AI-Powered Cell Painting in iPSC-Derived Hepatocytes for Liver Toxicity Profiling

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Introduction

Drug-induced liver injury (DILI) remains a key safety challenge. iPSC-derived hepatocytes (pixHep) provide physiologically relevant models, but conventional assays overlook subtle, heterogeneous responses. Using cell painting, we extract phenotypic features at both whole-image and single-cell levels to efficiently quantify morphological and functional diversity.

Cell Painting of iPSC-derived hepatocytes reveal heterogenous cell populations

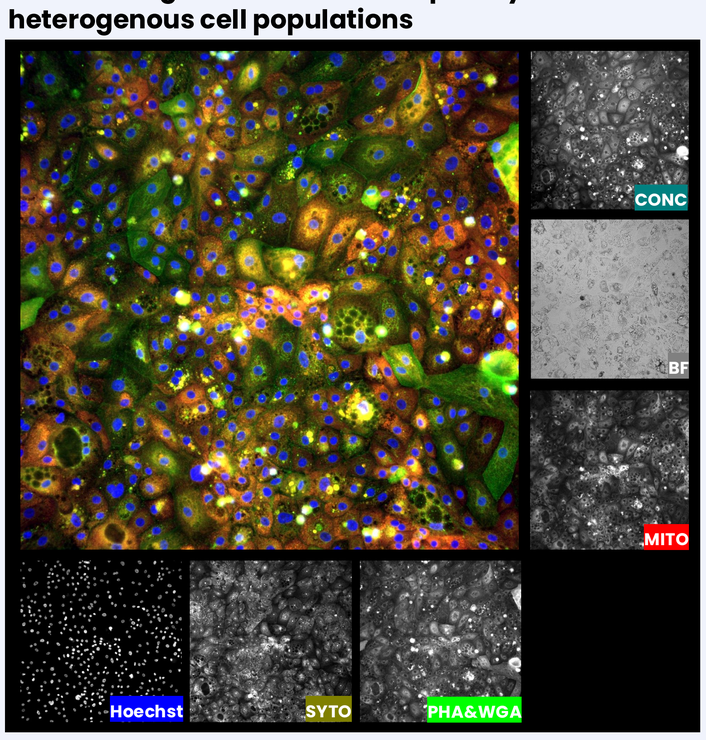

Figure 1: Cell painting of pixHeps. Merged HOECHST (blue), Phalloidin and WGA (green), and MITO (red) channels reveal extensive phenotypic diversity, including variation in cell size, vacuolation, and mitochondrial signal intensity. Grayscale panels show individual fluorescence channels and brightfield images used for downstream analysis.

Whole image feature extraction

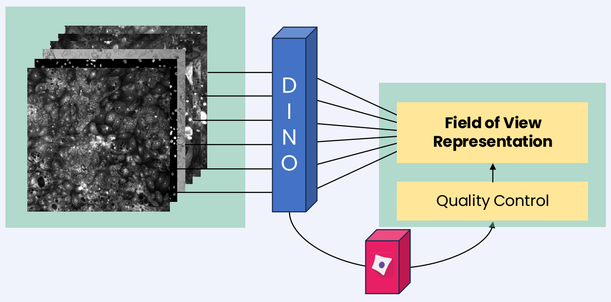

Figure 2a: DINOv3 extracts multichannel embeddings from multiple fields of views to capture overall well-level morphology and within-well heterogeneity.

Whole-image and single-cell features for toxicity modelling

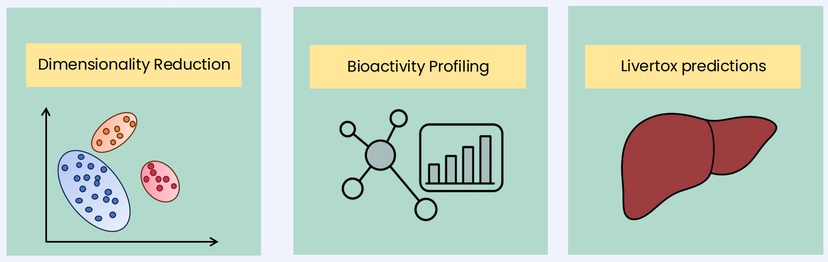

Figure 2c: Feature dimensionality reduction with PCA minimizes redundancy across image channels, producing compact representations of treatment responses. The resulting features capture deviations from negative controls and highlight sub-lethal bioactivity patterns relevant for functional Livertox prediction.

