Leveraging the Power of High Content Screening with StratoMineR™ for RAPIDtox: Cytochroma's Ethnically-Diverse, Predictive Liver Toxicity Platform.

Rapid iterative data mining from cell painting data for hepatic toxicology:

Learn how high content analysis and iPSC technologies can be combined for the development of predictive *in vitro* liver toxicity models. Check out how Cytochroma's screening platform using StratoMineR™ can facilitate the development of reliable biomarkers of toxicity risk and give an understanding of mechanisms of action.

Introduction

Cytochroma's mission is to accelerate drug discovery by identifying safer, novel medicines faster and more efficiently, without the use of laboratory animals. Cytochroma has an advanced ethnically diverse stem cell derived liver screening platform that can significantly cut the time and cost of bringing new drugs and vaccines to market, making essential medicine development, faster, and safer, with reduced cost. Cytochroma's products and screening service are produced and tested in an automated, animal-free manner that ensures highly reproducible results.

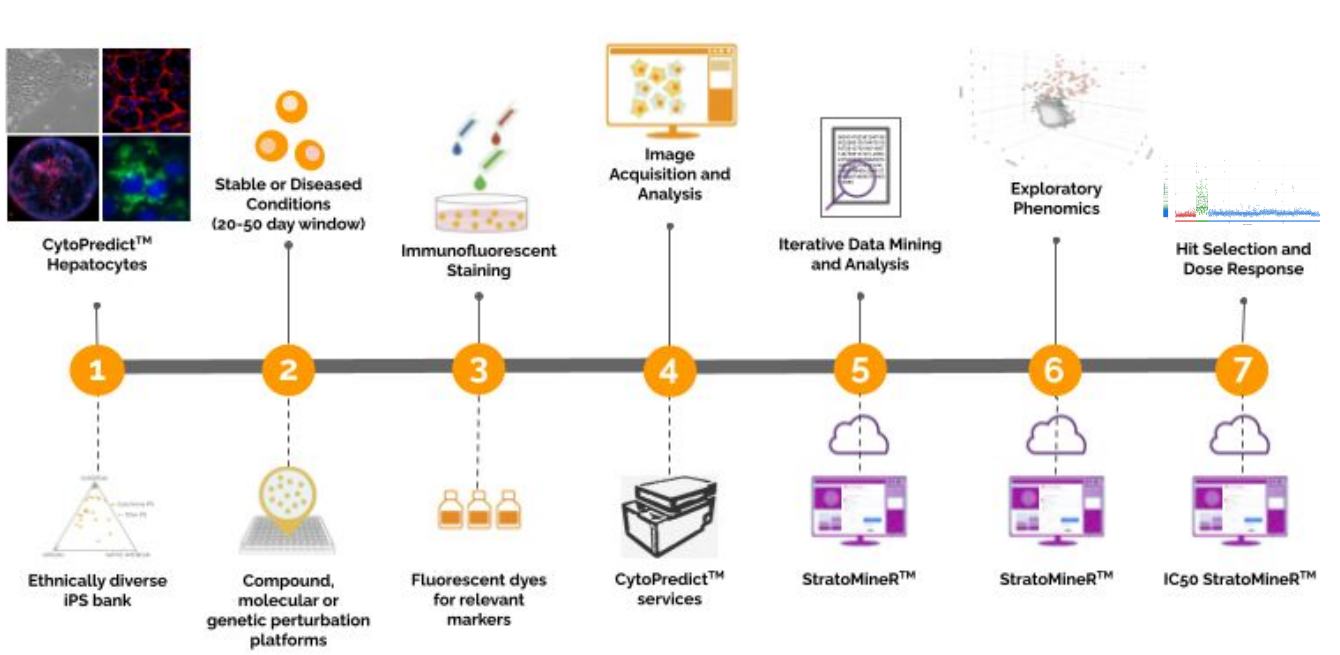


Figure 1. Overview of the predictive toxicology analytical workflow. Cytochroma identifies heterogeneous drug responses in the global population by using its proprietary cell-based ethnically diverse iPSC bank. After automated culturing and maturation of iPSC-derived liver models in multi-well plates (1), they are subjected to compound screening (2). An innovative tagging technology using relevant and specific markers enables direct visualisation (3). High throughput and high resolution automated image acquisition (4) of the liver models is carried out using Perkin Elmer's Opera Phenix system. Quantification of the images are subsequently analysed