AI-based analysis of complex biological phenotypes

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Introduction

Cell-based phenotypic assays have become an increasingly attractive alternative to traditional *in vitro* and *in vivo* testing in pharmaceutical drug development and toxicological safety assessment. The effectiveness of automated assays combined with advantages of machine-learning methods opens new opportunities to employ the power of AI to analyze complex multi-parametric datasets from screening and high-content imaging. In our studies, we used machine learning methods for analysis of morphological end points in complex cell models.

Characterization of complex processes like neuronal development or tumor growth are crucial for drug discovery and disease modeling. However, while high-content imaging is an efficient tool to capture phenotypic changes in complex cell morphology, quantitative image analysis is still a challenging task, due to multiple complex readouts, manifold changes in cell morphology, and the complexity of analysis algorithms used. Machine learning (AI)-based image analysis can address these challenges by reducing the effort and expertise required to capture and analyze morphological changes.

We demonstrate a workflow, which integrates a high-content imaging system with an AI-based image analysis platform to quantify complex biological phenotypes. We present two examples of AI-based image analysis of complex phenotypes: neurotoxicity assessment of compounds using human iPSC-derived neurons, and evaluation of dose-dependent efficacy of anti-cancer drugs in 3D spheroid assays.

Cell models

Neurotoxicity evaluation using iPSC-derived neurons

Primary human iPSC-derived neurons were received from Cellular Dynamics Int. (CDI, Madison WI). Cells (7.5K per well) were treated for 72 hours on laminin coated 384-well plates. Cells were stained with Calcein AM and Hoescht dye (Invitrogen, Carlsbad, CA) for 30 minutes prior to imaging on an ImageXpress® Micro Confocal High-Content Imaging System (Molecular Devices). Images were acquired using 10X objectives.

Effects on neurite outgrowth, complexity of networks, and viable cell number were assessed following 72 hours of exposure. Conventional phenotypic readouts included characterization of neurite outgrowth, branching, number of processes, and cell viability. Dose-response information was used for ranking chemicals according to their toxicity or safety.

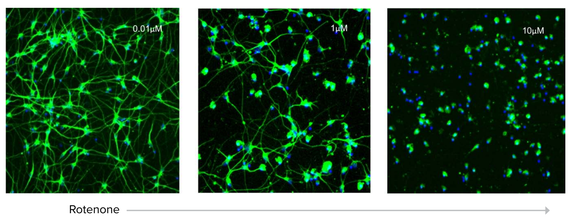


Figure 1. Human iPSC-derived cortical neurons were treated with indicated concentrations of rotenone for 72 hours, then stained with Calcein AM and Hoechst nuclear stain. Cells were imaged with ImageXpress Micro Confocal system at 20X magnification.

Evaluation of compound effects on 3D cancer spheroids

We optimized cell culture and high-content imaging methods to investigate effects of anti-cancer drugs on 3D spheroids formed from immortalized cancer cells (HCT116 colon carcinoma cell line). Spheroids were formed in ultra-low attachment (ULA) plates to initiate 3D spheroid formation. Cells (4000 per well) aggregated at the bottom of the wells, formed spheroids within 24-48 hours. Then cells were treated with different concentrations of compounds for five days. After that, spheroids were stained with Hoechst nuclear dye, Calcein AM (viability dye), Ethidium Homodimer (EtHD, dead cell dye), and imaged at 10X using ImageXpress Micro Confocal system with z-stack, 15 images 10 µm apart. Maximum projection images were evaluated for compound effects using high-content imaging.

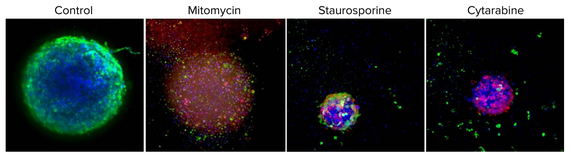


Figure 2. Human HCT 116 cells cultured as 3D spheroids were treated with indicated anti-cancer drugs (Mitomycin C 100 µM, Staurosporine 1 µM, Cytarabine 100 µM), and then stained with Hoechst nuclear dye, Calcein AM, and EtHD dead cell dye. Spheroids were imaged with ImageXpress Micro Confocal system at 20X magnification with z-stack. Maximum projection images presented.

