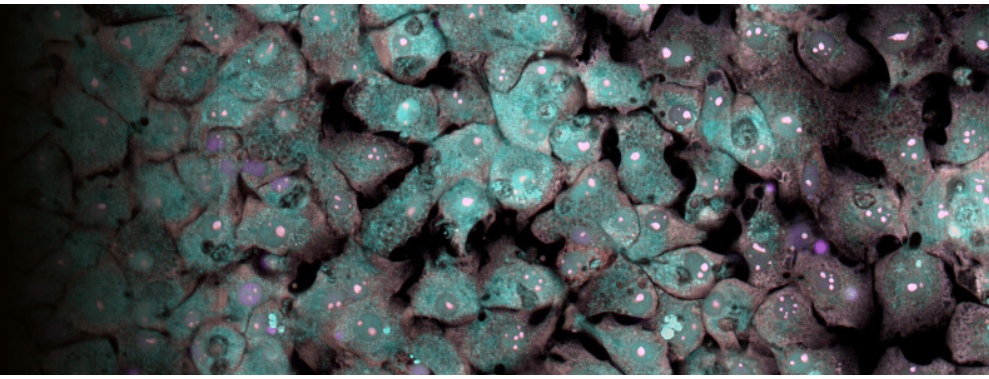
PHENOVISTA

APPLICATION NOTE

Cell Painting



WHAT IS CELL PAINTING?

Cell painting is a scalable, fluorescence imaging-based method that provides detailed, unbiased, comprehensive information about therapeutic candidates without *a priori* knowledge of their impact on cells. Cell painting is used in many drug-development campaigns and can drive forward your research through various applications, including mechanism of action (MOA) determination, toxicity profiling, and target identification.

Its power lies in its ability to generate very large, high-content datasets that contain thousands of metrics. Each cell type and treatment paradigm combination yields a unique, phenotypic signature, or fingerprint, that is compared to a library of fingerprints from reference compounds.

This means that you can compare thousands of metrics generated by your drug candidates with those of any number of reference compounds of your choice to see how your test articles stack up.

Organelle or Structure	Dyes
Nucleus	Hoechst
Endoplasmic reticulum	Concanavalin A (Alexa Fluor 488)
Nucleoli, cytoplasmic RNA	SYTO 14
F-actin	Phalloidin (Alexa Fluor 568)
Golgi, plasma membrane	Wheat germ agglutinin (Alexa Fluor 555)
Mitochondria	MitoTracker Deep Red FM

Table 1. *The typical cell-painting palette of six "paints".*

Cell painting is generally conducted using common, immortalized cells, but you can also adapt it to incorporate iPSC-derived or primary cells to generate clinically predictive information. The typical palette of dyes and their targets is shown in Table 1, but dyes can be interchanged to create new palettes that better suit the experiment's goals.

An example of the image-analysis process is outlined in Figure 1, in which A549 cells were stained with the typical palette. First, structures/organelles are identified, and regions of interest (ROIs) are determined. Specific features within those ROIs are identified and quantified, and various metrics of each feature are calculated. Combinations of those quantitative readouts are what comprise unique fingerprints.

