Evaluation of the performance of various cell dyes for Cell Painting assay

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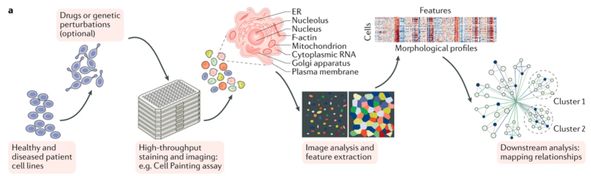
INTRODUCTION

Cell Painting, the most popular assay used for image-based profiling, involves staining plated cells with six small molecule dyes that mark the different compartments in a cell. Such profiles can then be used to discover connections between samples (whether different cell lines, different genetic treatments, or different compound treatments) as well as to assess changes in particular features resulting from each treatment. To better interrogate certain features, groups working with Cell Painting may wish to alter the standard dye panel while maintaining the ability to do overall profile clustering. In this study, we evaluate the performance of new dye products that can be used to observe live cell dynamics over time, to better separate channels, or to augment the traditional Cell Painting dye set.



(Chandrasekaran et al. 2020)

a) The typical workflow for generating image-based profiles using the Cell Painting assay.

b) Representative images from the Cell Painting assay. Six stains label eight cellular components, which are imaged in five channels.

METHODS

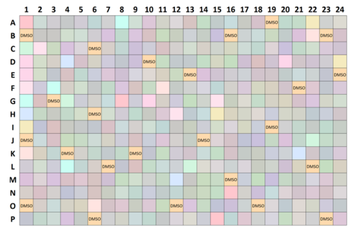
U2OS cells were perturbed with 90 different compounds based on the previously described JUMP-MoA2 plate and subsequently stained with one of the following dye sets: the standard Cell Painting (CP) dye panel from Revvity, the standard CP panel substituting PhenoVue 641 Mitochondrial with MitoBrilliant 646 from Tocris, the standard CP panel substituting PhenoVue 568 Phalloidin with PhenoVue 400LS Phalloidin from Revvity, or Saguaro ChromaLive plus Hoechst 33342. In one additional plate, cells were stained with ChromaLive dyes, imaged live, and then fixed and stained with the standard Cell Painting panel. Samples were imaged with a Revvity Opera Phenix microscope using 20x water objective, binning 2. The images for live cells (ChromaLive dyes) were taken in confocal mode; all others were captured in widefield. The features of the cells were extracted with Cell Profiler using standard protocols (Cimini et al, 2023). The extracted features from each of the dye sets were normalized with the negative controls (DMSO) and feature selected to create morphological profiles. The similarity of the profiles across the dye sets were compared using mean average precision (mAP) values, which indicate the degree of similarity between replicates of wells treated with the same compound. Higher mAP values indicate that all replicates are more similar to each other than to the controls.

For interactive, hoverable versions of all scatter plots:

http://broad.io/DyeSetEvaluationforCP

IMAGING RESULTS

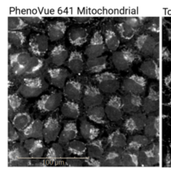
Plate Layout, color coded by unique compound



A set of 90 compounds was plated at 5mM in DMSO. This set is comprised of 45 compound pairs, each of which corresponds to a single mechanism of action. DMSO-only negative control wells were interspersed among the compound wells.

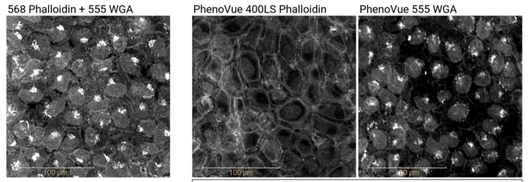
Cells were treated with a final concentration of 3µM of these compounds over 48h before live imaging or fixation.

Representative Images for Tocris MitoBrilliant™ 646



Revvity PhenoVue Dye Set with MitoBrilliant™ 646			
Fluorophore	Laser	Excitation	Emission
Hoechst 33342	405	350nm	461nm
PhenoVue 488	488	488nm	500-550nm
PhenoVue 555	561	561nm	570-630nm
PhenoVue 568	561	561nm	570-630nm
MitoBrilliant™ 646	**641**	**655nm**	**650-760nm**

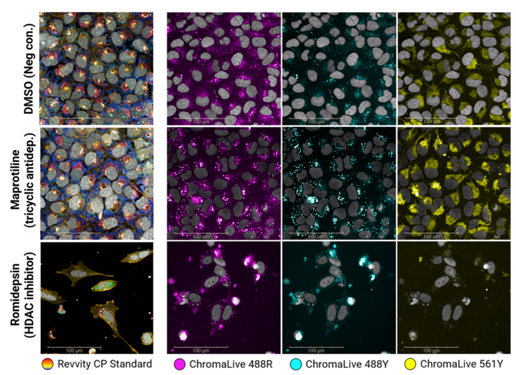
Representative Images for PhenoVue 400LS



In the original Revvity PhenoVue dye set for Cell Painting, Phalloidin and WGA are captured in the same channel (above). The use of PhenoVue 400LS Phalloidin allows Phalloidin-stained features to be captured separately from WGA-stained features.

