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

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Overview

Image-based profiling has proven to be useful in generating phenotypic profiles that help identify healthy and diseased cellular states, or predict drug mechanism of action [1].

The cell painting method has been popularized for generating such image profiles. It relies on fixed cells and multiple sample processing steps that can result in sample alterations, and the loss of crucial kinetic information.

Here, we present what is to our knowledge a first “live cell painting” compound screening study. A novel mix-and-read and non-toxic dye – which generates unique phenotypic fingerprints consistent with cellular phenotypes – was used to identify optimal treatment regime for a late-stage prostate cancer patient. Further image analysis using biologist-friendly AI tools helped predict drugs mechanisms of action.

Introduction

In response to the dire clinical scenario of a late-stage prostate cancer patient unresponsive to standard-of-care treatments, our study aimed to identify a viable treatment regime through high-content screening. Taking biopsy samples before and after the cancer evolved to the aggressive neuroendocrine phenotype, we performed three screens, including a unique 3D culture study.

Leveraging ChromaLive non-toxic dye and a linear classifier in Harmony software, we sought hit compounds common to all three screens. This innovative approach not only highlighted the value of our methodology for personalized medicine, but also showed the relevance of ChromaLive for phenotypic profiling in live cells, avoiding the pitfalls of fixed cell techniques.

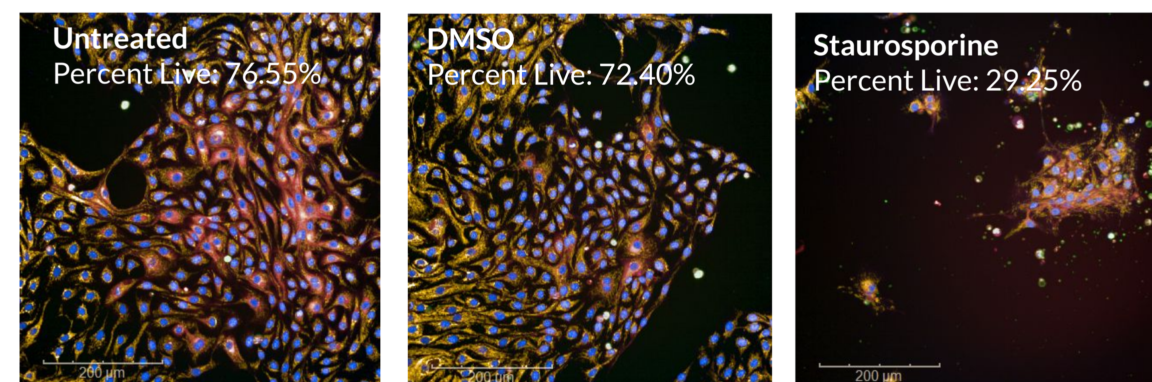


Figure 1. Live cell painting: linear classifier training images.
20x Water, Hoechst ChromaLive ChromaLive AnnexinV.
Images of patient-derived prostate cancer cells acquired on an Opera Phenix high-content imager.

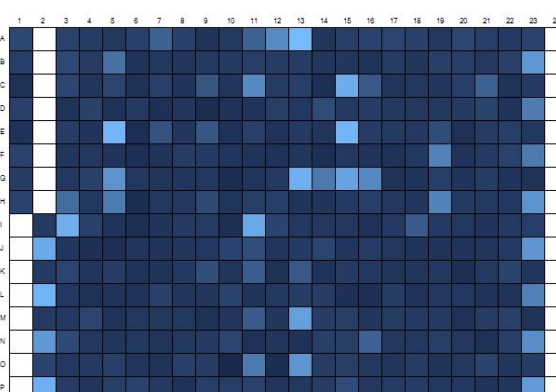


Figure 2. Example plate map from our high-content screening study.
Darker wells had a higher percentage of live cells as determined by a linear classifier built in Harmony software. *The light blue wells represented hit compounds, with a low percentage of live cells.*

Method – Screening in 3 Conditions

In our study, we employed a library of 1,508 compounds from the FDA-approved repertoire to conduct high-content screening on biopsy samples using ten 384-well plates. Each compound was screened in duplicate at a concentration of 1uM, and a total of three separate screens were performed. The first two screens used 2D monolayer cultures from biopsy samples both before and after neuroendocrine differentiation. Hit compounds identified from both screens were then tested in a final 3D culture high-content screen, using samples that had the neuroendocrine phenotype.

