Distinguishing Cell Lines by Phenotypic Profiling of the Nucleus

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1 Introduction

Although the intention of high content screening is to extract as much relevant information as possible, a large percentage of high content screening assays only analyze a small number of image based properties. This can result in the loss of valuable information. Nearly all screening approaches use a nuclear counterstain like Hoechst to aid in segmentation. Sometimes Hoechst total sum intensity distribution is also used to analyze cell cycle distribution, in particular G0/G1, S and G2/M populations. However, besides the cell cycle analysis, there is still more information that can be retrieved from the nuclear "counterstain". Here we show how additional information can be extracted from the nuclear stain by phenotypic profiling. Leveraging the high quality of images derived from either the PerkinElmer Opera Phenix™ High-Content Screening System or Operetta CLS™ High-Content Analysis System in combination with the advanced texture and morphology tools built into Harmony® High Content Imaging and Analysis Software, the nuclear staining can be used to differentiate cell lines from each other without any further staining or phenotypic markers.

2 Distinguishing HepG2 and NIH/3T3 Cells in Co-cultures

HepG2 liver and NIH/3T3 fibroblast cells were seeded into a PerkinElmer CellCarrier-384 Ultra Microplate either alone at different densities (1E4, 1.5E4, 2E4) or as co-cultures with different ratios (1:2, 1:1, 2:1) with a final cell number of 1.5E4. Prior to mixing, HepG2 cells were additionally stained with CellTracker Green CMFDA and NIH/3T3 cells with CellTracker Red CMTPX to validate the accuracy of the phenotypic classification. Next day cells were fixed, stained with Hoechst 33342 and imaged on an Opera Phenix HCS System using a 20x water immersion objective in confocal mode. A total of 9 fields per well were acquired.

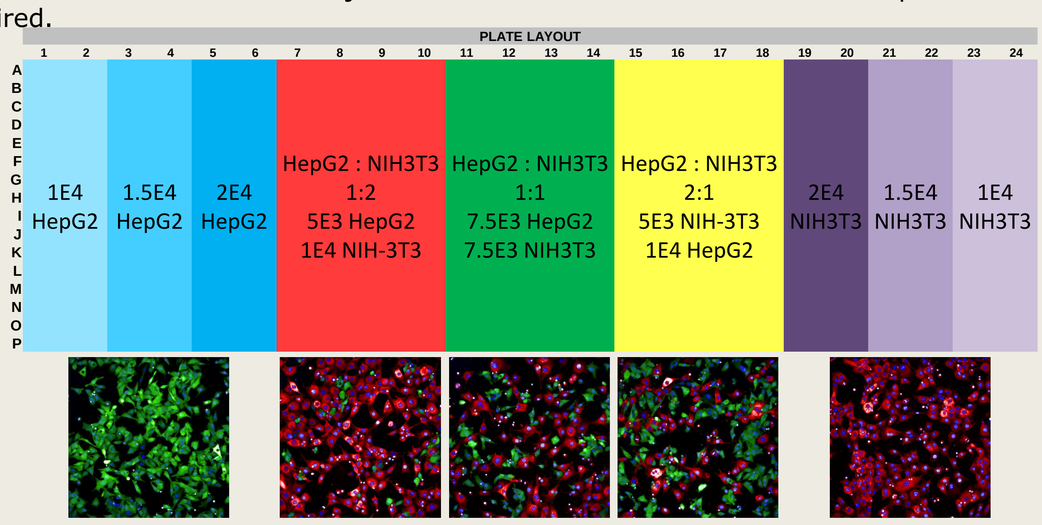

Figure 1: Plate layout and seeded cell numbers with representative images from the different areas below. Cells were stained with Hoechst 33342 and additionally HepG2 cells with CellTracker Green CMFDA and NIH/3T3 cells with CellTracker Red CMTPX .

For image analysis, Harmony High Content Imaging and Analysis Software was used. First nuclei were segmented and mitotic cells were excluded based on size and intensity features. Mitotic cells were eliminated from further analysis based on the assumption that these nuclei should have less discernable texture features than G0/G1, S and G2 nuclei. To phenotypically characterize the nuclei, advanced SER Texture and STAR Morphology parameters were calculated in addition to standard intensity and morphology parameters. In total 227 parameters were calculated for every nucleus. PhenoLOGIC™ machine-learning option on Harmony was then used to select the parameters best suited to discriminate between the two cell types. PhenoLOGIC enables supervised training by simply clicking on the respective objects to distinguish up to six different phenotypes.

