

Multomics profiling of the JUMP-MoA compound set.

Introduction

High-dimensional profiling technologies are increasingly used in drug discovery to characterize compound activity beyond single-target or single-endpoint measurements. Transcriptomic profiling captures changes in gene expression, providing molecular information about pathway activation, mechanism of action (MoA), and potential off-target responses. In parallel, cell painting uses multiplexed fluorescence imaging to quantify changes in cellular morphology across multiple organelles and cellular compartments, generating high-dimensional phenotypic profiles. Because these approaches interrogate different layers of cellular biology, they can provide complementary information about compound activity, with previous studies showing that some perturbations and pathways are better captured by one modality than the other¹.

The JUMP-Cell Painting Consortium (Joint Undertaking in Morphological Profiling) was established as a collaboration between the Broad Institute of MIT and Harvard, pharmaceutical companies, non-profit organizations, and technology partners, including Revvity, to advance morphological profiling and create large, standardized public datasets for drug discovery². The consortium generated cell painting resources spanning chemical and genetic perturbations and developed benchmark compound sets for evaluating profiling technologies and computational approaches. One of them, the JUMP-MoA resource, was designed as a compact 384-well benchmark containing 90 compounds representing 47 annotated MoA classes.



Building on this framework, JUMP-MoA compounds were profiled in U2OS and HepG2 cells using MERCURIUS™ DRUG-seq for 3' mRNA profiling and MERCURIUS™ Total DRUG-seq, which provides information across full-length total RNA transcripts, together with cell painting for morphological profiling. The resulting datasets were evaluated individually and after multiomics integration to determine their ability to resolve compound and MoA relationships using unsupervised clustering and supervised classification.

Methods

Experimental design

U2OS and HepG2 cells were treated with 80 compounds from the JUMP-MoA collection, representing 47 annotated MoAs. DMSO was included as control. Compounds were tested at 3 μ M for 48 h, consistent with the JUMP-MoA recommended concentration and the compound-treatment duration established during optimization of the JUMP cell painting protocol.

Of the 47 MoAs, 14 were represented by a single compound, while 33 were represented by compound pairs. Most treatments were analysed with four replicates. Eight of the single-compound MoAs were represented by eight replicates, and selumetinib, a MEK inhibitor, was also analysed with eight replicates (Figure 1). Matched compound treatments were profiled by cell painting and by transcriptomic workflows using MERCURIUS™ DRUG-seq and MERCURIUS™ Total DRUG-seq.

Transcriptomic and cell painting profiling

Transcriptomic profiles were generated using MERCURIUS™ DRUG-seq and MERCURIUS™ Total DRUG-seq. The principal single omics versus multiomics analyses presented here

focused on MERCURIUS™ Total DRUG-seq. Libraries were sequenced on an Element Biosciences AVITI™ System using a paired-end read configuration, where Read 1 had 28 bp (sample barcode + UMI) and Read 2 had 100 bp (RNA sequence). The sequencing depth was approximately 4 million read pairs per sample for the HepG2 cells and 6 million read pairs per sample for the U2OS cells. Transcriptomic data were processed and normalized prior to downstream analysis.

Cell painting data were filtered and normalized using established procedures for morphological profiling prior to downstream analysis.

Data integration and analysis

Transcriptomic and cell painting profiles were first evaluated independently. Multiomics integration was then performed using regularized generalized canonical correlation analysis (RGCCA), a multiblock statistical framework designed to identify relationships between multiple sets of variables measured on the same samples³. Integrated representations were compared with the individual transcriptomic and morphological datasets.

Uniform Manifold Approximation and Projection (UMAP) was used to visualize relationships among treatments and MoA classes. Clustering performance was quantified using silhouette scores, enabling comparison of cell painting, transcriptomic, and integrated profiles. Supervised machine-learning models were used to predict mechanism of action from cell painting and transcriptomic profiles. Classification performance was evaluated across 7 ML models, and accuracy was averaged across models⁴. For the per-MoA comparison, mean classification accuracy for each mechanism of action obtained from cell painting was plotted against that obtained from MERCURIUS™ Total DRUG-seq.