using Harmony software with advanced segmentation capabilities (5). The high content output from image analysis contains multi-parametric data and is then further analysed using StratoMineR™ (6) developed by Core Life Analytics. This software platform allows for rapid high content data mining and analytics that Cytochroma uses to discover unique phenotypic signatures as well as novel hepatotoxic hits (7). StratoMineR™ also allows for the use of machine learning to handle growing multi-parametric data for upscaling of the high content screening (HCS) platform. This will allow us to actively engage in a data-driven iterative loop to rapidly find novel hepatotoxic compounds, especially as Cytochroma scales up.

Cytochroma's monolayer liver models are differentiated, mature and functional, making them ideal for toxicity testing.

To date, more than 160 compounds have been tested in quadruplicates at multiple concentrations ranging from 0.75 µM to 200 µM. 48 hours after drug induction, an established Cell Paint method is used for direct visualisation using fluorescent tags. Hoechst 33342, Syto14 (CellMask), Phalloidin/ AlexaFluor and Mitotracker Deep Red are used.

Images were acquired using the Perkin Elmer High Content Opera Phenix Imaging System, and analysed by Harmony software v4.9, evaluating > 350 features based on intensity, texture, size, and morphology.

The output of the Image quantification is uploaded into StratoMineR™ for HC analysis (see Workflow)

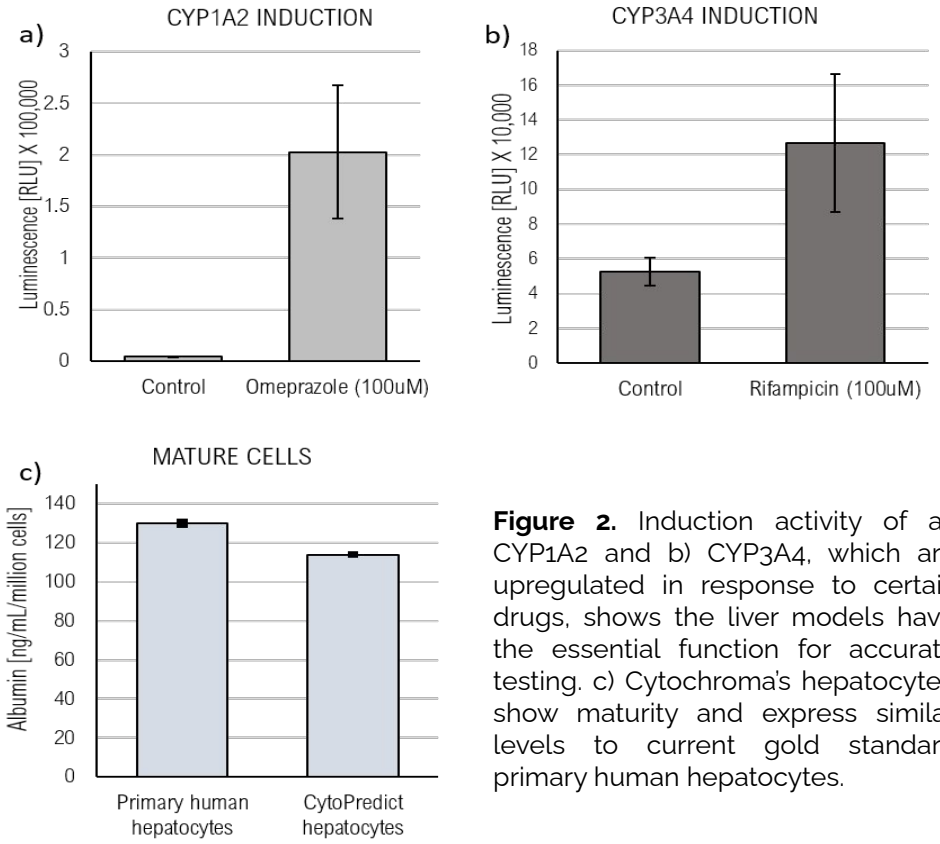


Figure 2. Induction activity of a) CYP1A2 and b) CYP3A4, which are upregulated in response to certain drugs, shows the liver models have the essential function for accurate testing. c) Cytochroma's hepatocytes show maturity and express similar levels to current gold standard primary human hepatocytes.