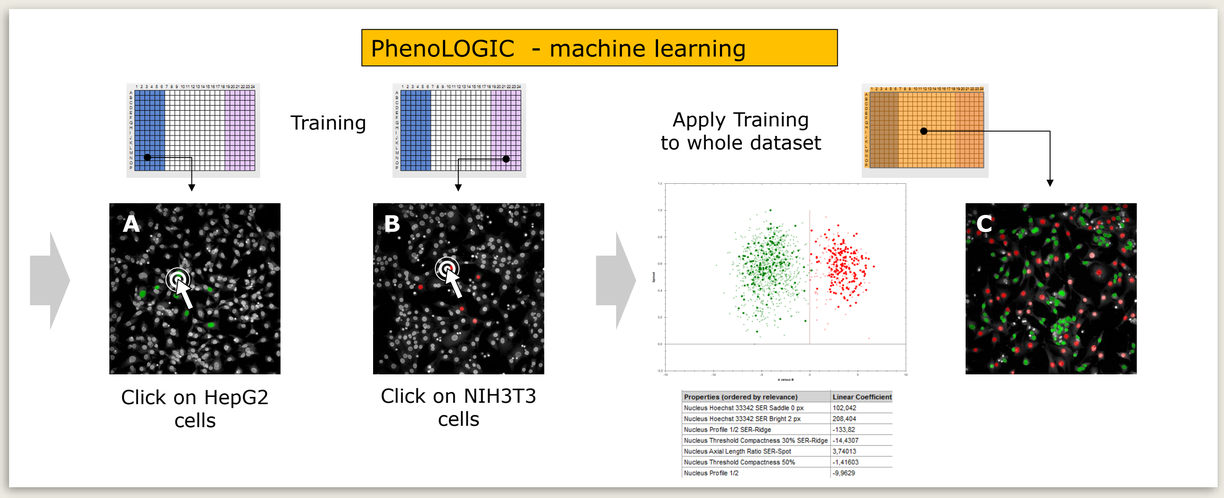

Figure 2: Identifying two cellular phenotypes using the interactive training mode PhenoLOGIC machine learning. In "Training" mode single cells can be clicked to teach the software to identify the different cell types in "single cultures" (A and B). Once cells for each class are marked, the "Training" can be applied to the whole data set. The software combines the most meaningful parameters (shown in table below the scatter plot), whether it's two, three, four or, as in this case, seven, to achieve accurate classification of the two cell types (panel C). Note how advanced SER and STAR features dominate the selection over classical morphology parameters.

To check the accuracy of the linear classification, the CellTracker intensity in a perinuclear region was calculated. If a cell was classified as one cell type but the respective CellTracker intensity was below a defined threshold the cell was counted as a "falsly assigned" cell. The results of the classification are shown in the bar graphs below.

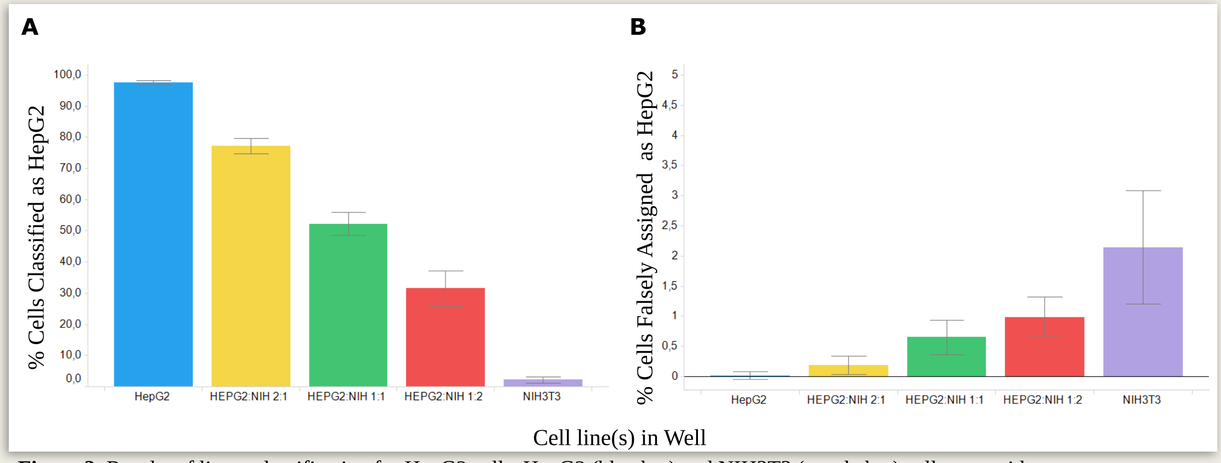

Figure 2: Results of linear classification for HepG2 cells. HepG2 (blue bar) and NIH3T3 (purple bar) cells were either cultured individually or as co-cultures at different ratios (yellow, green and red bars). (A) The graph shows the percentage of cells that were classified as HepG2 by linear classification. (B) Based on the CellTracker staining, cells that were falsely assigned as HepG2 were identified and the percentage of these falsely assigned HepG2 cells is shown. About 2% of the cells were falsely assigned in pure NIH/3T3 wells and less than 1% in co-cultures. n=96 wells for single cultures, n=64 wells for co-cultures, error bars represent ± one standard deviation.

