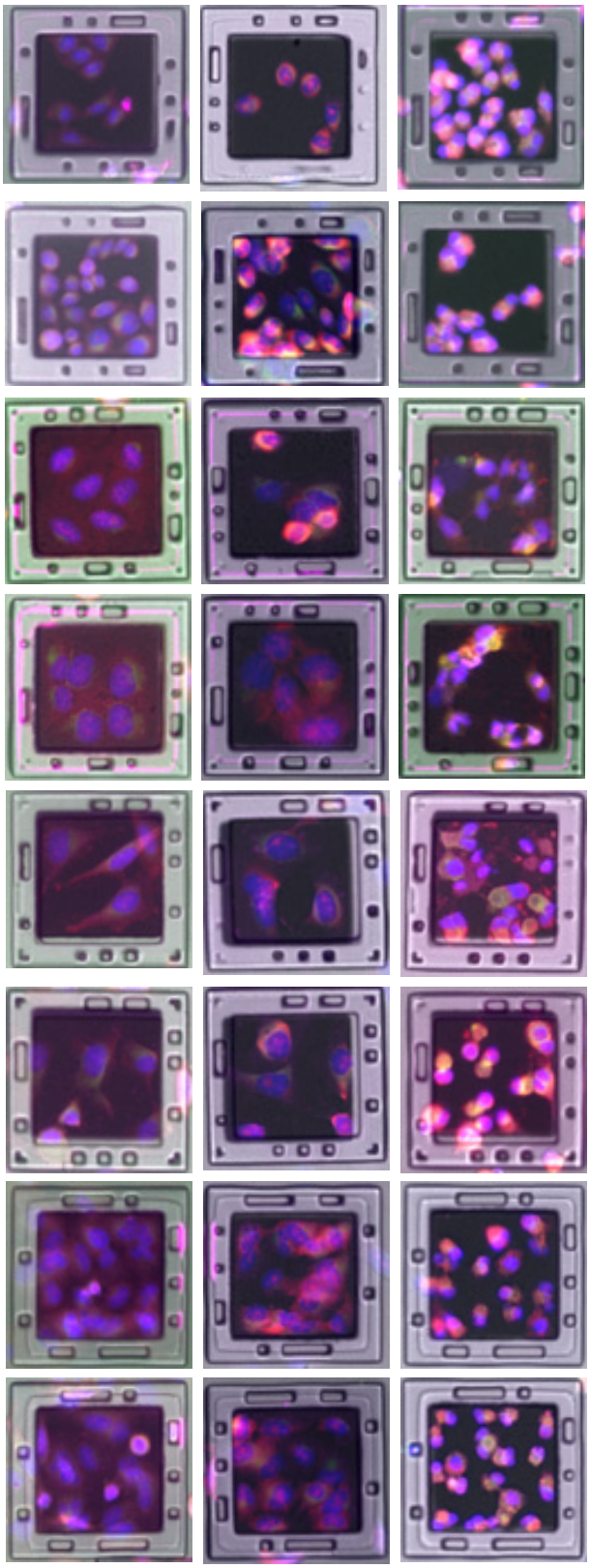

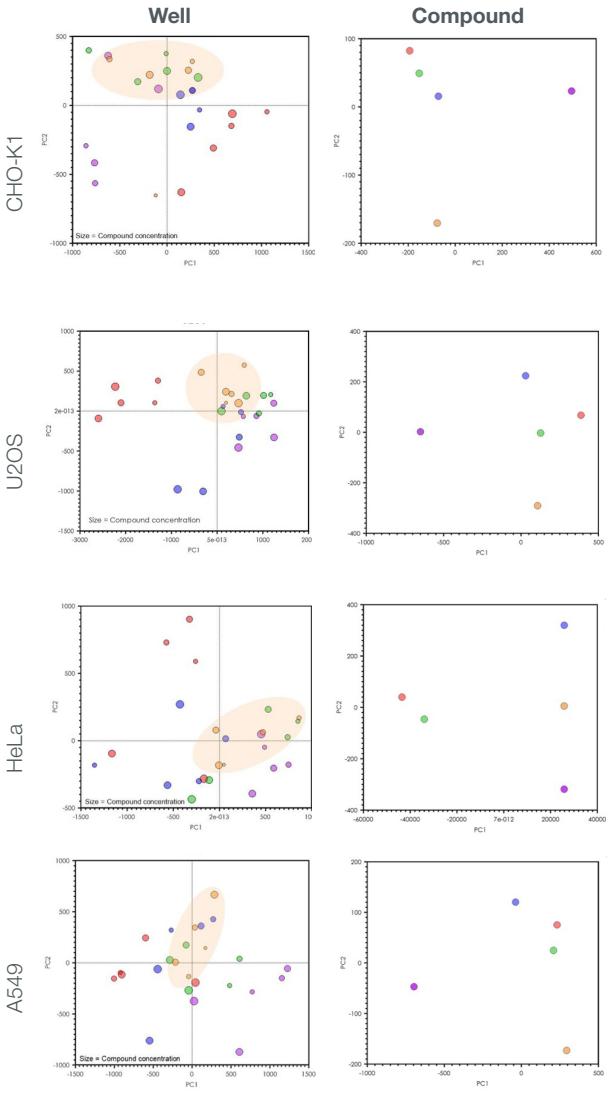

*Etoposide increases red staining in nucleus and Cytochalasin D causes cell rounding

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