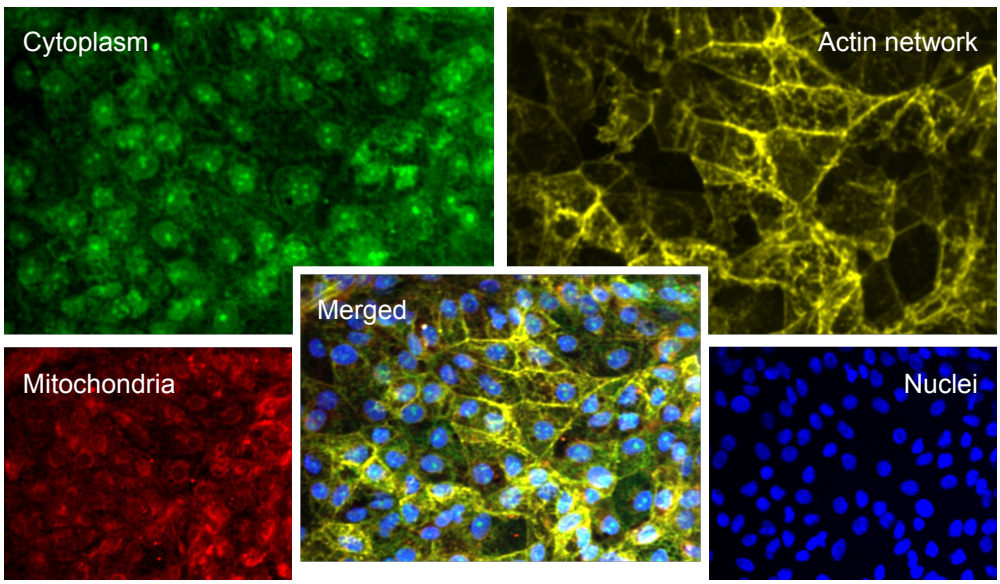


Figure 3. Images on the right are from control wells during Cell Paint toxicity assay. Images were acquired using the Opera Phenix Imaging System. Example of each channel is shown, with a composite image in the middle of nuclei, cytoplasm, actin network and mitochondria.