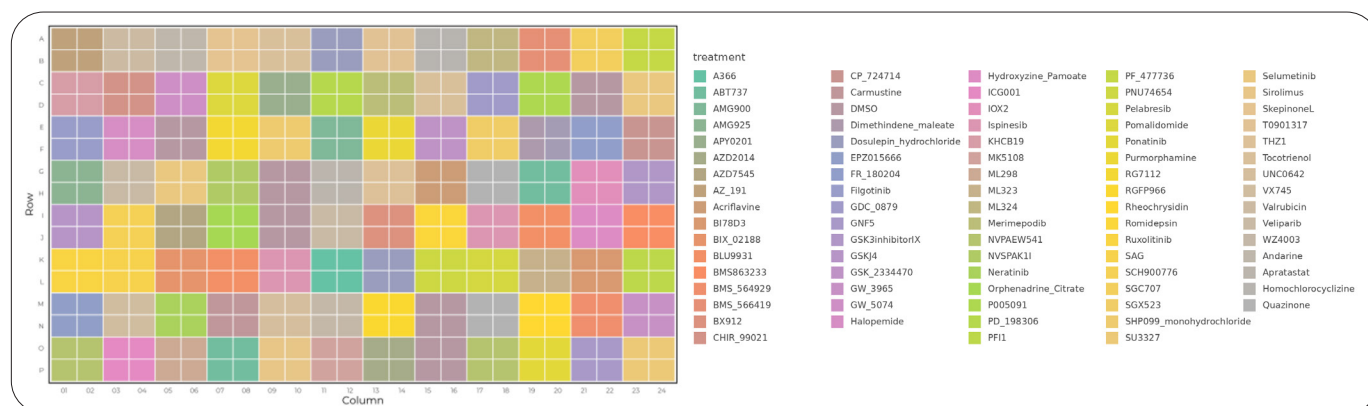


Figure 1: JUMP-MoA compound plate map. Layout of the 384-well plate used for compound profiling. Each color represents a distinct compound, with repeated colors indicating replicate wells distributed across the plate. The compound identity corresponding to each color is shown in the legend.

Results

Transcriptomics and cell painting show distinct clustering patterns

UMAP visualization was used to compare clustering of MERCURIUS™ Total DRUG-seq, cell painting, and integrated profiles in HepG2 and U2OS cells. The individual transcriptomic and cell painting datasets showed distinct clustering patterns, with clustering performance varying

between the two cell types and profiling approaches. Integration of the transcriptomic and cell painting datasets produced a combined representation of the compound profiles (Figure 2).

