Simplified workflow for phenotypic profiling based on the Cell Painting assay

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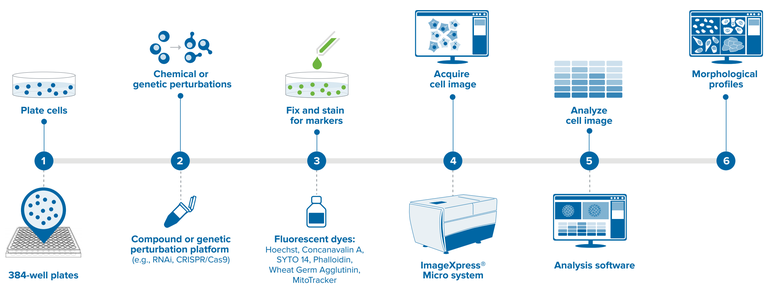
Introduction

Multiparametric high-content screening approaches, such as the Cell Painting assay, are increasingly being used in many applications ranging from drug discovery programs to functional genomics screening[6]. The Cell Painting assay uses up to six fluorescent dyes to label and visualize a variety of organelles at the single-cell level[3]. All the features extracted from the assay give unique cellular "signatures" that characterize any given cell. In addition, insights into the mechanism of action may be gained by comparing the phenotypic profiles of novel compounds with those reference compounds[3]. Most biologists are familiar with the methods used in the Cell Painting assay protocol. However, the sheer volume of data generated from these experiments require additional computational tools to extract meaningful information.

Here, we present a complete workflow for the Cell Painting assay that can be easily implemented. Using this approach, we find that cells treated with the same compound show similar phenotypic profiles. Hierarchical clustering analysis grouped highly toxic compounds such as paclitaxel and rotenone together. Chloroquine and tetrandrine, both of which affect autophagy[2,4], were also found in the same cluster. These results demonstrate that the proposed workflow is a viable and robust approach for performing high-content phenotypic profiling.

Methods



Overview of the workflow for image-based profiling. Details for each step listed below.

1. U2OS cells (ATCC) were seeded at 2000 cells per well.
2. Eleven compounds were tested in quadruplicate wells in a seven point 1:3 dilution series along with suitable controls. Compounds used: Ca-074-Me, CCCP, chloroquine, cytochalasin D, etoposide, latrunculin B, rapamycin, rotenone, staurosporine, paclitaxel and tetrandrine.
3. Twenty four hours after compound treatment, cells were stained with the cell painting dyes using the protocol by Bray et al[1]. The following dyes were used: MitoTracker Deep Red, wheat-germ agglutinin/Alexa Fluor 555, Concanavalin A/AlexaFluor 488, phalloidin/Alexa Fluor 568, SYTO14 and Hoechst 33342.
4. Image acquisition was performed on the ImageXpress® Micro Confocal High-Content Imaging System (Molecular Devices) using a 20X Plan Apo objective, with the following filters ex/em: DAPI 377/447, FITC 475/536, TRITC 543/593, TexasRed 560/624, Cy5 631/692
5. IN Carta™ Image Analysis Software was used for image analysis. 280 measurements that were selected include parameters related to intensity, texture, shape, spatial relationship, and co-localization scores.
6. Cell-level data was uploaded into StratoMineR™ (https://www.corelifeanalytics.com/, Core Life Analytics) for further data analysis. Briefly, quality control, plate normalization, data transformation, and feature standardization was applied. Principal component analysis (PCA) was used to reduce the dimensionality of the data set. Further downstream analyses such as hit selection and cluster analysis were performed based on the principal components and the phenotypic distance score derived.

Results

Modeling angiogenesis

In our model assay, U2OS cells were treated with 11 compounds for 24 hours and then processed according to a previously published protocol[1]. Cells were then imaged on the ImageXpress Micro Confocal system (Figure 1).