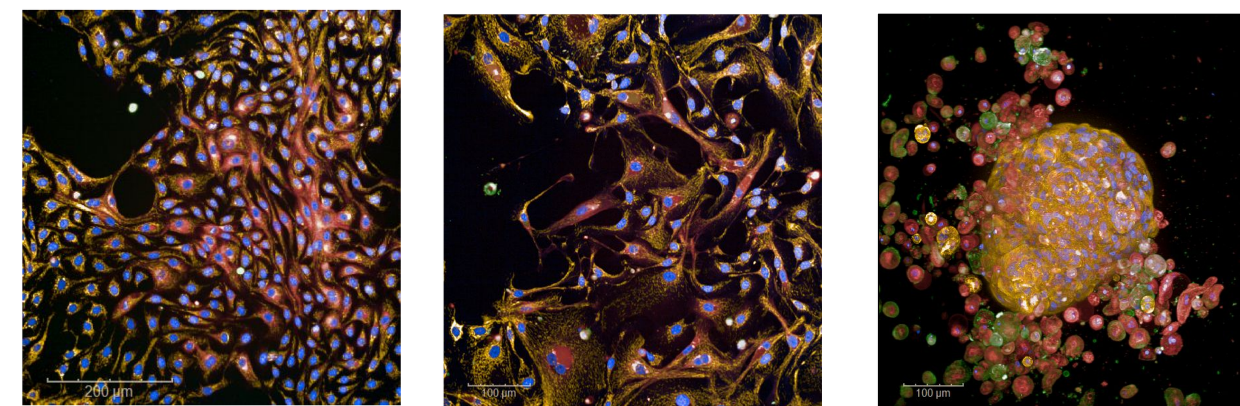
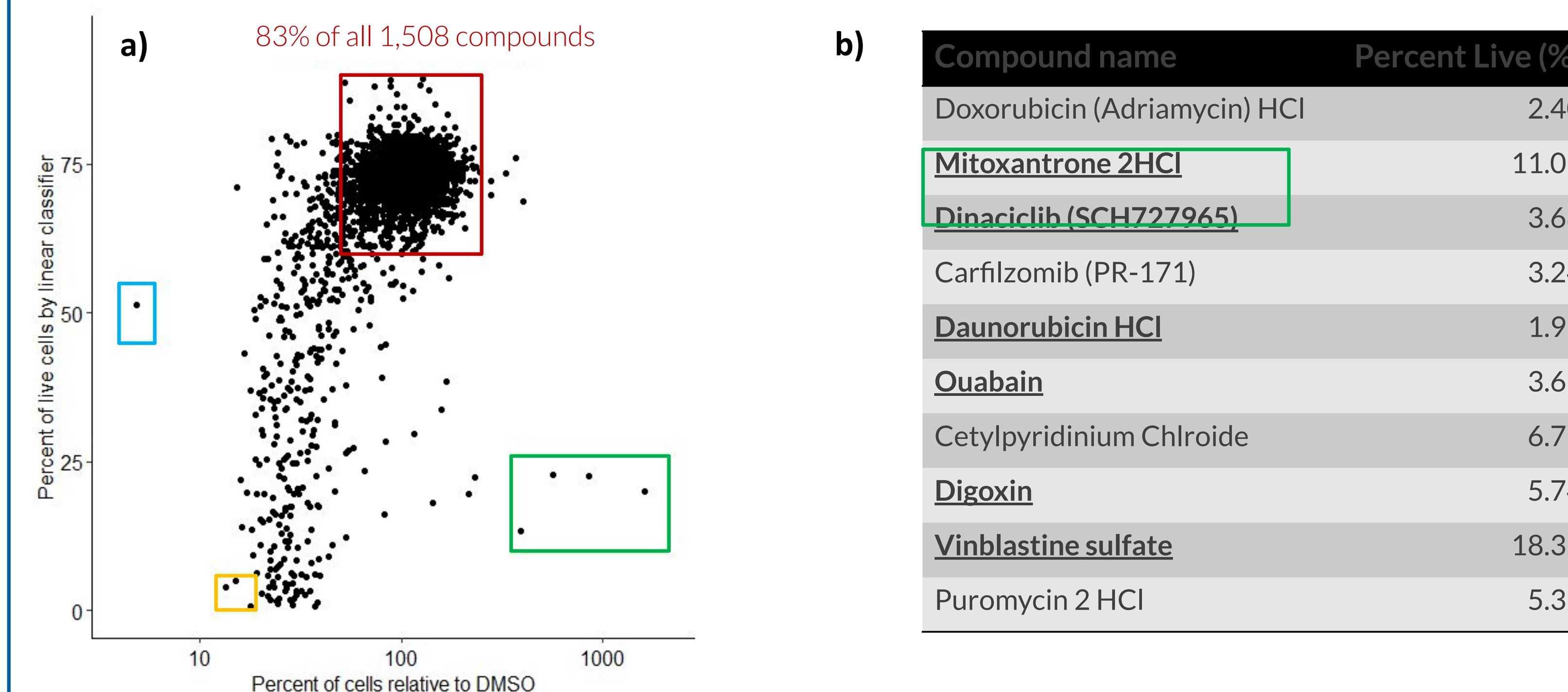