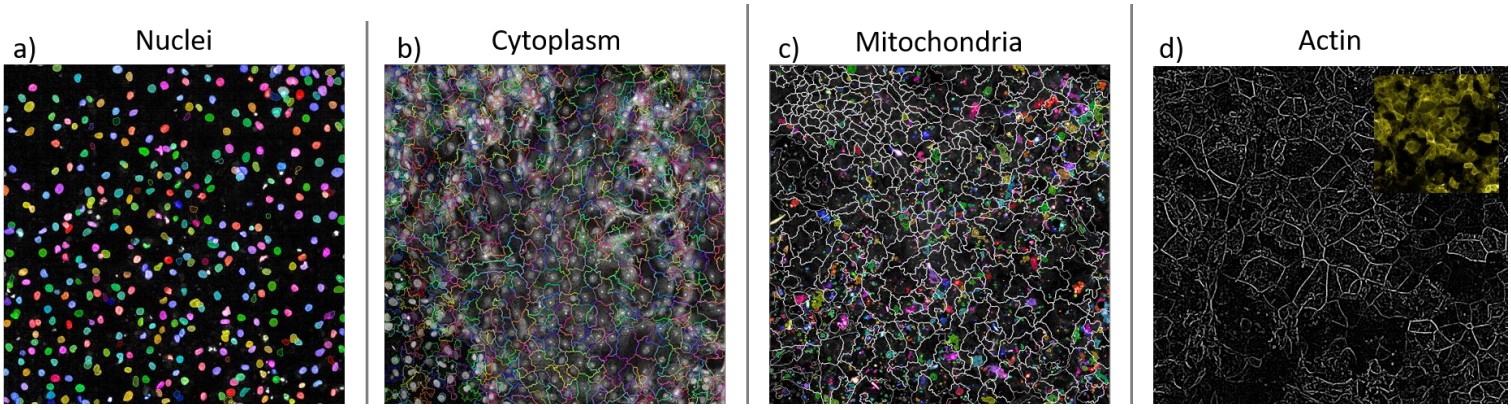
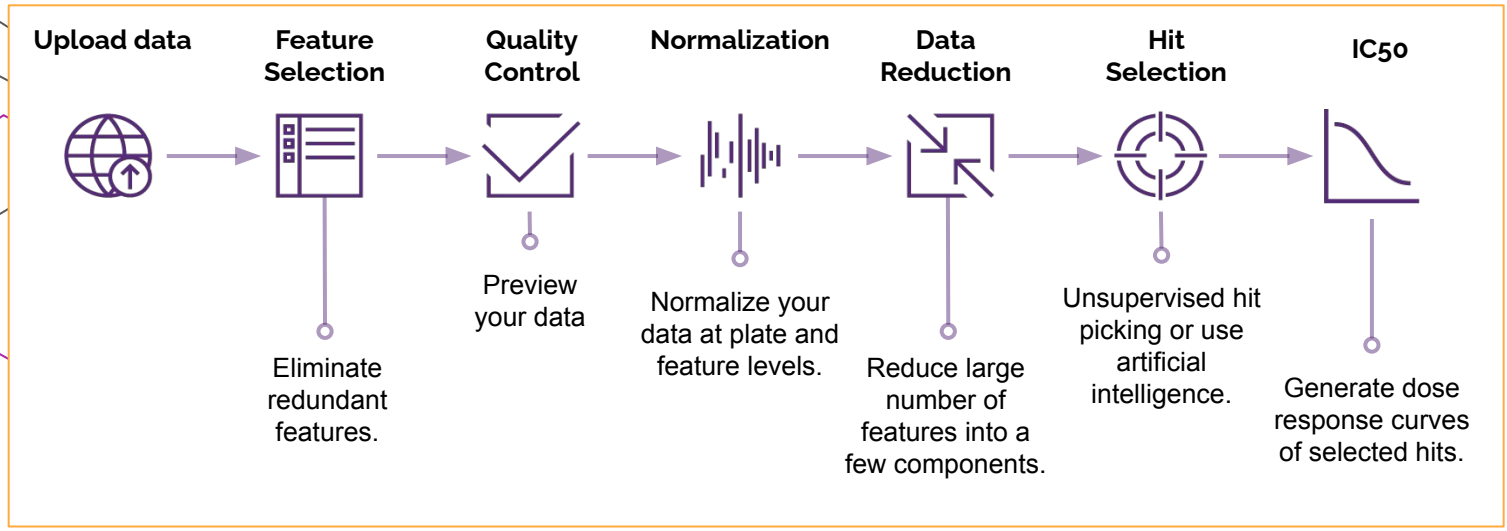


Figure 4. Feature extraction and segmentation using Harmony 4.9 software for calculating STAR morphology. Example, axial and radial distribution of mitochondria were measured, as well as symmetry, profile, and threshold compactness. a) Shows the nuclei segmentation using optimised thresholding parameters. b) Cell outline, calculated using Syto14 and Phalloidin/Alexa Fluor tags. c) Segmentation process for calculating mitochondria within defined cells. d) Using STAR morphology function to calculate key texture properties from actin staining, such as 'ridges'. The insert top right shows the original image.