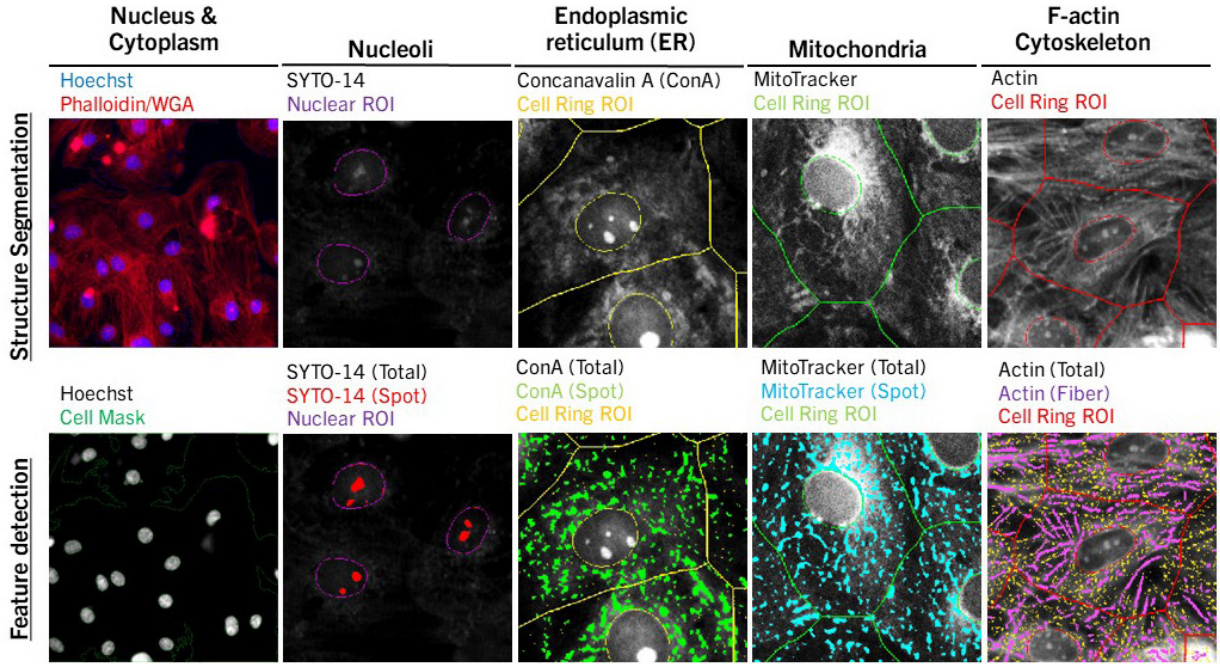


Figure 1. *Representative images of A549 cells stained with the typical, cell-painting palette undergoing image analysis.*

Identifying Structural Toxicants Using Cell Painting in Human, iPSC-derived Hepatocytes

Images of iPSC-derived hepatocytes treated with a library of compounds and stained with the typical, cell-painting palette.

Figures 3 and 4 depict the Uniform Manifold Approximation and Projection (UMAP) and dendrogram-heat map of the dataset, respectively. In the UMAP, dots that cluster closely have similar phenotypic signatures, and those that are separate have the most unique fingerprints. In the heat map, the darker the color, the more dissimilar the fingerprints are. Both representations of the data indicate that unique, phenotypic signatures are induced by bafilomycin A, cytochalasin D, and a high concentration of podophyllotoxin. Furthermore, treatment with different concentrations of the same compound can lead to different phenotypic profiles.

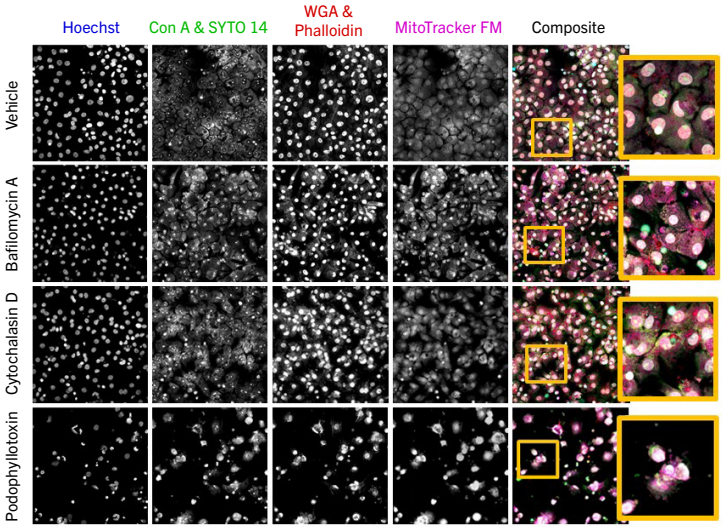


Figure 2. *Representative images of stained hepatocytes. Treatment with various compounds induced clear, phenotypic differences.*

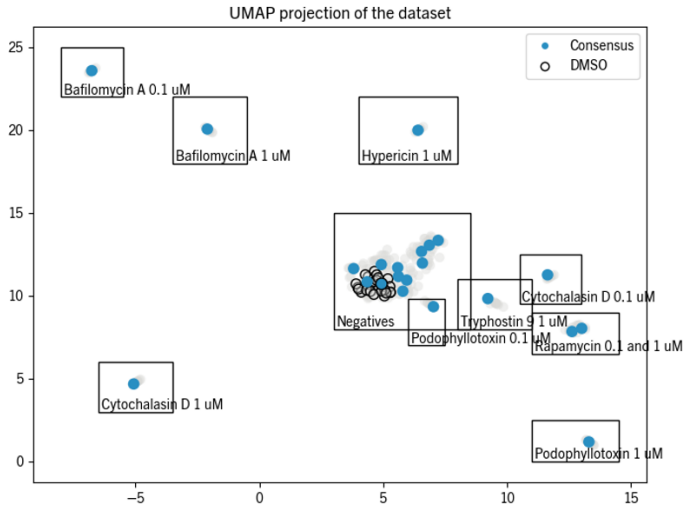


Figure 3. *UMAP of cell painting of hepatocytes indicates that unique phenotypic fingerprints are induced by treatment with bafilomycin A, cytochalasin D, and podophyllotoxin.*