Instrument

ImageXpress Micro Confocal High-Content Imaging System with water immersion option

Using artificial intelligence for analysis of complex phenotypes

Principles of machine learning for imaging

Using pattern recognition or AI for image analysis relies primarily on defining groups of images or image regions that are different from each other due to experimental conditions or manual observation. The process of training a pattern recognition model based on these differences is automated, so it is not necessary to describe this process or be familiar with its algorithms and parameters like it is in conventional image analysis.

We illustrate two different types of AI in scoring dose-response relationships. The first is for categorical classification to differentiate live cells, dead cells, and debris. This AI is used for scoring assays based on cell viability. The second set of AIs are trained based on concentrations of compounds in the assays, with one AI trained per compound. This second set of AIs assigns a continuous variable score to each cell (or "block" in the case of spheroids) based on its average similarity to the cells treated with each of the different drug concentrations. There is no *a priori* selection of which image properties are considered in calculating this score or in classifying cells. The set of numerical image descriptors computed for each cell or image region is always the same, and it is part of the automated AI training process to determine which descriptors are most informative for a given imaging problem.

As implemented in this study, the cell detection and segmentation used ViQi's proprietary algorithm utilizing information from all of the image channels and performing multi-resolution cell detection followed by a high-dynamic range local segmentation. The detected objects are further classified by an AI step that uses a set of descriptors that consist of pixel intensity statistics, shape descriptors and texture descriptors for each channel (fluorescence, brightfield, etc.) in the image.

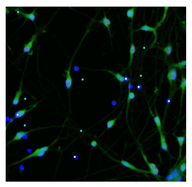
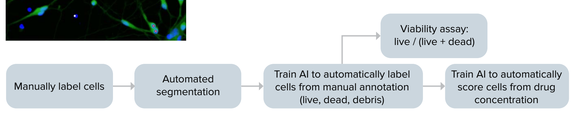


Figure 3. Example manual annotation of object types. To train an AI to discriminate object types, the user manually clicks on a subset of objects and labels them as dead cells (white), live cells (orange) and debris (cyan). Several objects of each class (~5-10) are identified in several images (~2-5) covering the range of imaging conditions and experimental manipulations.



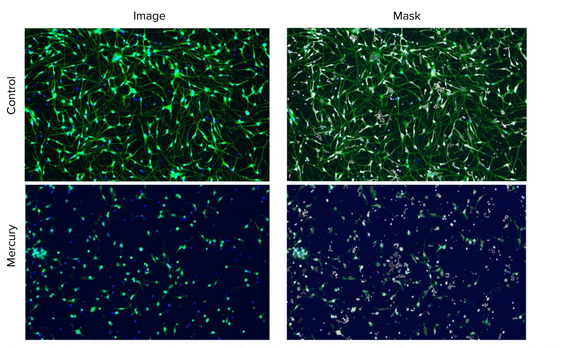


Figure 4. Example views of untreated (DMSO, top) and treated (0.3 µM mercury, bottom) cells. The grey areas on the right indicate the segmentation masks determined automatically by the ViQi cell segmentation algorithm.

Phenotypic analysis of spheroids

Individual cells within spheroids are typically more difficult to distinguish, and it was preferable to base a response score on the behavior of groups of cells rather than exclusively on cells in isolation. Instead of segmenting cells, the maximum intensity projection (MIP) image was broken up into equal-sized contiguous blocks or tiles. A small set of tiles containing cells were manually labeled to train an AI, which was then used to label the remaining blocks as spheroid or non-spheroid. Only the spheroid-containing blocks were used for training the second set of AIs based on compound concentrations.

