

Introduction

- Drug-induced nephrotoxicity remains a leading cause of clinical trial failures and post-market withdrawals, with renal proximal tubule epithelial cells (RPTECs) being particularly vulnerable due to their role in drug secretion and reabsorption.
- Cell painting, a multiplex fluorescence microscopy technique, captures hundreds of cellular features (including morphology, organelle distribution, and cytoskeletal architecture) to generate "phenotypic fingerprints" of drug-induced effects.
- Here, we employed the cell painting assay to evaluate drug-induced nephrotoxicity in human RPTECs, and integrated cell viability assays in renal tubuloids to develop predictive nephrotoxicity models, enabling unbiased toxicity profiling beyond conventional viability assays.

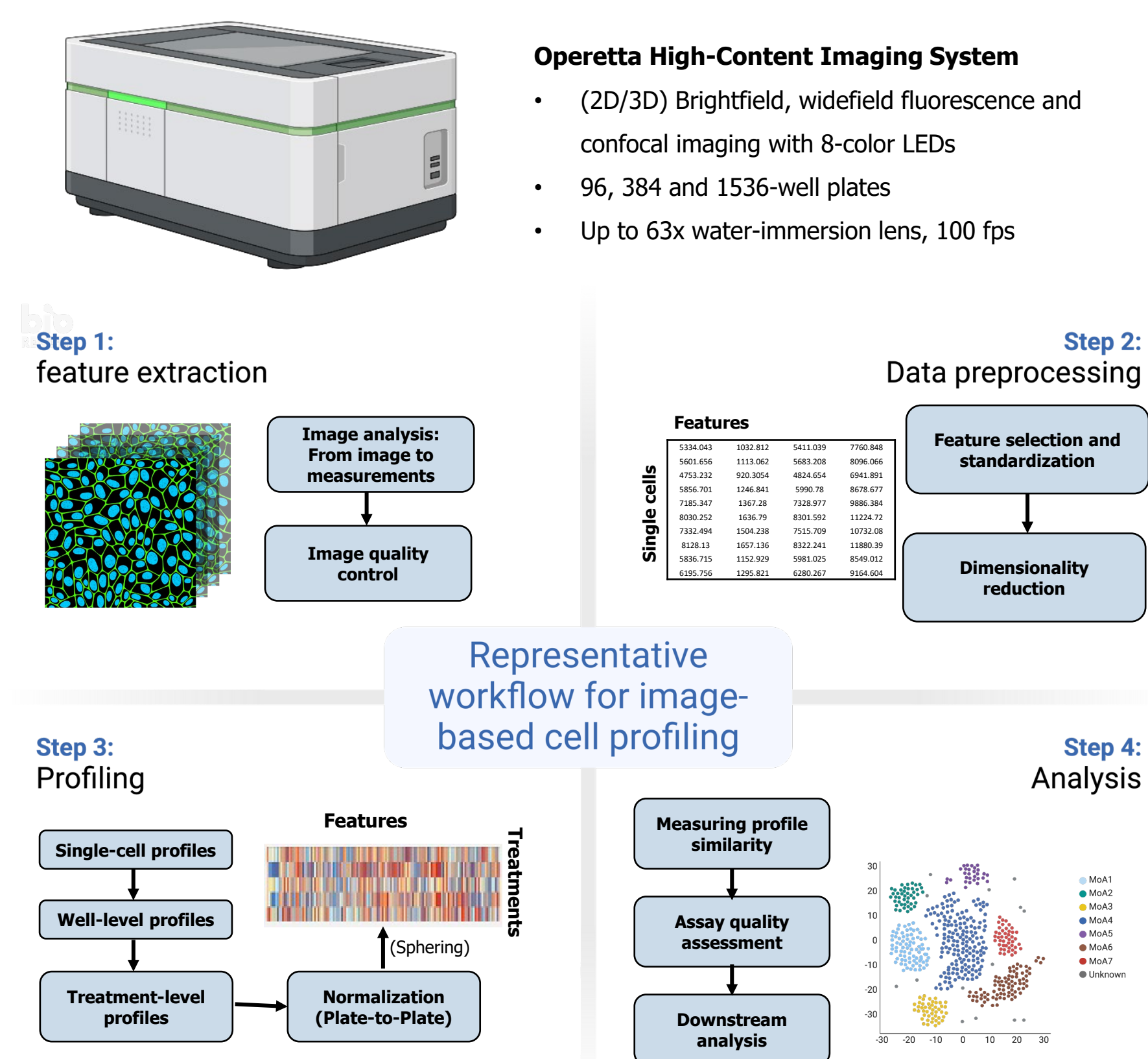
Methods

- Cell Painting Assay:** Human RPTEC cells were treated with compounds for 48 hours. Then cells were stained with a panel of 6 fluorescent dyes to target distinct cellular compartments: nuclei (Hoechst 33342), plasma membrane + Golgi apparatus (PhenoVue Fluor 555 - WGA), mitochondria (PhenoVue 641 Mitochondrial Stain), endoplasmic reticulum (PhenoVue Fluor 488 - Concanavalin A), Nucleoli (PhenoVue 512 nucleic acid stain), and actin filaments (PhenoVue Fluor 568 - Phalloidin). Fluorescent images were acquired using a high-content imaging system (Operetta CLS, PerkinElmer) at 40x magnification.
- Tubuloids derived from human adult kidney were cultured and treated with compounds for 7 days, cell viability assays (CellTiter-Glo) were conducted, and IC₅₀ values (concentrations inducing 50% cell death) were calculated.

Conclusion

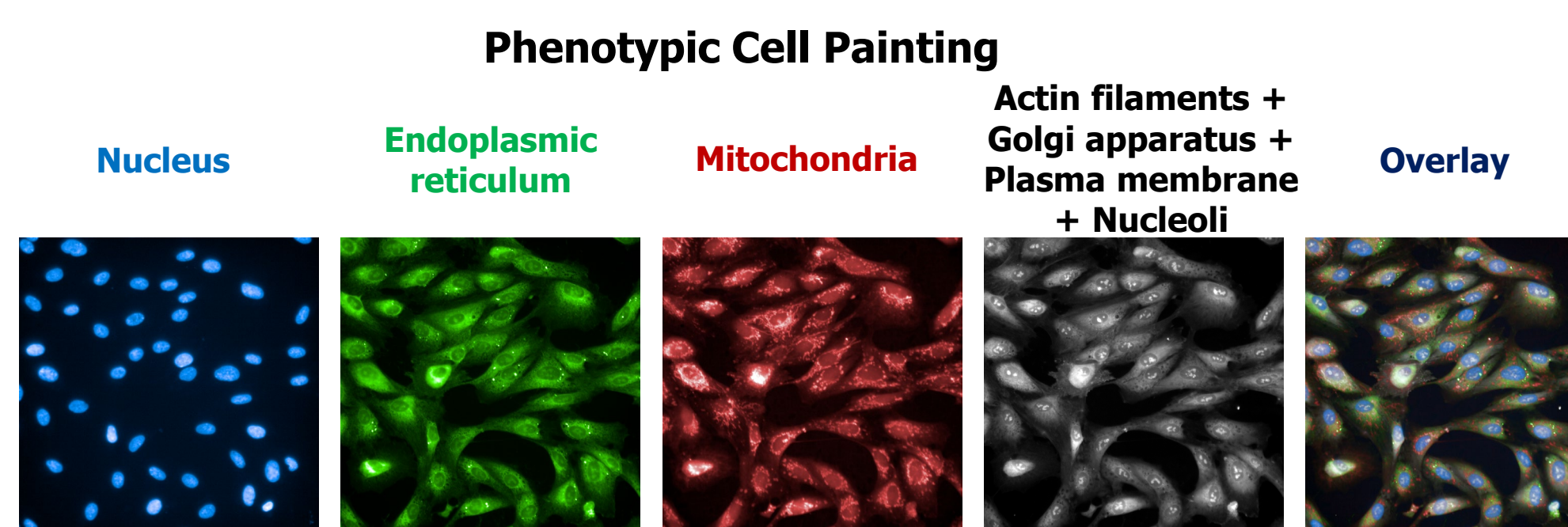
- The cell painting assay captures subtle phenotypic alterations, including altered mitochondrial intensity, endoplasmic reticulum fragmentation, and actin cytoskeleton disorganization.
- Cell painting revealed distinct phenotypic clustering between nephrotoxins and non-nephrotoxins, and aggregate phenotypic changes assessed by Euclidean distance were applied for phenotypic profiling.
- 3D human tubuloid models exhibit good sensitivity and specificity in differentiating nephrotoxic from non-nephrotoxic compounds.