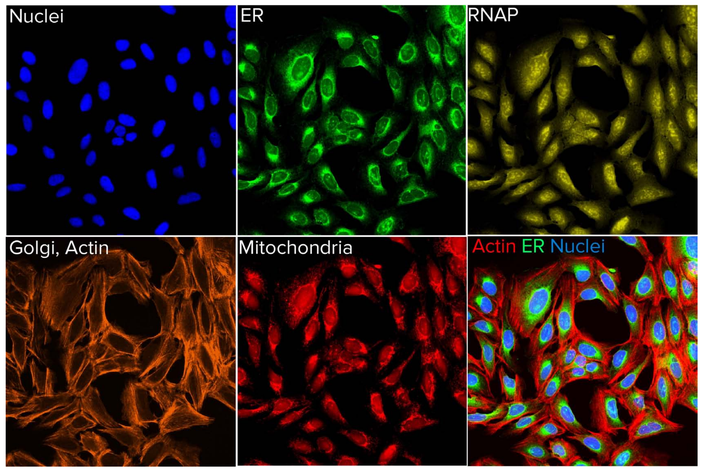


Figure 1. Cell Painting assay. Cells were compound treated, stained, and then imaged. Example image of each acquired channel from a control well is shown. The last panel shows a composite image consisting of actin, ER (endoplasmic reticulum), and the nuclei.

Feature extraction

Using IN Carta software, the image analysis routine can be adjusted to achieve robust detection of cells and organelles (Figure 2). Deep learning semantic segmentation module (SINAP) may be used to improve detection of challenging features. Pretrained deep learning models are available for the detection of nuclei or cells. Alternatively, users can train new models based on their specific objects of interest.

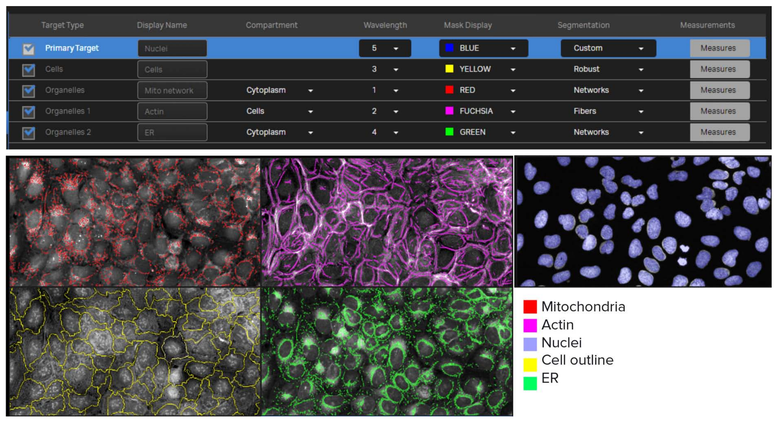


Figure 2. Feature extraction in IN Carta software. A) An analysis protocol was created in the IN Carta software to segment the various cellular structures. Here, we used the built-in nuclei model to achieve robust segmentation of nuclei across all treatments. Other cellular features such as cytoplasm compartment, actin filament network, ER, and mitochondria were also segmented. Measurements related to intensity, texture, colocalization and shape are selected during the analysis setup. We selected a total of 280 measurements for cells and subcellular structures in the protocol. B) Example images with feature mask overlay in IN Carta software.

Data analysis workflow

Measurements from the IN Carta software were uploaded into HC StratoMineR for further data analysis[5] (Figure 3).

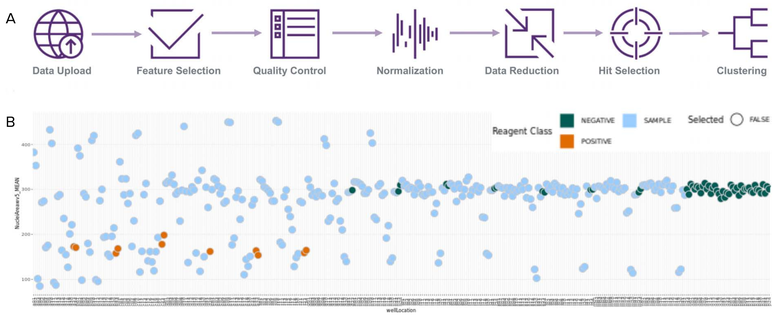


Figure 3A–B. Using HC StratoMineR for data analysis. A) HC StratoMineR is a web-based platform which guides users through a typical workflow in analysis of high-content multi-parametric data. B) Scatter plot from the QC step. The x-axis represents all samples, y-axis represents the selected feature (nuclei area).