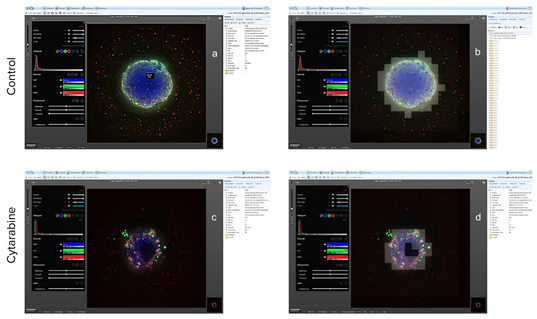


Figure 5. Example views of untreated (a,b) and treated (c,d) spheroid images. The untreated image (a) shows an overlay of manually scored locations separating centroid from non-centroid. The right hand images (b and d) show a mask overlay of blocks classified as spheroid. Only these blocks are used by a dose-response AI.

Results: Effects of neurotoxic compounds

As a next step, we tested a set of neurotoxic compounds that have suspected toxic effects on the nervous system, including established drugs used for treatment of cancer or environmental substances.

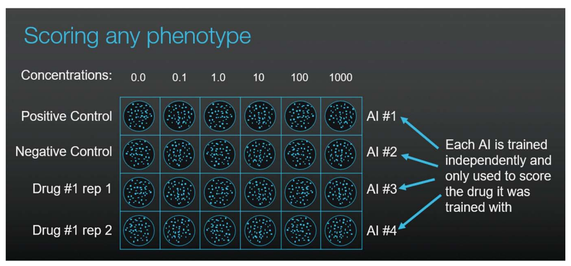


Figure 6. The dose-response experiment sets up different AIs per compound where each concentration is defined as a separate class. Here we will have four AI models with six classes each. Therefore the AI's job is to find the cell attributes that best separate the observable effects of these concentrations.

Effects of neurotoxic drugs

To test if the assay can identify chemicals for their potential neurotoxic effects, we tested a panel of 23 compounds that represent different classes of drugs, known to cause peripheral neuropathy (cancer drugs), and selected environmental substances including methyl mercury, rotenone, flame retardants, and pesticides. For neurotoxicity tests, neurons were treated with compounds for 72 hours. Some substances caused severe disintegration of neural networks and cell death; selected drugs caused mostly moderate perturbations.

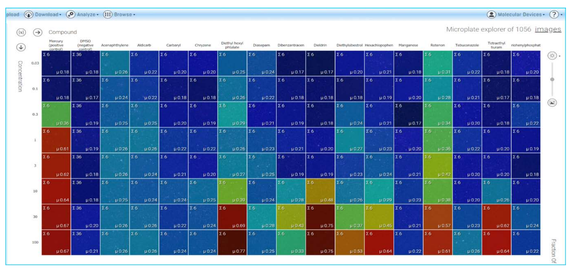


Figure 7. The compounds and concentrations are displayed with a heatmap overlay representing viability (fraction of live cells) scored by the machine learning model.

Multi-parametric assessment of compound effects

Analyzing compounds based solely on detecting compound-specific phenotypic changes results in clear differences between compounds known to have positive neurotoxic effects (Mercury, Rotenone) and compounds known not to have significant effects on neurons (DMSO, Tebuconazole). In this assay, it is possible to discriminate the intermediate effects of methyl mercury compared to the more classical bi-phasic response for rotenone.

The box plots below show the distribution of response scores produced by the AIs grouped by concentration for each drug. The AI scores individual cells, visible as individual points for outliers, with quartiles of the distribution represented by the box and whiskers, and the median indicated with a line.

When an AI detects no response (DMSO and Tebuconazole below), the scores are not distributed around "0.0", but are in the middle of the concentration range. Each cell's response score is based on a weighted probability distribution for, or similar to, the concentration classes. When the classes are not distinguishable, the relative probability is the same for each concentration, making the score equivalent to averaging the concentrations.