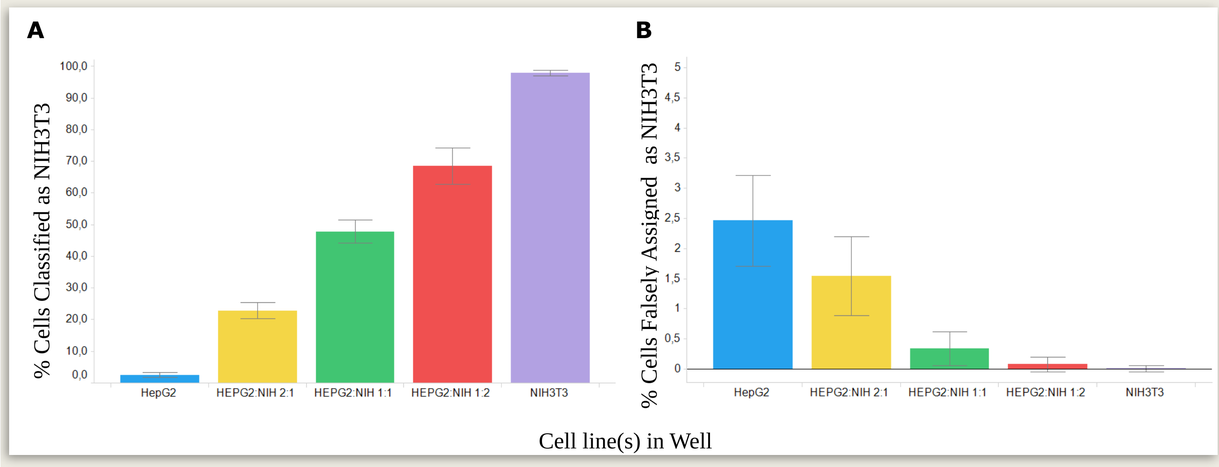

Figure 3: Results of linear classification for NIH/3T3 cells. HepG2 (blue column) and NIH/3T3 (purple column) cells were either cultured individually or as co-cultures at different ratios (yellow, green and red bars). (A) The graph shows the percentage of cells that were classified as NIH/3T3 by linear classification. (B) Based on the CellTracker staining, cells that were falsely assigned as NIH/3T3 were identified and the percentage of these falsely assigned NIH/3T3 cells is shown. About 2.4% of the cells were falsely assigned in pure HepG2 wells and less than 1.5% in co-cultures . n=96 wells for single cultures, n=64 wells for co-cultures, error bars represent ± one standard deviation.

3 Distinguishing Seven Cell Lines

To test the model further, seven different cell lines, NIH/3T3, MDCK, HeLa, MCF7, A549, HepG2 and HT1080 were seeded into a CellCarrier-384 Ultra Microplate (3 columns = 48 wells per cell line).
The next day the cells were fixed and stained with Hoechst 33342. Imaging was done on an Operetta CLS High-Content Analysis System using a 20x water immersion objective in confocal mode. The image analysis was conducted similarly to that for the co-culture experiment but without the counter staining of the CellTracker dyes. Additionally, this time the whole set of parameters was subjected to a principle component analysis (PCA). The PCA was done using High Content Profiler™.

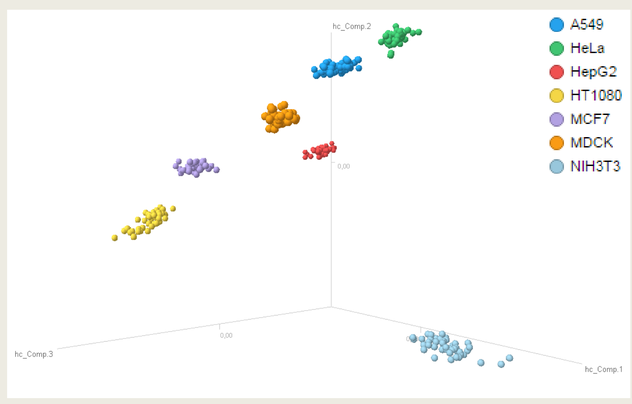

Figure 4: Three dimensional Principle Component Analysis (PCA) of the phenotypic profiling of seven different cell lines. The whole set of parameters calculated in Harmony was subjected to PCA. As can be seen in the figure the seven cell lines form independent clusters. This clearly shows that the calculated parameters are sufficient to distinguish the cell lines from each other.

