Live Cell Painting: Drug Responses in Human Primary Patient Cells with a New Nontoxic Dye

Betty Li[1], Wiebke Schormann[1], Stanley Liu[1], Ilya Goldberg[2], Teresa Findley[2], Ibrahim Bilem[3], David W. Andrews[1].

[1]Sunnybrook Research Institute, [2]ViQi Inc., [3]Saguaro Technologies, Inc.

Overview

- High content imaging is widely used in phenotypic profiling, which helps identify healthy and diseased cellular states or predict drug mechanism of action [1].
- Live Cell Painting, using ChromaLive dye, enables screening of compound libraries in biologically-relevant culture conditions.
- ChromaLive is a mix-and-read and non-toxic dye that provides unique phenotypic fingerprints consistent with cellular phenotypes.
- In this study, ChromaLive was used to identify optimal treatment regime for a late-stage prostate cancer patient, and to predict drug mechanisms of action [2].

Introduction

In this study, a set of three screens was conducted on biopsy samples, taken before and after cancer progression to the aggressive neuroendocrine phenotype, in a Live High-Content Screening assay, using ChromaLive.

Image-based profiles analysis, done with a linear classifier in Harmony software, revealed hit compounds in all three screens, emphasizing the value of ChromaLive in HTS imaging for current needs in personalized medicine and phenotypic profiling.

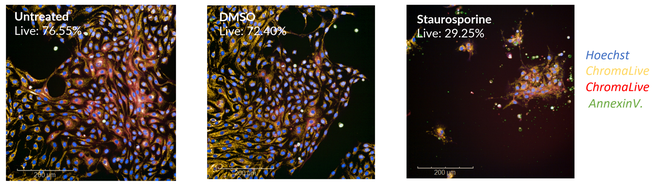


Figure 1. Live cell painting: linear classifier training images *(20x Water).*
Patient-derived prostate cancer cells imaged with an Opera Phenix HCI.

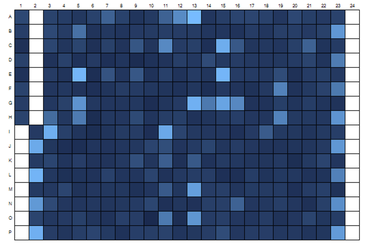


Figure 2. Representative plate map of the compounds screen used in this study.
The light blue wells correspond to hit compounds, with a low percentage of live cells, as determined by a linear classifier built in Harmony software.

Method – Screening in 3 Conditions

A library of 1,508 FDA-approved-compounds was used to run three separate high-content screens. The first two screens were performed in 2D monolayer cultures on biopsy samples, harvested before and after neuroendocrine differentiation. Identified Hit compounds identified were then tested on neuroendocrine phenotype organoids. Each compound was screened in duplicate at concentrations of 1uM and 10uM.

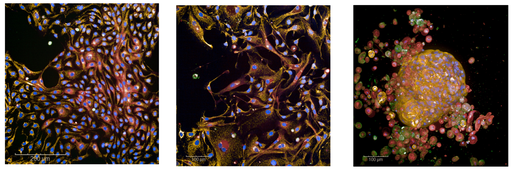
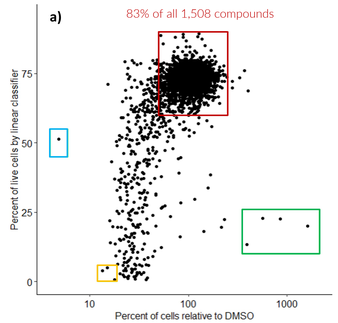


Figure 3. Representative images of ChromaLive-stained cells in each 3 screens.
Patient-derived prostate cancer cells before (left image) and after (middle) neuroendocrine differentiation. Right image depicts untreated 3D organoid in neuroendocrine state.

Identifying a Functioning Treatment

Analysis of the percentage of live cells along with cell count in each sample helped identify 9 different drugs (4a), that were then tested in 3D cultures to generate a list of candidate treatments (4b).