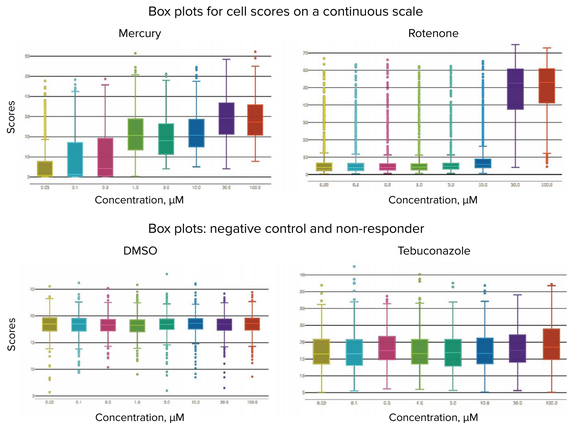


Figure 8. Automatically computed neurotoxic responses to strong neurotoxicants Methyl Mercury and Rotenone, DMSO and non-responder. The box plot shows effects of different doses as determined by ViQi's AI system.

Results: Effects of anti-cancer compounds on morphology of 3D cancer spheroids

In the different cell assay model, we tested a selected set of anti-cancer drugs. As before, we have trained AI models per compound to best separate the observable effects of drug concentrations using the blocks belonging to spheroids.

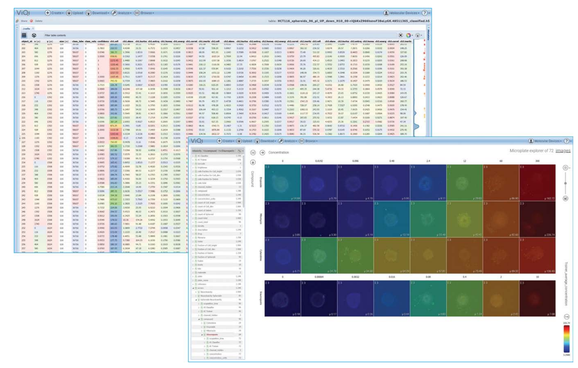


Figure 9. Table of numeric descriptions of texture and intensity features used in AI training. Each row represents a single tile in the image. The results for the four compounds assayed are represented with a heatmap reporting the average phenotypic effect for the spheroid tiles reported by the AI.

Dose-dependent assessment of compound effects in 3D spheroids

Maximum intensity projection (MIP) images of 3D spheroids treated with compounds were analyzed by AI. This assay shows promise for an automated high-throughput assessment of anti-cancer compounds in spheroids. ViQi's AI system automatically scores thousands of parameters describing small sub-regions in spheroid MIP images and computes average response scores per concentration.

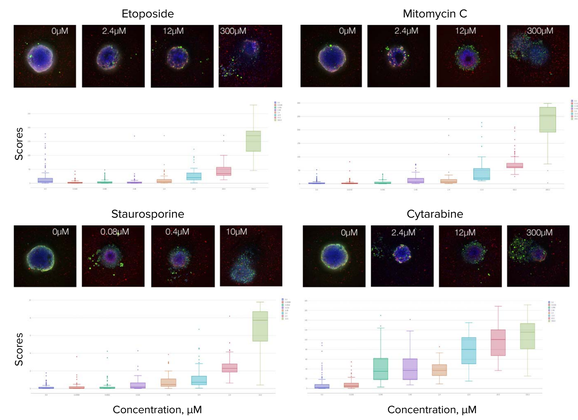


Figure 10. Automatically computed spheroid responses to four anti-cancer compounds: Etoposide, Mitomycin C, Staurosporine, and Cytarabine. Each sub-figure shows exemplar projection images of spheroids with four different doses including control and maximum dose. The box plot shows effects of those doses as determined by ViQi's AI system.

Conclusion

AIs can be used to greatly simplify the image analysis tasks necessary to assay cellular responses to compound treatments. These AI-based assays can be quantitative and highly sensitive. They can be based on target phenotypes such as cell viability, or they can be performed open-ended, reporting on any change induced by the compound treatments without *a priori* knowledge of what a response might be, or whether there will be a response at all.

The ability to automate the training of AI models to perform these assays enables biologists to use these powerful tools without the expert knowledge needed to optimize these models and tailor them for specific image analysis tasks.

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