Time-Series Inspired Modeling of Dose-Response Dynamics for More Sensitive Prediction of Toxicity from Cell Painting Data

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ABSTRACT

Toxicity remains one of the major challenges in the drug discovery process, with an estimated 30% of drug candidates failing during development due to toxic effects[1]. This highlights the ongoing need to improve current toxicity assessment methods in order to detect harmful effects as early and accurately as possible.

Despite recent technological advances, the cell based assays currently used for testing compound toxicity have significant sensitivity limitations. Typically, the concentrations at which a toxicity effect is detected in in vitro assays greatly exceed the concentrations required to elicit toxicity in a real organism. This makes the resulting calculations for IC50 or PoD (Point of Departure) highly unreliable in in vivo studies, calling for the development of new methods capable of capturing the toxicity at lower concentrations.

To address these challenges, we've adapted an eXplainable Convolutional neural network for Multivariate Time Series classification (XCM)[2] by treating concentrations as time points to analyze Cell Painting assay data. We selected this method to address the nature of increasing doses, which can mimic temporal progression. Additionally, using a CNN-based approach enables analysis of the importance of both features and concentrations through the Grad-CAM method[3].

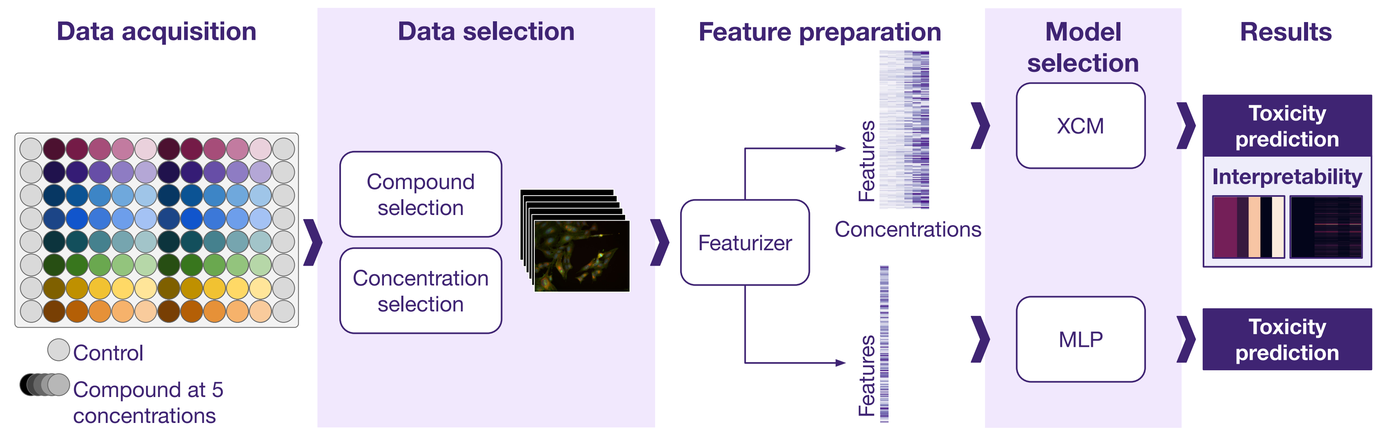
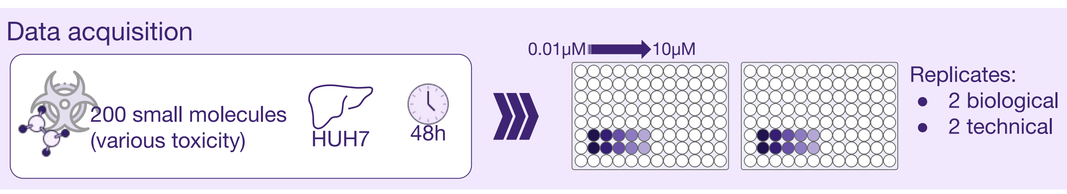


Figure 1 Prior to image acquisition, HUH7 cells were treated with compounds at 5 concentrations and stained using Cell Painting[4] protocol. In the next step, images corresponding to selected compounds and concentrations were featurized using CellProfiler[5]. Then, we applied classifiers, XCM[2] and MLP to capture the phenotypic effect of the early onset of various types of cellular toxicity. XCM additionally provides an interpretation of the results.

