Al-based analysis of complex biological phenotypes

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 and endpoints 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 provides an efficient tool to capture phenotypic changes in complex cell models, quantitative image analysis is still a challenging task due to multiple complex readouts, manifold changes in cell morphology, and the complexity of analysis algorithms. Using 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 isolated from Cell line Detroit 515 [28]. Cells (75K per well) were treated for 72 hours on laminin coated 384-well plates. Cells were stained with Calcium AM and Hoechst dye (Invitrogen, Carlsbad, CA). After 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.

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 (HT1376 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), and imaged at 10X using ImageXpress Micro Confocal system with a stack of 15 images 10 μm apart. Maximum projection images were evaluated for compound effects using high-content imaging.

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 bins. A small set of tiles containing cells were manually marked to train an AI, which was then used to label the remaining blocks as spherical or non-spherical. Only the spheroid-containing blocks were used for training the second set of AIs based on compound concentrations.

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 to 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 assay, 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 descriptors 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 Vقي’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 that uses a set of descriptors that consist of pixel intensity statistics, shape descriptors and feature descriptors for each channel (fluorescence, brightfield, etc.) in the image.

Figure 3. Example manual annotations 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 (purple), live cells (orange), and debris (peach). Several objects of each class (T5–T7) are identified in several images (T2–T6) covering the range of imaging conditions and experimental manipulations.

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.

Scoring any phenotype
Each AI is trained independently and only used to score the drug that it was trained with.

Effects of neurotoxic drugs
In the case of cell viability, this is done by comparing the numerical score assigned to a cell or group of cells to a set of thresholds. The score is then used to determine if a cell can be considered viable (score above threshold), dead (score below threshold), or debris (score in between thresholds).

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 a more automated high-throughput assessment of anti-cancer compounds in spheroids. Vقي’s AI is capable of scoring thousands of parameters describing small sub-regions in spherical MP images and computes average response scores per concentration.

Multi-parametric assessment of compound effects
Analysing 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 biphasic response for rotenone.

In the dose-response experiment, we set up different AIs per compound where each is named after the compound. Each AI is trained to find the cell attributes that best separate the observable effects of these concentrations.

Conclusions
As can be greatly simplified, the image analysis tasks necessary to assay cellular responses to compounds treatments. These AI-based analyses can be quantitative and highly sensitive. They can be targeted to target phenotypes such as cell viability or signal pathway changes, or can be performed open-ended, reporting on any change induced by the compound treatments without prior knowledge of what a response might be. 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.