Single-Cell feature extraction

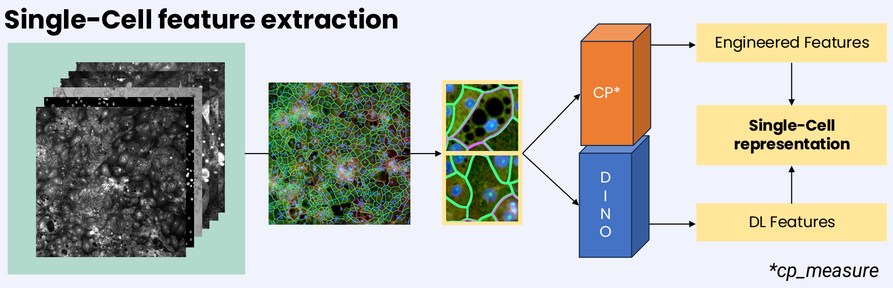

Figure 2b: pixHep images contain overlapping cells with variable size and diffuse boundaries. To achieve accurate instance segmentation, a custom Cellpose–SAM model was trained. Per-cell features were derived from the original channels and masks, combining interpretable morphological metrics with dense (per pixel) DINOv3 embeddings.

Single-Cell modeling

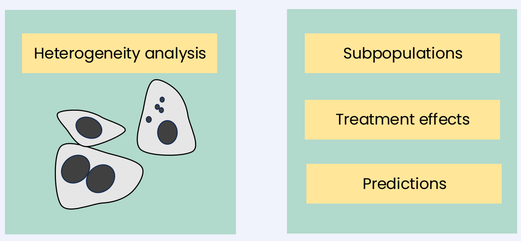

Figure 2d: Single-cell analysis captures shifts in cell subpopulations following treatment, providing a more granular view of phenotypic responses that supports improved prediction and mechanistic interpretation.

Data generation

pixHeps were treated with 24 DILI-related compounds spanning a range of hepatotoxic risk (11 high, 5 low, 1 none, and 7 ambiguous concern). Treatments were applied across eight concentrations (0–250 µM). After 48 hours, cell viability (ATP content) and Cell Painting assays were performed.

Morphological landscape of DILI Compounds

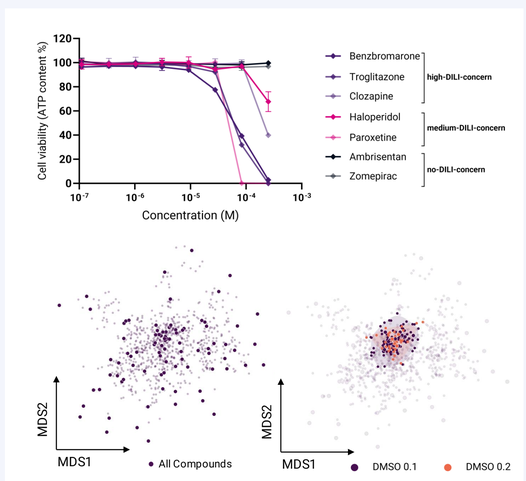

Figure 3a: Top: Cell viability dose–response curves show that most DILI-related compounds reduce ATP content only at the highest concentrations. Bottom: Morphological profiling across all compounds reveals distinct phenotypic landscapes; DMSO controls (purple/orange) cluster tightly within this space. Using well-level morphological features, compound identity was predicted with 69% balanced accuracy (leave-one-out analysis averaged across all pairs).