Mitoxantrone was clinically identified as the most appropriate treatment, leading to positive clinical outcomes, eight weeks post-treatment. This work highlights the translational impact of our methodology.



b)

Compound name	Percent Live (%)
Doxorubicin (Adriamycin) HCl	2.40
Mitoxantrone 2HCl	11.03
Dinaciclib (SCH727965)	3.68
Carfilzomib (PR-171)	3.24
Daunorubicin HCl	1.91
Ouabain	3.63
Cetylpyridinium Chlroide	6.77
Digoxin	5.74
Vinblastine sulfate	18.33
Puromycin 2 HCl	5.32

Figure 4. a) Scoring 1,508 drugs with a linear classifier trained to identify live vs dead cells.
Hit compounds identified with a combination of low cell count and low percentage of live cells (yellow). Most compounds (83%) had no significant impact (red), one compound was found to form persister cell populations (blue), and some compounds (green) were discarded due to significant debris that were scored as dead cells. **b) List of hit compounds from the 3 screens.** *Underlined compounds were hits in all 3 screens.*

Predicting Drug Mechanisms of Action

ChromaLive has also been shown to provide valuable insight into drug mechanism of action (MoA) using two AI-driven image analysis tools (Harmony software and ViQi autoHCS). Interestingly, consistent results were obtained with both methods, which demonstrates the robustness of ChromaLive at predicting drug MoA.

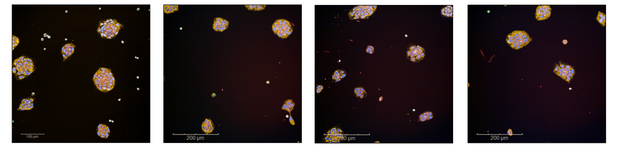
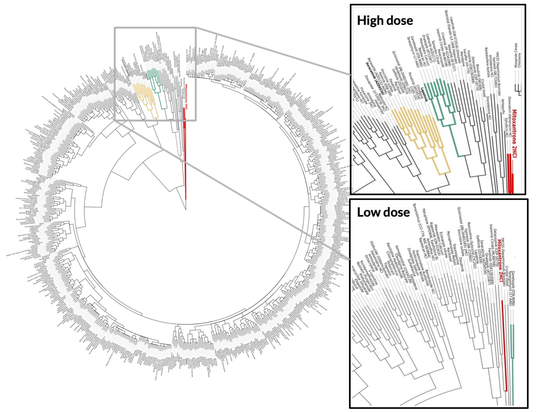


Figure 5a. Representative images from the 20 kinase inhibitors identified with a linear classifier built on Harmony Software. Example of compounds with similar image-based profile (cell colonies), which were found to act as receptor tyrosine kinase inhibitors.



1. **Chemotherapeutic**
   Mitoxantrone
   Doxorubicin
   Epirubicin
2. **HSP90 inhibitors**
   Onalespib
   Ganetespid
   Tanespimycin
3. **RTK inhibitors**
   Lapatinib
   Gefitinib
   DasatinibPelitinib
   Saracatinib

Figure 5b. Phenotypic clustering for 643 compounds at high dose using ViQi AutoHCS AI analysis platform: Clusters correspond to compounds sharing similar MOA. *Dendrograms (left) and insets (right) were generated by an AI trained to discriminate compound-induced phenotypes, based on ChromaLive staining. Cluster 1 (red): Independent replication using ViQi AutoHCS, highlighting compounds identified by the linear classifier (Fig. 5a). Notable overlap includes topoisomerase inhibitors doxorubicin, epirubicin, and mitoxantrone.*

Conclusion

This study highlights the value of ChromaLive as a "live cell painting" assay in high-throughput screening. By eliminating the need for washing and fixation steps, this new approach overcomes challenges associated with sample alterations, and enables the detection of drug-induced transient-effects.

To the best of our knowledge, this is the first “live cell painting” proof-of-concept screening study. This study of 1,500 FDA-approved compounds, performed on live patient-derived cells, emphasizes the potential of ChromaLive for advancing personalized medicine and drug discovery efforts.

References

1. Zoffmann, S. et al. Machine learning-powered antibiotics phenotypic drug discovery. Sci. Rep. 9, 5013 (2019).