Improving the robustness of Cell Painting with a near-infrared label and advanced image and data analytics

Introduction

Image-based phenotypic profiling approaches, such as the widely-used Cell Painting assay, use high-content imaging along with multiparametric readouts to study biological, genetic, and chemical perturbations in cells. This increasingly popular method is being used in applications ranging from drug discovery programs to genomic screening studies. In the standard Cell Painting assay, eight cellular compartments are captured in five imaging channels. This broad staining of the cell enables multiple morphological perturbations to be monitored and quantified at single-cell resolution. Limitations of the published cell painting assay include the limited availability of suitable dyes and the ability of imaging systems to give adequate spectral separation. Moreover, such a highly multiplexed assay requires additional resources and computational infrastructure to support, process, and interpret the data at scale.

Here, we sought to improve on the assay by taking advantage of the ImageXpress® Confocal HT.ai High-Content Imaging System which is equipped with a NIR light source. We swapped out Alexa Fluor 568 Phalloidin for Alexa Fluor 750 Phalloidin which allows for the cytoskeleton to be distinctly separated from the Golgi compartment. For manageable image and data analyses, we used the IN Carta® Image Analysis Software for feature extraction, followed by StratominR for data analysis. To determine if our approach demonstrated any improvements over standard Cell Painting protocols, we compared the phenotypic distance score between images acquired with five channels and those acquired with six channels. We found up to 49% increase in distance score for cells treated with a subset of compounds. These results show that using separate imaging channels for the Golgi and cytoskeleton increases the sensitivity of the Cell Painting Assay and more robustly represents the cellular phenotypic profiles.

Methods

1. U2OS cells (ATCC) were seeded at 2000 cells well per.
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. Cells were stained using the protocol by Bray at al. For the modified protocol, phalloidin/ Alexa Fluor Plus 750 (Thermo Fisher) was used instead of phalloidin/Alexa Fluor 568.
4. Image acquisition was performed on either the ImageXpress Confocal HT.ai (laser based) or ImageXpress Micro Confocal (LED based) high-content imaging system (Molecular Devices) using a 20X Plan Apo objective. The filters used are indicated in Table 1.
5. In Cell Image Analysis Software was used for image analysis. Measurements that were selected include parameters related to intensity, texture, shape, spatial relationship, and co-localization scores.
6. Cell-level data was uploaded into StratominR (https://cla.stratominr.com/index.php, 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.

Table 1. Stains used in the Cell Painting assay and filter sets used for detection of cellular compartments.

Results

Image acquisition

The cell painting assay uses six fluorescent markers to simultaneously label eight cellular compartments and then captures them in five imaging channels. The Golgi apparatus and cytoskeleton are both imaged using the same filter set (Table 1, Figure 1). Resolution of these subcellular structures is typically carried out in the image analysis step. In our model assay, U2OS cells were treated with 11 compounds for 24 hours and then processed according to a previously published protocol. Cells were then imaged using five imaging channels (Figure 1).

Figure 1. Cell Painting assay Examples of cells stained with dyes for the cell painting assay using previously published protocols. Note that golgi and actin structures are acquired in the same channel (AGP). Actin staining is represented by the filamentous structures while the golgi is not easily distinguished by eye.

Table 2. Change in distance score (%)

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Conclusions

• Our results demonstrate improvement in sensitivity to the Cell Painting assay with the use of a near-infrared laser. In this case it allowed for the development of an assay that is more sensitive in the separation of golgi phenotypes.

• The extran laser will also allow for the extension of the standard Cell Painting assay through the addition of project-specific biomarkers.

References