DATASET



METHODS

Toxicity types

Hepatotoxicity (DILI)	Cardiotoxicity (Cardio)	Genotoxicity (AMES)

Additional subset on non-toxic compounds was used for reference as inactives.

Computational methods

MLP	XCM	Anomaly score
Multi Layer Perceptron	eXplainable Convolutional neural network for MTS classification	out-of-distribution detection

MTS - Multivariate Time Series

Training data selection

All compounds vs. compounds with high Anomaly score	All concentrations vs. highest concentrations (only for MLP)	Single- vs. multi-label training

RESULTS

- Our initial study, conducted on a small dataset of Cell Painting images (200 compounds), demonstrates the feasibility of using time series model for early toxicity detection and dose–response estimation.
- In this study, the XCM method was applied to track changes in individual image features as a function of compound concentration (analogous to time points) (Fig. 1).
- A comparison between the XCM and MLP methods revealed differences in predictive performance, depending on the type of toxicity and the training setup (Table 1).
- Further analysis of individual compounds highlights the complementary nature of different toxicity prediction approaches vs cell count and anomaly detection (Fig. 2).
- Importantly, the XCM method enables biological interpretation by identifying image features that drive model decisions (Fig. 3A). It also shows the potential to detect early signs of toxicity in cellular phenotypes (Fig. 3B).

Method	XCM			MLP			
	All	Only active	Multilabel	All	Only active	Multilabel	All conc.
DILI	0.507	0.560	0.582	0.585	0.579	0.573	**0.601**
Cardio	0.577	0.644	0.532	0.605	**0.662**	0.622	0.580
AMES	0.509	0.632	**0.637**	0.535	0.601	0.634	0.611

Table 1 The table presents the average ROC AUC scores achieved by different modeling approaches for predicting the toxicity types analyzed in this study. "**All**"- Models trained on the full set of compounds; "**Only active**"- Models trained only on compounds that showed phenotypic changes at any tested concentration, based on Anomaly score; "**Multilabel**"- Models trained to predict all toxicity types simultaneously. For XCM, all tested concentrations were used in training, whereas for MLP models were trained on the highest concentration with an exception of "**All conc(entrations)**". The results indicate that the optimal modeling approach varies depending on the toxicity type, with different strategies yielding the best performance for different endpoints.