Revvity PhenoVue Dye Set with PhenoVue 400LS Phalloidin			
Fluorophore	Laser	Excitation	Emission
Hoechst 33342	405	350nm	461nm
PhenoVue 400LS	**405**	**405nm**	**570-630nm**
PhenoVue 488	488	488nm	500-550nm
PhenoVue 555	561	561nm	570-630nm
PhenoVue 641	641	640nm	650-760nm

Representative Images for Saguaro ChromaLive

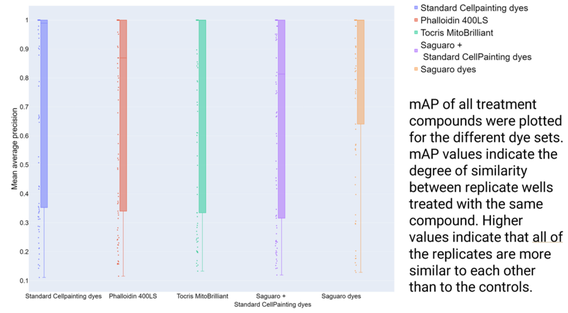


The effects of certain compounds, such as the tricyclic antidepressant maprotiline, are better observed with the live dye set from Saguaro. Other compounds, such as the HDAC inhibitor romidepsin, are similarly distinguished from control by the Saguaro live dye set and the standard Revvity Cell Painting Set. Other examples can be seen in the mAP scatterplot at right.

Saguaro ChromaLive Dye Set			
Fluorophore	Laser	Excitation	Emission
ChromaLive 488-Yellow	**488**	**488nm**	**500-630nm**
ChromaLive 488-Red	**488**	**488nm**	**630-750nm**
ChromaLive 561-Yellow	**561**	**561nm**	**570-630nm**
Hoechst 33342 (optional)	405	350nm	461nm

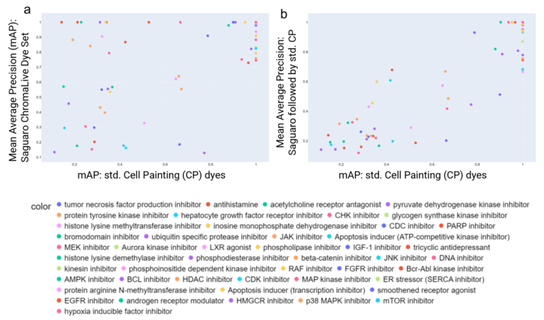
ANALYSIS RESULTS

Overall mean average precision values (mAP) across dye sets



mAP of all treatment compounds were plotted for the different dye sets. mAP values indicate the degree of similarity between replicate wells treated with the same compound. Higher values indicate that all of the replicates are more similar to each other than to the controls.

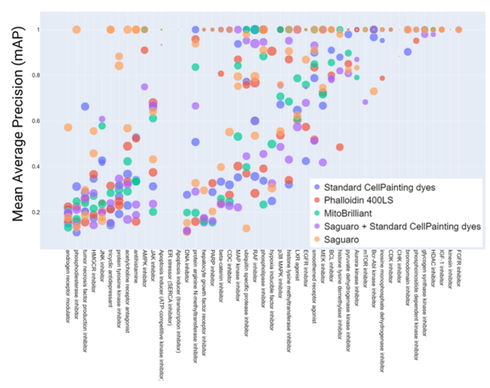
Compared to standard Cell Painting dye set, features measured from Saguaro live cell dyes have different performance characteristics for different MoAs



a) mAP performance per MoA using standard Cell Painting features vs live cell dye features. Performance per MoA is not often well correlated between plates with these feature sets.

b) mAP per MoA of Cell Painting features between plates which have (Y axis) or have not (X axis) previously been treated with live cell dyes and been imaged for live cell dyes over the course of compound treatment. Per-MoA performance is much more tightly correlated between these plates.

Mean average precision (mAP) based on compound MoA



Mechanism of Action (2 compounds per MoA)

Mean Average Precision values for all the compounds and all dye sets analyzed here, sorted by compound MoA. Since cell toxicity can be a major driving phenotype, the size of the markers represents the average number of cells present in the replicates.

KEY CONCLUSIONS

- All tested dye sets allow for good replicate identification with the 90 compounds on the JUMP-MoA2 plate.
- Dyes designed for live cell imaging show a different performance profile across different MoAs than standard Cell Painting dyes or their variants.

FUTURE DIRECTIONS & CONSIDERATIONS

- This work only tests 90 compounds from 45 mechanism of action classes. Ideal dyes for the biology of interest, as well as staining conditions, should always be confirmed for particular treatments and samples.
- This work only tests the ability to reliably distinguish compound-treated wells from the negative controls. Further experiments are needed to test generalization across mechanism of action and the ability to tie specific features to specific phenotypes.

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