4 Effect of Histone Modification Inhibitors

Out of the seven cell lines used above, HeLa, HepG2 and NIH/3T3 were seeded into CellCarrier-384 Ultra Microplate and the next day treated with different concentrations of either the histone deacetylase inhibitor Trichostatin A (TSA) or the histone acetylase transferase inhibitor HAT II (plate layout and treatment are shown in Figure 5A). After 24h incubation, cells were fixed and stained with Hoechst 33342. A z-stack of five planes at 3µm distance was acquired on an Opera Phenix HCS System using a 20x water immersion objective in confocal mode. A total of 9 fields per well were acquired. The images were analyzed as maximum intensity projections similar to the experiments above but in addition Haralick texture features were extracted. The whole set of calculated parameters was subjected to PCA.

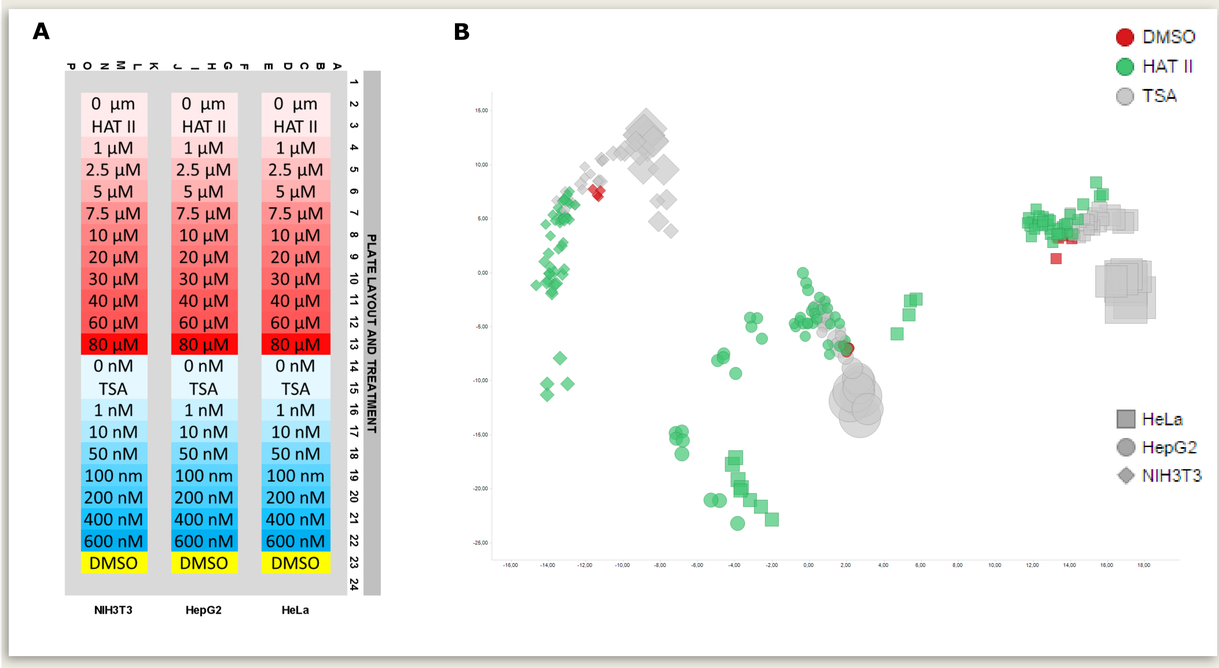

Figure 5: Effect of HADC and HAT inhibitors analyzed by phenotypic profiling of the nuclei. HeLa (square), HepG2 (circles) and NIH/3T3 (diamonds) were treated with increasing concentrations of TSA or HAT II (**A**) . After calculating SER Texture, STAR Morphology and Haralick Texture the parameters were subjected to PCA. In accordance to their opposite effects on DNA packaging the different treatments scatter into different directions from the DMSO control. The size of the marker indicates the respective concentration.

6 Summary

The Hoechst staining of cells' nuclei contains a plethora of information that can be used for much more than just aiding in segmentation during image analysis. It can be used to distinguish individual cell lines based on phenotypic HCS features in addition to other markers or to support the analysis of compounds altering DNA modifications. These are examples which demonstrate how High Content Screening provides much more information from your samples. These same principles and strategies can be applied to other fluorescent labeling approaches for phenotypic characterization. The prerequisite though is a combination of high quality images and powerful image analysis tools to process deep information.

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