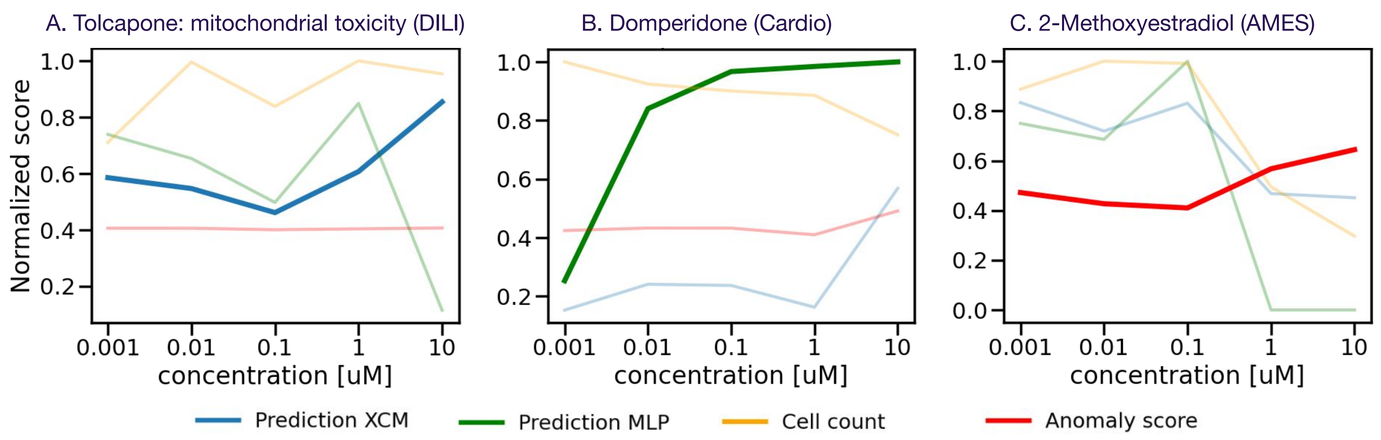


Figure 2 Different cases illustrate the complementary nature of the methods used in the study. A) For tolcapone, a compound associated with hepatotoxicity, the XCM method successfully captured the early changes in cell morphology, while both the MLP and anomaly detection methods failed to do so. B) In the case of domperidone, only the MLP method was able to predict its cardiotoxicity. Notably, both XCM and MLP predicted toxicity for compounds that did not induce any cell death. C) Here, the performance of both XCM and MLP closely correlates with cell count, which is also reflected by anomaly detection—an unsupervised method for identifying significant changes in cellular morphology. These findings highlight the potential of XCM and MLP methods for predicting toxicity independently of cell viability.

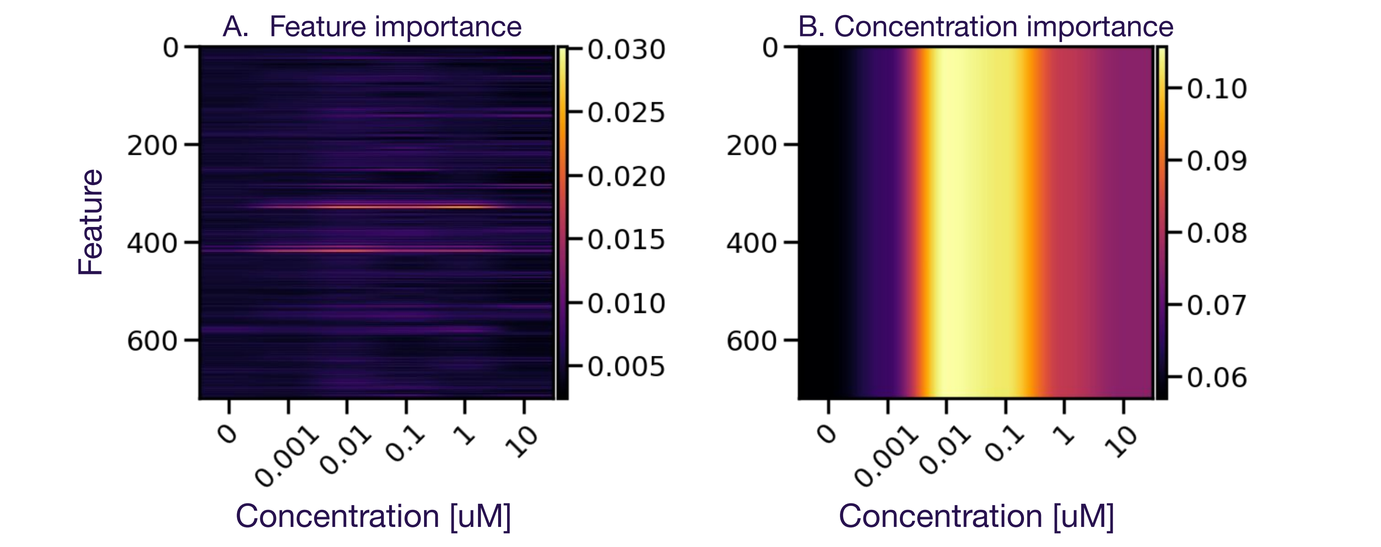


Figure 3 Interpretation modules in the XCM method. A) importance of individual input features across varying concentrations, highlighting those most influential in driving the model's prediction. B) cumulative importance of each concentration, indicating that the model begins detecting relevant signals at lower concentrations.

CONCLUSIONS

- **The eXplainable Convolutional Neural Network for Multivariate Time Series classification (XCM) is a valuable approach for detecting the early onset of toxicity in Cell Painting images.**
- **In addition, the method enables interpretation of results by highlighting image features driving model decisions.**
- **These promising initial findings warrant further investigation with larger datasets and more comprehensive exploration of time series architectures.**

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