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

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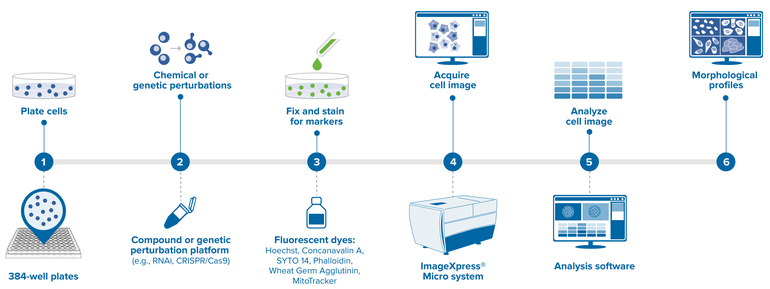
Introduction

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 StratoMineR 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



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. Cells were stained using the protocol by Bray et al[1]. 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 Carta 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 StratoMineRTM (https://cla.stratominer.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.

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[1,2]. Cells were then imaged using five imaging channels (Figure 1).

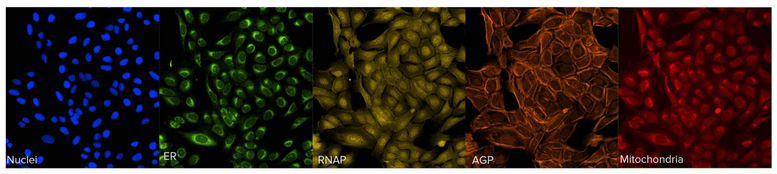


Figure 1. Cell Painting assay Examples of cells stained with dyes for the cell painting assay using previously published protocols. Note that golgi and actin structures are acquired in the same channel (AGP). Actin staining is represented by the filamentous structures. However, the golgi is not easily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

Cell Compartment	Dye	Ex/Em of dye	Filter used (LED)	Filter used (Laser)
Nucleus (DNA)	Hoechst 3342	350/461	DAPI 377/447	DAPI 405/452
Endoplasmic Reticulum (ER)	Concanavalin A – AlexaFluor488 conjugate	495/519	FITC 475/536	FITC 467.5/520
Nucleoli (RNA)	SYTO14	516/549 (DNA) 521/547 (RNA)	Cy3 531/593	YFP 520/562
Golgi Apparatus (AGP)	Wheat germ agglutinin AlexaFluor555 conjugate	555/565	Texas Red 560/624	TRITC 555/598
F-actin (AGP)	Phalloidin AlexaFluor568	578/600	Texas Red 560/624	
F-actin	Phalloidin AlexaFluor750	758/784		Cy7 725/794
Mitochondria (mito)	MitoTracker Deep Red	644/665	Cy5 631/692	Cy5 638/692