1 General Workflow for Cell Painting Assay



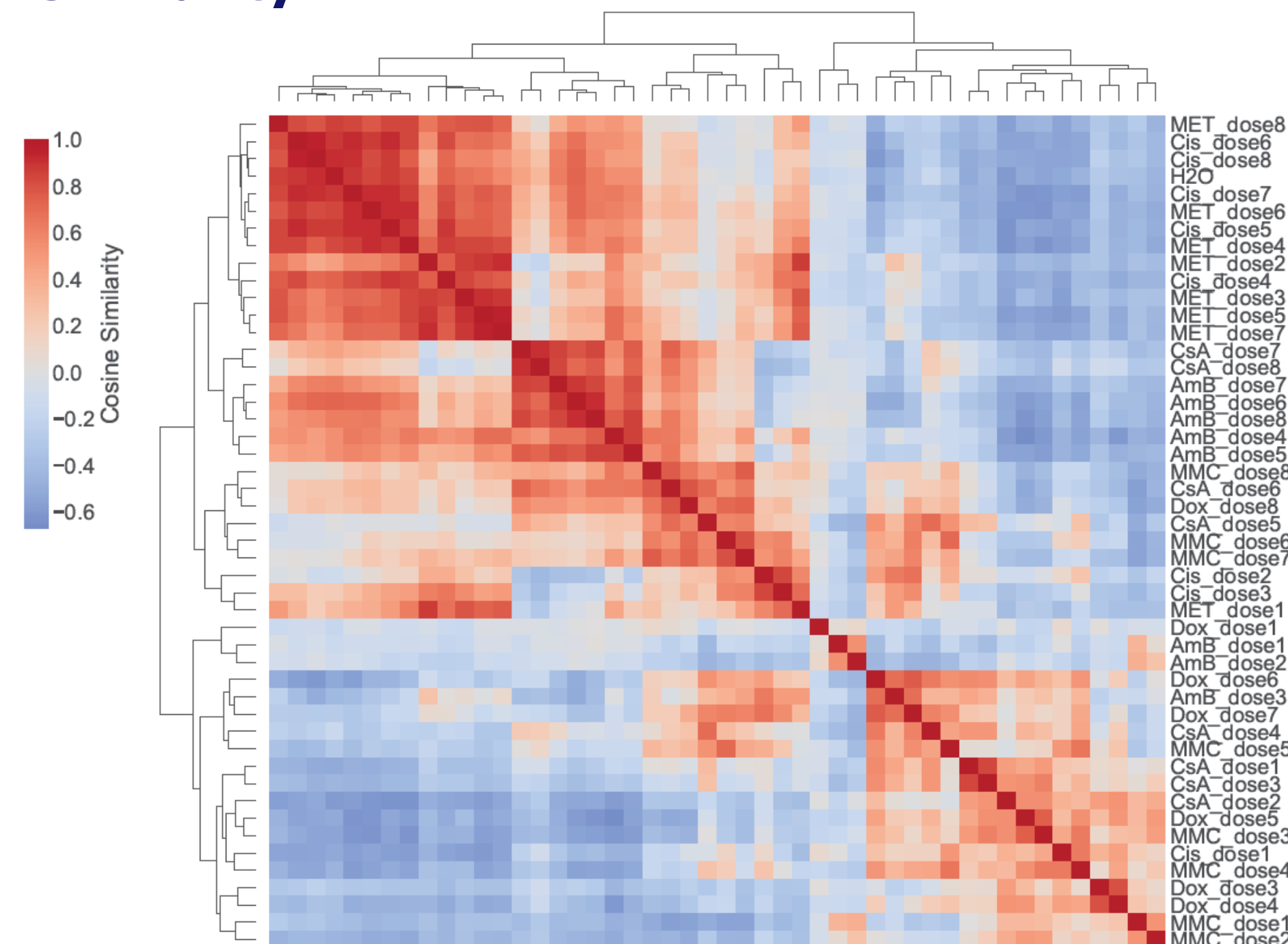
2 Cell Painting Panel on human RPTEC cells and Representative Images