Figure 3. Example images of cells stained with ChromaLive in each 3 screens.
Patient-derived prostate cancer cells before (left image) and after (middle) neuroendocrine differentiation. Right image is an example image of untreated 3D organoid in neuroendocrine state.

Identifying a Functioning Treatment

Analysis of the percentage of live cells in each sample relative to a DMSO control helped identify a series of hit compounds, which were refined down to 9 different drugs by cross-examination with cell count in each sample (4a). These 9 hit compounds were then tested in 3D cultures to generate a list of candidate treatments (4b). Clinical considerations guided the selection of mitoxantrone, and eight weeks post-treatment, the patient exhibited a positive response, highlighting the translational impact of our methodology.



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 are found 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 as they presented 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

In pursuit of the more comprehensive phenotypic information that ChromaLive nontoxic dye provides, we employed two automated AI image analysis tools to predict compound mechanisms of action. First, a simple linear classifier was trained on Harmony software to identify compounds with similar image-based profiles (figure 5).

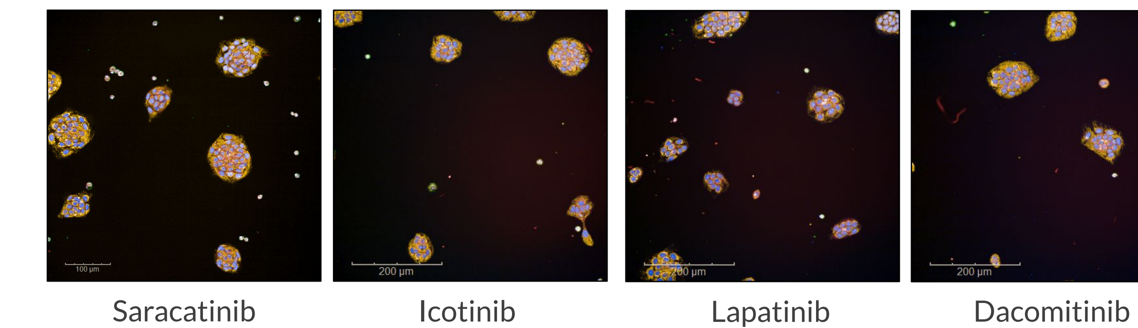


Figure 5. Example images from the 20 kinase inhibitors identified with a linear classifier. Four example compounds which result in compact cell colonies are depicted. These compounds were then found to act as receptor tyrosine kinase inhibitors.

Second, we ran a similar analysis, but used ViQi AutoHCS AI analysis platform, a cloud-based tool that automatically analyzes large microscopy image sets.