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

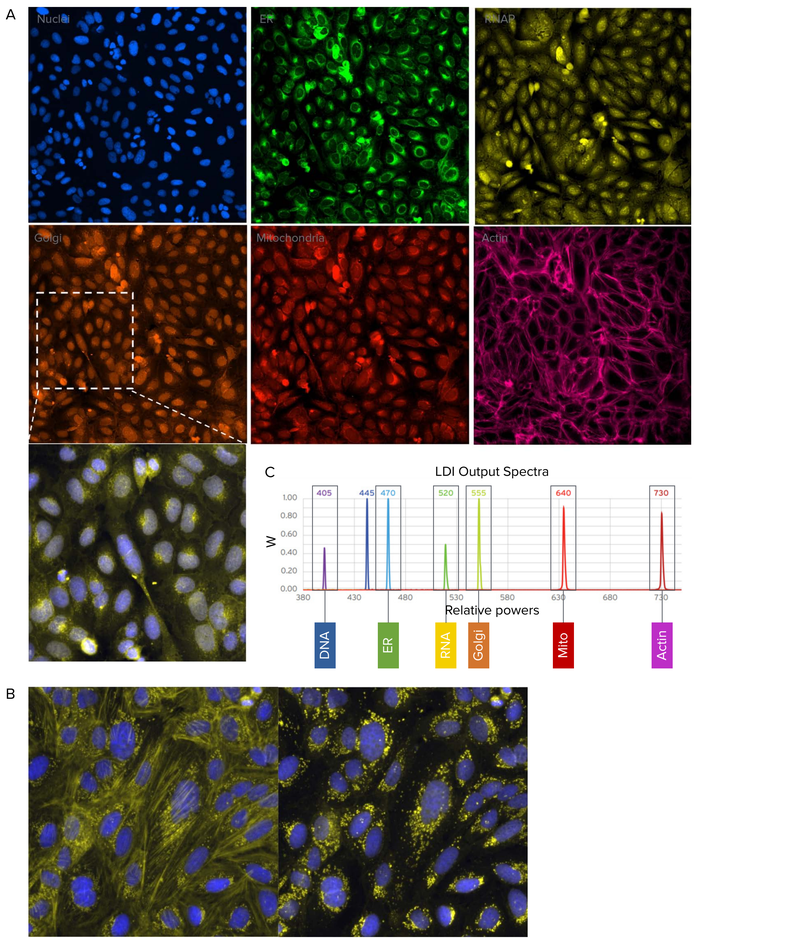


Figure 2. Cell painting assay modified Modified protocol using far-red light source to improve spectral separation in the Cell Painting assay. A) Phalloidin Alexa Fluor 750 was used to label actin in the modified protocol. Example images from a control well is shown. Inset (white box) is zoomed in and shown as a composite of the nuclei (blue) and golgi (yellow) channel. Golgi punctae are clearly seen distributed in a mostly perinuclear organization. B) Images of cells treated with tetrandrine. On the left is a reconstructed image of the actin and golgi channel merged (yellow). Right shows the image with only golgi (yellow) and nuclei (blue). The effects of tetrandrine on golgi distribution is clearer with the modified protocol (right). C) Laser light source and their relative powers shown. Detection of cellular compartment with their respective light source indicated.

Results

Feature Extraction

Using IN Carta software, the image analysis routine can be adjusted to achieve robust detection of cells and organelles (Figure 3). Deep learning semantic segmentation module (SINAP) may be used to improve detection of challenging features.

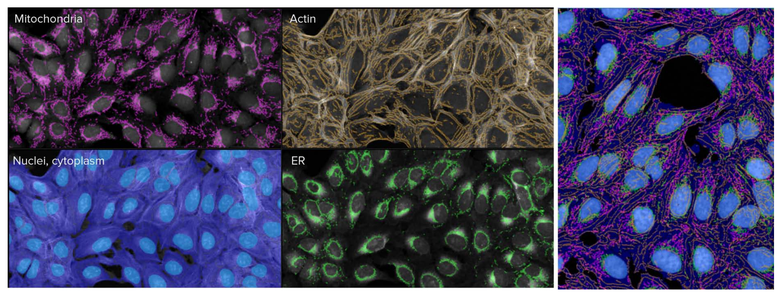
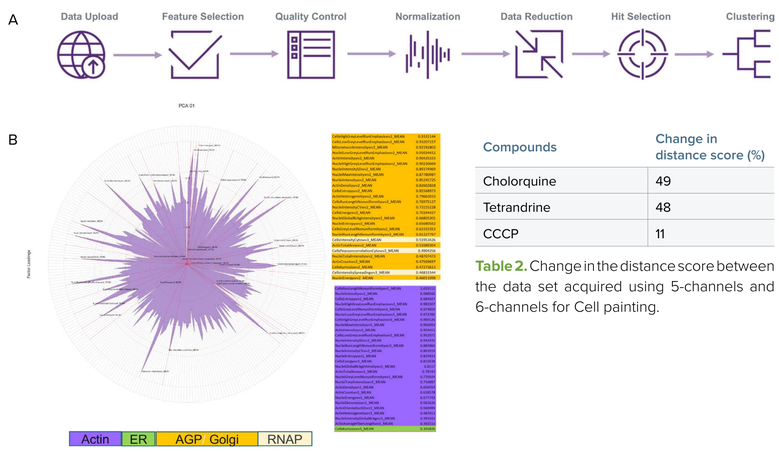


Figure 3. Feature extraction in IN Carta software Analysis was done in the IN Carta software to segment the various cellular structures. Here, the built-in nuclei model was used to achieve robust segmentation of nuclei across all treatments. For images acquired in six channels, 487 features were extracted per cell. Shown here are example images with feature mask overlay in IN Carta software.

Data analysis

Measurements from IN Carta software were uploaded into HC StratoMineR, an intuitive cloud-based data analytics platform for further data analysis[3] (Figure 4). Principal component analysis (PCA) was carried out on the data extracted from the standard Cell Painting assay vs the modified assay. Interestingly, measurements from the AGP channel make up most of the feature components in PCA 1 from 5-channel images. However, measurements from the actin channel make up most of the dominant features in PCA1 from the modified 6-channel images. This suggests that the modified assay can improve resolution of phenotypic profile between the actin and golgi components.

To quantify the differences between the two assays, we compared their phenotypic profiles based on their distance scores. This score represents the phenotypic distance a sample is from the negative control which can be used for hit selection in screening assays. We found an increase in the distance score for compounds known to affect the golgi pathway (Table 2). 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.



Compounds	Change in distance score (%)
Cholorquine	49
Tetrandrine	48
CCCP	11

Table 2. Change in the distance score between the data set acquired using 5-channels and 6-channels for Cell painting.

Figure 4. 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) PCA (generalized weighted least squares) was used to reduce the data to 15 components. Feature contributions to PCA1 is shown as a polar plot. Features color coded according to the cellular compartment they represent. PCA1 features from 5 channel (top) and 6 channel (bottom) cell painting acquisition. Note the differences in the feature contributions.

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 extra channel will also allow for the extension of the standard Cell Painting assay through the addition of project-specific biomarkers.

References

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3. Omta WA, et.al., *Assay Drug Dev Technol.* 2016 Oct;14(8):439–452.

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