Target	Dye
Mitochondria	PhenoVue 641 Mitochondrial Stain
Plasma membrane + Golgi apparatus	PhenoVue Fluor 555 - WGA
Endoplasmic Reticulum	PhenoVue Fluor 488 - Concanavalin A
Actin filaments	PhenoVue Fluor 568 - Phalloidin
Nucleus	PhenoVue Hoechst 33342 nuclear stain
Nucleoli	PhenoVue 512 nucleic acid stain



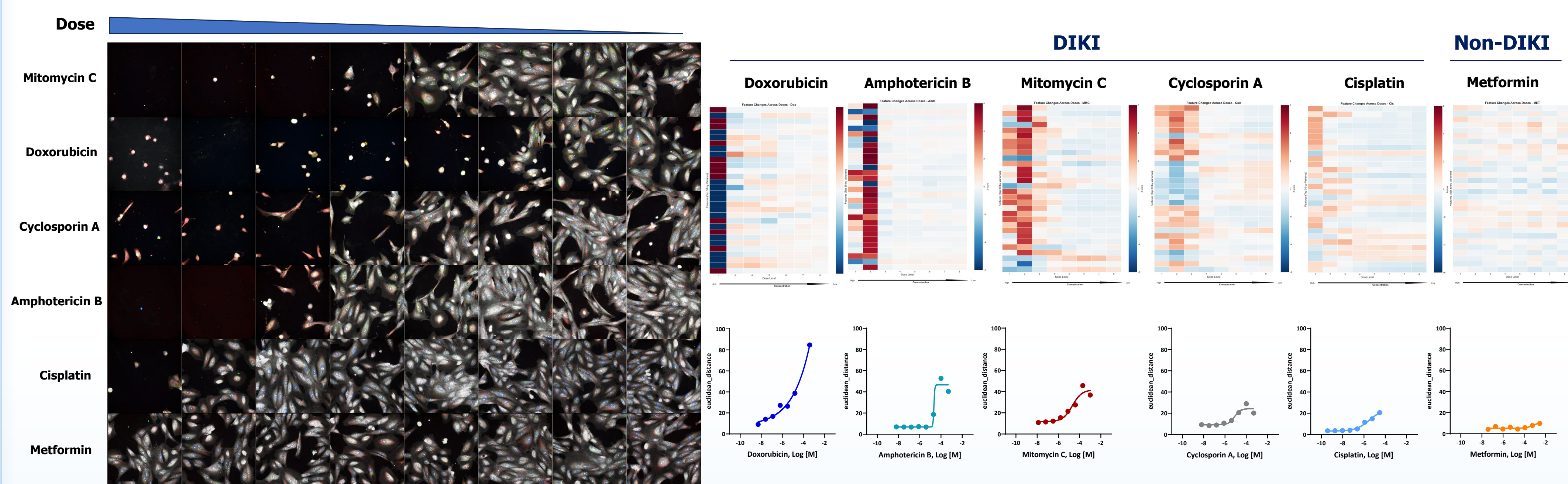
Summary of the fluorescent dyes used in the Cell Painting assay and representative images of vehicle (H₂O)-treated RPTECs. The table lists each dye, its primary cellular target, and the channel acquired on the Operetta CLS high-content imaging system. The panel of images illustrates the distinct subcellular features captured under basal conditions, corresponding to the fluorescent channels used for phenotypic profiling.