This library of fingerprints can be used as references to various ends when characterizing a drug candidate. For instance, if the drug candidate has a fingerprint similar to that of cytochalasin D:

- MOA determination: The drug candidate may also act by inhibiting actin polymerization.
- Toxicity Profiling: The drug candidate is likely extremely toxic.
- Target Identification: The drug candidate may also target cytoskeletal filaments.

In this single experiment, we directly compared and simultaneously measured compounds' effects on thousands of metrics, producing a broad and comprehensive assessment of each compound on hepatocytes with unparalleled efficiency – a feat that is effectively impossible and infinitely more costly if attempted with *in vivo* models or typical immunofluorescence-based experiments.

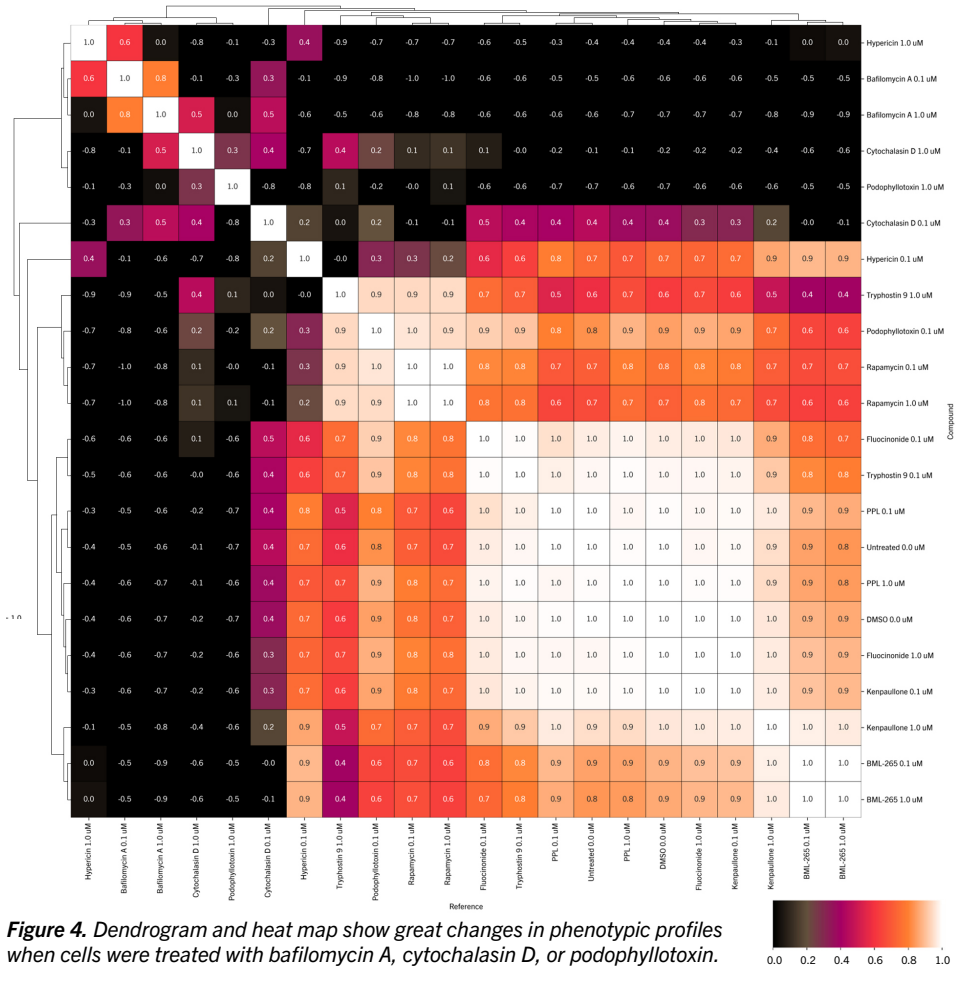


Figure 4. *Dendrogram and heat map show great changes in phenotypic profiles when cells were treated with bafilomycin A, cytochalasin D, or podophyllotoxin.*

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