Phenomics capture subtle sublethal morphological changes

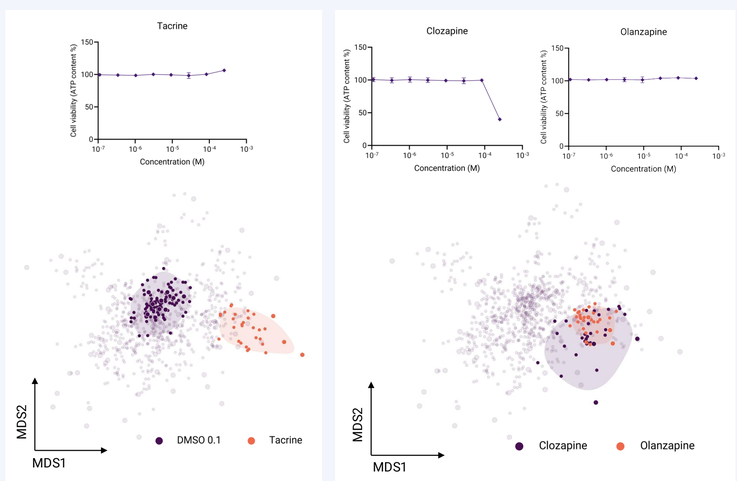

Figure 3b: Left: The DILI compound Tacrine shows minimal change in cell viability but forms a distinct cluster in morphological feature space, indicating sub-lethal phenotypic effects. Right: Clozapine (purple) and Olanzapine (orange), two structurally similar antipsychotics with differing DILI risk, occupy overlapping morphological regions. Nevertheless, well-level morphological features distinguished the two treatments with 65% balanced accuracy at sub-lethal doses.

Single-Cell morphological landscape reveal distinct cellular morphologies

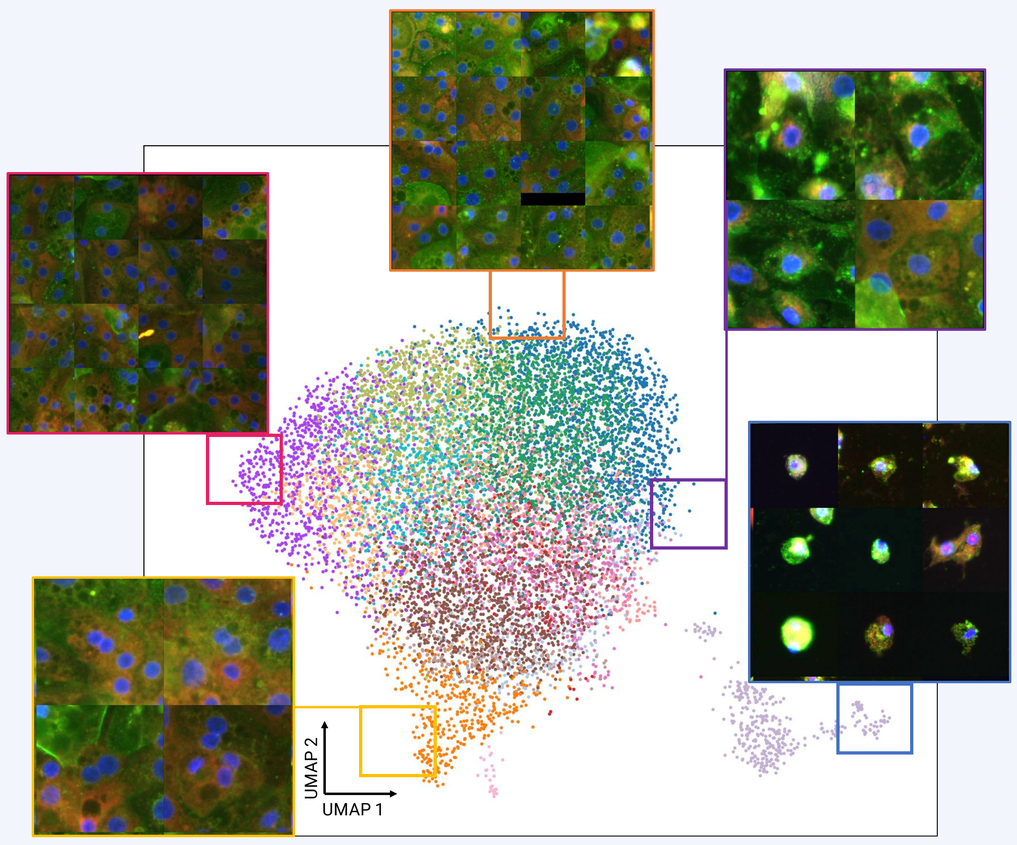

Figure 3c: Single-cell analysis of pixHeps reveals several distinct morphological axes among both healthy and dying cells, reflecting inherent phenotypic heterogeneity within the population and between treatments.

Conclusions

- pixHeps showed greater morphological complexity than conventional liver lines, and DINOv3 captured image-level variance without segmentation.
- Single-cell analysis using a custom Cellpose–SAM model was effective, with aggregated features matching DINOv3 in detecting treatment responses.
- Both methods revealed subtle, consistent phenotypic changes unseen by eye or viability assays, supporting Cell Painting's sensitivity and potential for DILI prediction.
- Expanding the compound set with more perturbations will enable clinical DILI prediction and mechanistic insight into hepatotoxic responses.

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