Results

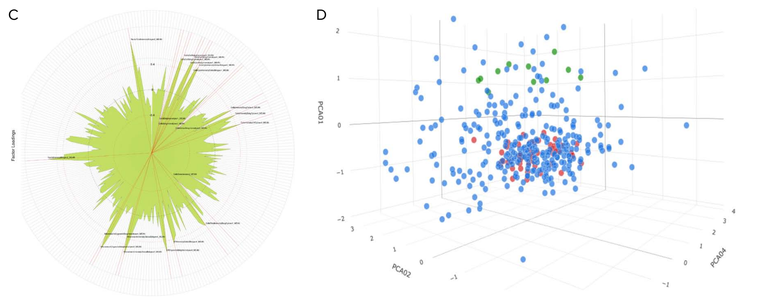


Figure 3C–D. Using HC StratoMineR for data analysis. C) Principal component analysis (generalized weighted least squares) was used to reduce the data to 15 components. Feature contributions to PCA4 is shown as a polar plot. D) 3D scatter plot shows interactions between data points in relation to three different PCAs.

Comparison of phenotypic profiles – Clustering

The distance score is calculated based on selected principal component scores. This score represents the phenotypic distance a sample is from the negative control which can be used for hit selection in screening assays. Subsequently, hierarchical cluster analysis can be performed based on selected features or components.

Cells treated with the same compounds are clustered together (Figure 4A, cluster 9, 10). Compounds known to have similar cellular effects were also clustered together. The actin polymerization inhibitors, cytochalasin D and latrunculin B were found together in cluster 6. Tetrandrine and choloroquine, both of which have effects in the autophagy pathway[2,4], were also clustered together (Figure 4B).

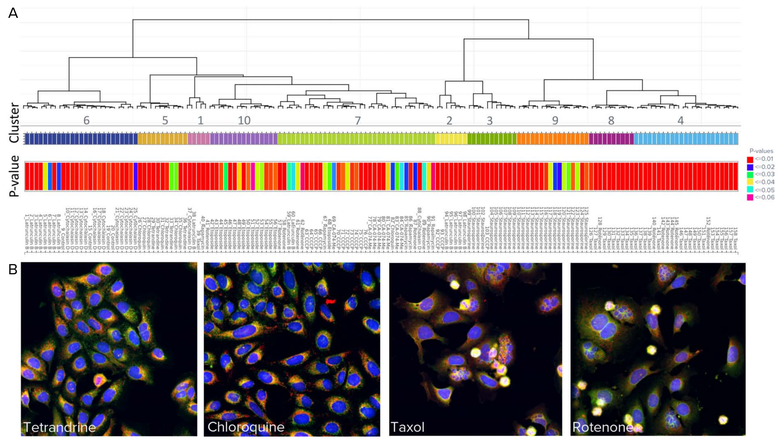
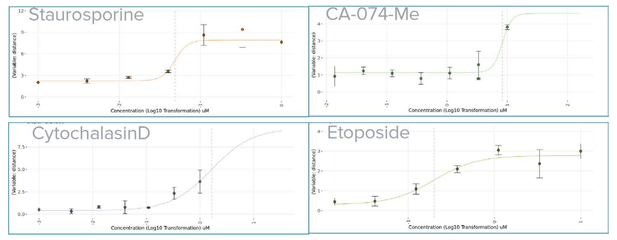


Figure 4. Cluster analysis. A) A dendrogram that represents hierarchical relationships is shown. Wells belonging to the same cluster (numbered) are represented by colored bars. P-values based on the distance score are shown for each well. Note that cluster 9 consists of only staurosporine treated cells, whereas cluster 10 consists of only etoposide treated cells. B) Examples of compound-treated cells belonging to some of the clusters are shown. Cluster 5 consists of tetrandrine- and chloroquine-treated cells. Note the increased number of ER punctae in both wells. Cluster 4 consists of rotenone- and paclitaxel-treated cells. Note the presence of blebbing in some of the cells belonging in these wells, suggesting cytotoxic effects.

Dose-response modeling based on phenotypic profiles



EC values:
Staurosporine 0.05 µM,
CA-074-Me 8.2 µM,
cytochalasin D 1.6 µM,
etoposide 0.2 µM.

Figure 5. Dose-Response curve. The phenotypic distance score obtained from the hit selection step was used to plot the dose response curve for each compound in IC StratoMineR. Y-axis represents the phenotypic distance, x-axis represents the concentration.

Conclusion

- Our results demonstrate the feasibility of using ImageXpress Micro Confocal system, IN Carta software, and StratoMineR for image-based profiling assays.
- The IN Carta software combines ease of use with more advanced feature segmentation options (SINAP) to allow robust segmentation of cellular features.
- The StratoMineR platform allows non-expert users to rapidly carry out phenotypic data analysis with its intuitive guided workflow.

References

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