4 Clustering of Compounds by Phenotypic Profile Similarity



Hierarchical clustering heatmap of compounds based on similar cellular responses in human RPTEC. Pairwise similarity between the phenotypic profile of each compound-dose combination was computed using cosine similarity, as represented by the color scale. The dendrogram illustrates the clustering of conditions with analogous multiparametric phenotypic responses.

3 Dose-Dependent Morphological Feature Changes of Six Compounds in RPTEC Cells



Dose-response profiling of six compounds using the cell painting assay.

Left panel. Composite fluorescence images of RPTECs treated with escalating doses of 6 compounds for 48 hours. Images are overlaid multi-channel views from the Cell Painting assay, visualizing phenotypic changes across a toxicity spectrum from potent nephrotoxins.

Right panel. Heatmap of the top 30 most variable phenotypic features across compound concentrations (arranged from high to low, left to right). Values are row-normalized to visualize relative change. Corresponding dose-response curves, showing aggregate phenotypic change (Euclidean distance) relative to vehicle control for each compound. DIKI: Drug-induced kidney injury.

5 The Human Tubuloid Assay can Predict DIKI Risk



Compound	Category	Literature		Experimental	
		C _{max} μM*	IC ₅₀ μM	IC ₅₀ μM	IC ₅₀ /C _{max}
Mitomycin C	DIKI	1.77	0.016	0.009	
Carboplatin	DIKI	67.3	15.7	0.2	
Oxaliplatin	DIKI	4.37	3.6	0.8	
Cisplatin	DIKI	7.47	2.4	0.3	
Rifampicin	DIKI	12.2	14.8	1.2	
Pentamidine	DIKI	0.9	3.0	3.3	
Zoledronic acid	DIKI	0.97	8.6	8.8	
Vancomycin	DIKI	38	150	3.9	
Tenofovir	DIKI	0.93	>125	>100	
Gentamicin	DIKI	15.7	2045.5	130.3	
Omeprazole	DIKI	3.18	83.9	26.4	
Tetracycline	DIKI	3.67	46.8	12.7	
Doxorubicin	DIKI	0.04	0.004	0.1	
Cyclosporin A	DIKI	0.21	4.0	18.8	
Amphotericin B	DIKI	1.52	4.8	3.1	
Ifosfamide	DIKI	199	5143.0	25.8	
Adefovir	DIKI	0.07	>250	>100	
Tacrolimus	DIKI	0.03	20.2	673.2	
Phenacetin	Non-DIKI	38.4	1290.5	33.6	
Ciprofloxacin	Non-DIKI	12.1	>200	>100	
Lisinopril	Non-DIKI	0.09	>1000	>100	
Isoproterenol HCl	Non-DIKI	0.95	245.5	258.4	
Mannitol	Non-DIKI	43.1	>1000	>100	
Metformin	Non-DIKI	12.4	>3000	>100	
Thiamine	Non-DIKI	6.8	>2000	>100	
Dexamethasone	Non-DIKI	0.68	>1000	>100	
Atropine	Non-DIKI	0.09	375.4	4171.4	
Pindolol	Non-DIKI	0.2	>1000	>100	

*C_{max} values are from Schulz, M. et al., Crit Care.(2012)16: R136

Sensitivity: 77.8%
Specificity: 90.0%

Left panel. Tubuloids derived from human adult kidney formed tubular structures and expressed key epithelial markers, including Ki-67, ZO-1, and PAX8.

Right panel. Cell viability of human tubuloids following 7-day compound exposure was determined using the CellTiter-Glo assay. Compounds with an IC₅₀/C_{max} ratio < 100 were considered DIKI-positive.