StratoMineR™ Workflow



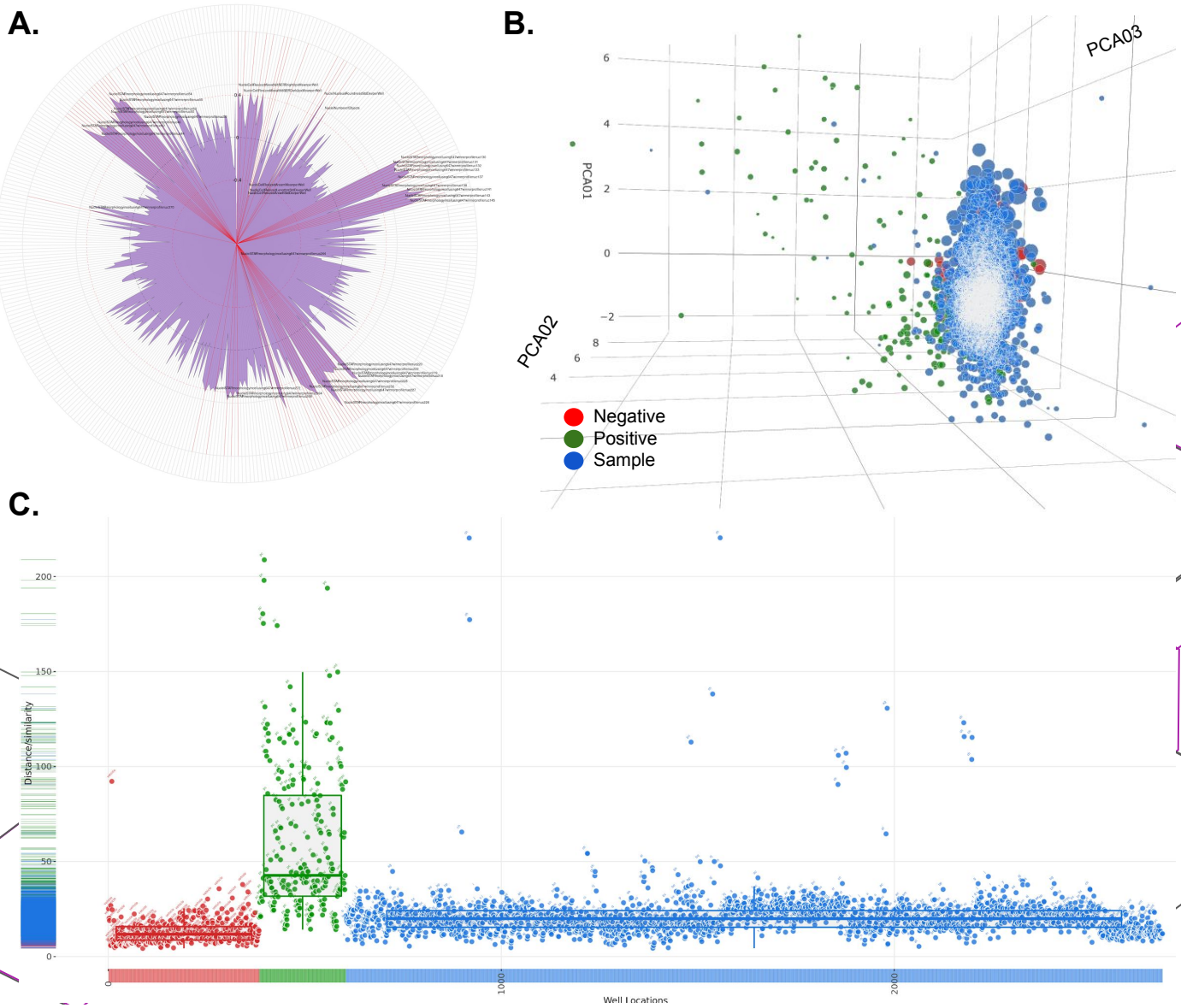


Figure 4: **A**. Due to the large number of features, StratoMineR™ performs dimensionality reduction to reduce the complexity of the data. This is useful for three critical reasons: 1) reducing computational load, 2) reduces redundancy, and 3) it reveals the biology behind the data by highlighting important features. StratoMineR can also suggest the number of principal components to use, in this case 8 PCAs). A spider plot showing one of the principal components, with features that have significant loadings on the principal component. **B.** Visualizing data points in 3D in relation to 3 components or features is particularly useful as it helps Cytochroma to identify interactions between data points in relation to each other. This is useful to identify samples (blue dots) that clustered with positive controls (green dots) or negative controls (red dots), and may therefore have similar MOA. **C.** Unsupervised hit selection calculates Euclidean distance scores for all wells calculated from the median of the negative controls with $p < 0.05$. Hit rate of 59.84% with 1149 hits.

