ARTIFICIAL INTELLIGENCE CAPTURES STRUCTURAL TOXICITY IN HUMAN IN VITRO CELL MODELS

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Abstract

In vitro human cell models, such as cells differentiated from induced pluripotent stem cells (iPSCs) have been increasingly used to evaluate toxicity in early drug development.

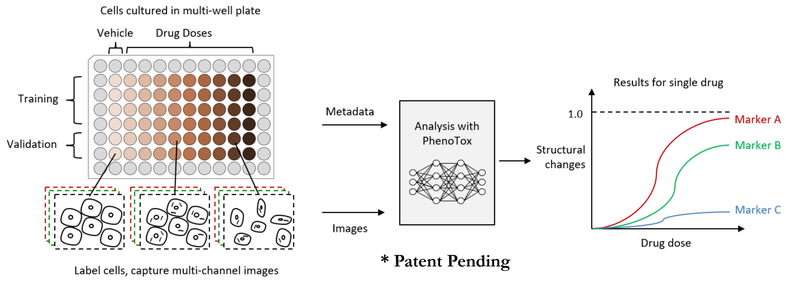
Current cell-based assays focus on cell damage through nuclear count or mitochondrial deficiency, or functional defects such as arrhythmia or prolonged contraction time in cardiomyocytes. There is a need for new assays that can capture structural changes in cells, which may complement existing assays, improve sensitivity, and better predict clinical toxicity.

We propose a novel method, PhenoTox, which uses artificial intelligence (AI) to capture drug-induced structural changes in cell cultures that relate to toxic drug effects. Our tool provides a scalable, high-throughput approach to quantify structural changes that occur prior to the onset of noticeable cell damage or death.

Method: PhenoTox

The input to PhenoTox is a collection of images of cells labeled and imaged at multiple doses and/or time-points for the drugs of interest and a reference set of images with only the vehicle applied.

PhenoTox uses deep learning to quantify drug-induced structural changes and generates a plot or heatmap of z-factors, depicting the doses and timepoints at which structural changes have happened and how strongly they differ from control.



*** Patent Pending**

PhenoTox has the following features:

- measures subtle or complex drug-induced structural changes at the single-cell level that occur prior to noticeable cell damage or death,
- agnostic to imaging modality, enabling the interrogation of any cellular structure or protein that relates to mechanistic properties,
- compatible with types of culture plates, enabling the measurement of structure as well as function from the same cell populations.

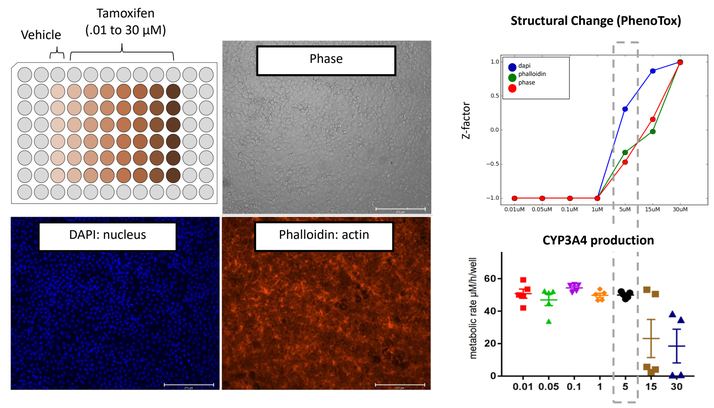
PhenoTox is More Sensitive than CYP Production for Tamoxifen-Treated Hepatocytes

Cells from CDI were cultured on 96-well plates and treated with 7 doses of Tamoxifen in addition to vehicle only as the control.

After 48 hours of drug treatment, cells were fixed and stained with DAPI and phalloidin. Bright-field and fluorescence images were collected at 12 non-overlapping locations per well.

Images were analyzed by PhenoTox. For each drug dose being tested, 4 wells were allocated for training and 2 wells were allocated for validation.

PhenoTox shows structural changes for all 3 channels for Tamoxifen which correlated with CYP3A4 production. Structural changes were detected at one dose lower (5uM) than was seen in loss of CYP3A4 production. No structural change was detected for Aspirin.



PhenoTox Is Complementary to Contractility Assays for Cardiomyocytes

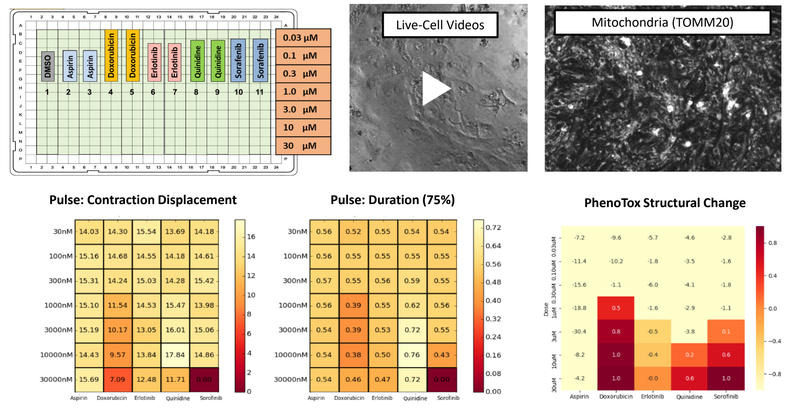
Cells from CDI were cultured on 384-well plates and treated with 7 doses of 5 compounds, in addition to vehicle only as the control.

After 48 hours of drug treatment, brightfield videos with duration of 8-seconds were captured at each well, and then cells were fixed and stained with TOMM20. Fluorescence images were captured at 9 non-overlapping locations per well.

Videos of beating cardiomyocytes were analyzed using Pulse for measuring contractility.

Fluorescence images were analyzed by PhenoTox. For each drug dose being tested, 6 wells were allocated for training and 2 wells were allocated for validation.

PhenoTox shows structural changes that correlate highly with contractility changes. No structural change was detected for Aspirin.



Conclusion

- PhenoTox reliably detects dose-dependent structural changes in iPSCs.
- PhenoTox results correlated highly with functional assays, including CYP3A4 production for hepatocytes and contractility for cardiomyocytes.
- Structural changes were detected at one dose lower (5uM) than was seen in loss of CYP3A4 production, which was confirmed in repeat experiments.

Links and Disclaimer

- PhenoTox analysis was performed with PhenoLearn: **www.phenolearn.com**
- Contractility was analyzed using Pulse: **www.pulsevideoanalysis.com**
- **Disclaimer**: The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be considered to represent any agency determination or policy.

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