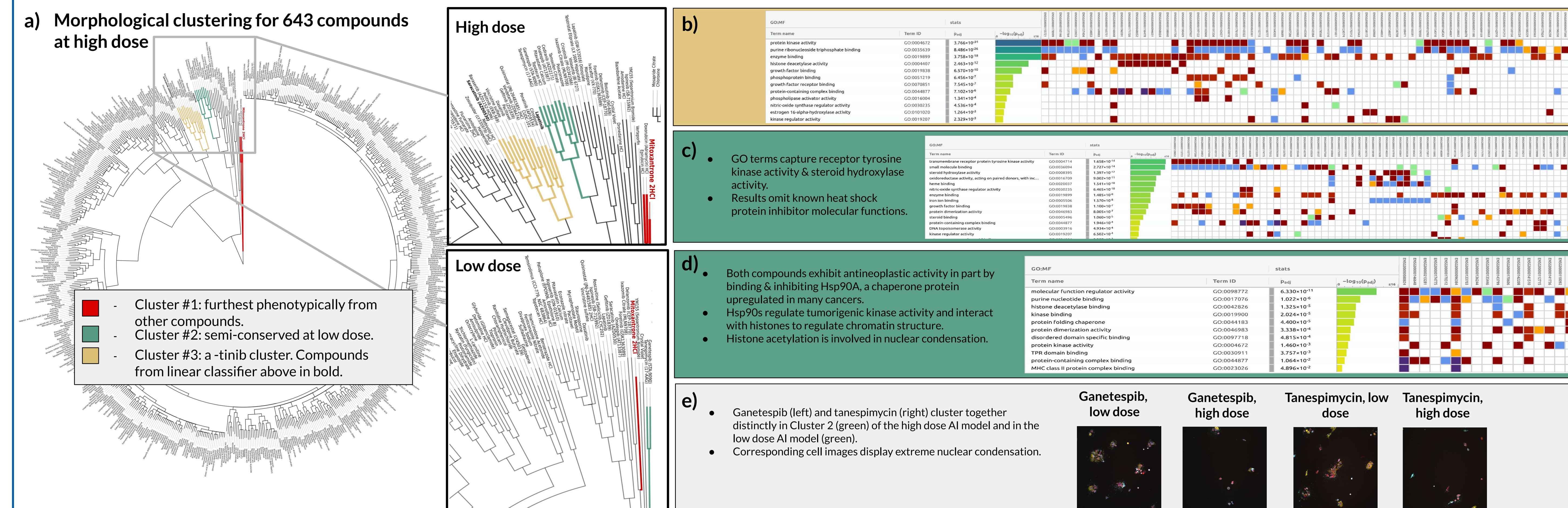


Figure 6. ViQi AutoHCS identifies morphological clusters which correspond to shared compound mechanism of action (MOA). ***a)*** *Dendrograms (left) and insets (right) were generated by an AI trained to discriminate phenotypes induced by compound treatments based on fluorescent images of treated cells. Cluster 1 (red): Independent replication using ViQi AutoHCS, highlighting compounds identified by the linear classifier (Fig. 5). Notable overlap includes topoisomerase inhibitors doxorubicin, epirubicin, and mitoxantrone.* ***b-e)*** *Functional enrichment results are shown for selected clusters with a similar phenotype. Target genes for clusters of compounds were identified with STITCH, and used for gene enrichment analysis with g:Profiler.* ***b)*** *Cluster 3 (gold) contains tyrosine kinase inhibitors including the two compounds identified above by the linear classifier (saracatinib, lapatinib).* ***c)*** *Cluster 2 (green) at high dose shows an enrichment of tyrosine kinase activity and steroid hydroxylase activity.* ***d)*** *Conserved compound pair Ganetespib and Tanespimycin (green at high and low dose) shows enrichment for HSP proteins. This MoA is not picked up at high dose (c) despite the cluster containing several known HSP90 inhibitors.* ***e)*** *Images of compounds in (d) show nuclear condensation.*

Conclusion

Our "live cell painting" approach, utilizing a novel mix-and-read, non-toxic dye, represents a significant advancement in high-content screening. By eliminating the need for washing and fixation steps, we overcome challenges associated with sample alterations, and enable the detection of dynamic phenotypic changes in live cells.

The identified treatment regime for a late-stage prostate cancer patient and the phenotypic profiling study we performed highlights the practical implications of our approach, emphasizing its potential 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).
2. ViQi AutoHCS: https://viqiai.com/autohcs

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