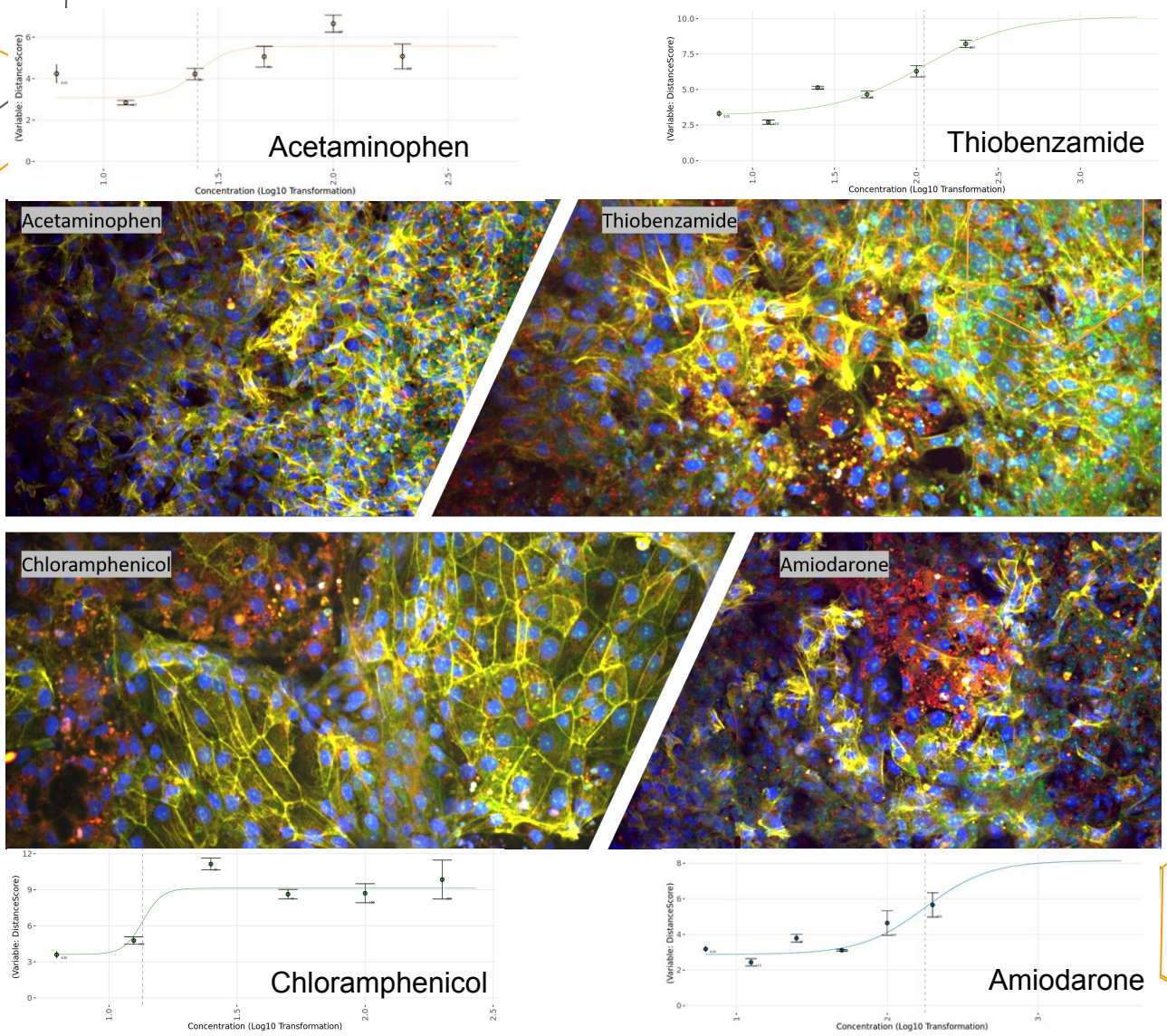


Figure 5: Using phenotypic distance score ranking from the hit selection step, Cytochroma can identify key compounds of interest as well as plot dose response curves for each compound. The Y-axis represents the phenotypic distance taking into account all eight principal components. The x-axis represents the concentration. IC values (uM): Acetaminophen = 25, Thiobenzamide = 111, Chloramphenicol = 13.5, Amiodarone = 178.

Conclusions

We show a streamlined workflow for high-throughput toxicity screening using StratoMineR™ as a tool for phenotypic screening. The unlimited source and scalable production of liver models, coupled with the automated, established, and scalable toxicity assay allows Cytochroma to screen multiple compounds in mature, polarized and stable models that are physiologically representative with enhanced sensitivity. StratoMineR™ is a powerful and intelligent data analytics tool that allows Cytochroma to perform deep analyses on phenotypic features across multiple experiments. The highly representative data outputs can reduce the failure rates that are currently seen going from preclinical to clinical development.

Acknowledgements

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