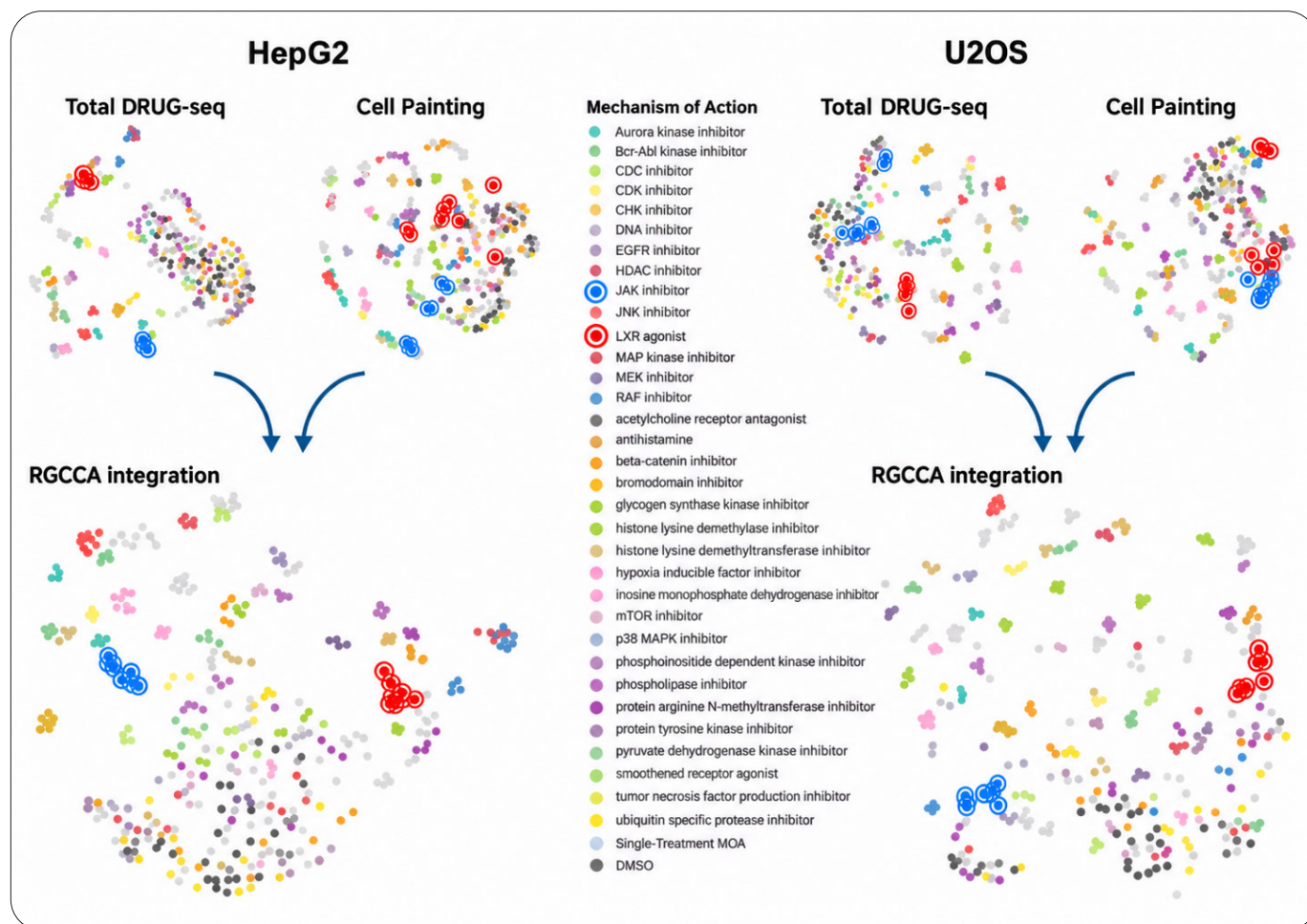


Figure 2: UMAP visualization of compound profiles in HepG2 and U2OS cells generated by MERCURIUS™ Total DRUG-seq, Cell Painting, and RGCCA-based multiomics integration. Each point represents a compound treatment, coloured according to annotated mechanism of action. Selected MoA classes, including JAK inhibitors and LXR agonists, are highlighted. In both cell types, the integrated RGCCA representation shows visually clearer separation of several compound classes, consistent with the quantitative clustering analysis presented in Figure 3.

Integration of transcriptomic and cell painting data improves clustering

Silhouette-score analysis was used to quantitatively compare clustering performance across the individual and integrated datasets. In HepG2 cells, both 3' mRNA MERCURIUS™ DRUG-seq and MERCURIUS™ Total DRUG-seq, which provides full-length total RNA transcript information, showed higher clustering performance than cell painting, with MERCURIUS™ Total DRUG-seq achieving the highest silhouette scores among the single omics approaches. In U2OS cells, MERCURIUS™ Total DRUG-seq also clearly outperformed cell painting, whereas 3' mRNA MERCURIUS™ DRUG-seq showed lower clustering performance. Integration of MERCURIUS™ Total DRUG-seq and cell painting further improved clustering in both cell types, with a particularly pronounced improvement in U2OS cells (Figure 3). These results show that clustering performance depends on both cell type and profiling modality and support the complementarity of transcriptomic and morphological profiling in this dataset.

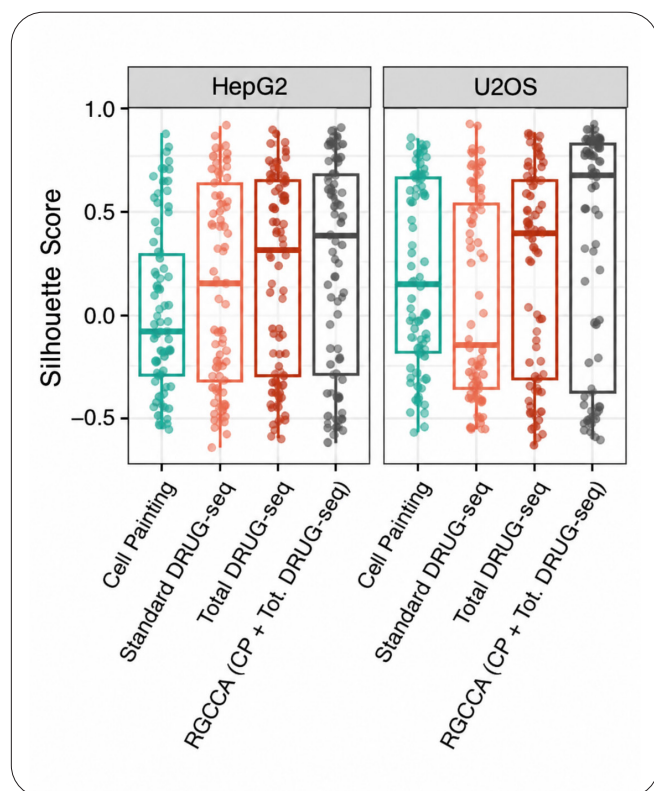


Figure 3: MoA-related clustering performance across Cell Painting, MERCURIUS™ DRUG-seq, MERCURIUS™ Total DRUG-seq, and integrated RGCCA profiles. Silhouette scores are shown for HepG2 and U2OS cells. Points represent individual observations, and boxplots summarize the score distributions. Higher scores indicate better separation of compounds by mechanism of action. Integrated RGCCA profiles showed the strongest overall clustering performance.

Transcriptomic profiling shows strong supervised classification performance

Supervised classification was subsequently used to evaluate MoA prediction using the individual profiling modalities and integrated datasets (Figure 4A). In U2OS cells, MERCURIUS™ Total DRUG-seq achieved a median per-MoA classification accuracy of approximately 80 %, based on accuracies averaged across 7 ML models. Integration of Total DRUG-seq with cell painting further increased median MoA classification accuracy in U2OS, consistent with complementary information provided by transcriptomic and morphological profiling. In contrast, integration did not improve classification performance in HepG2 cells (Figure 4B).

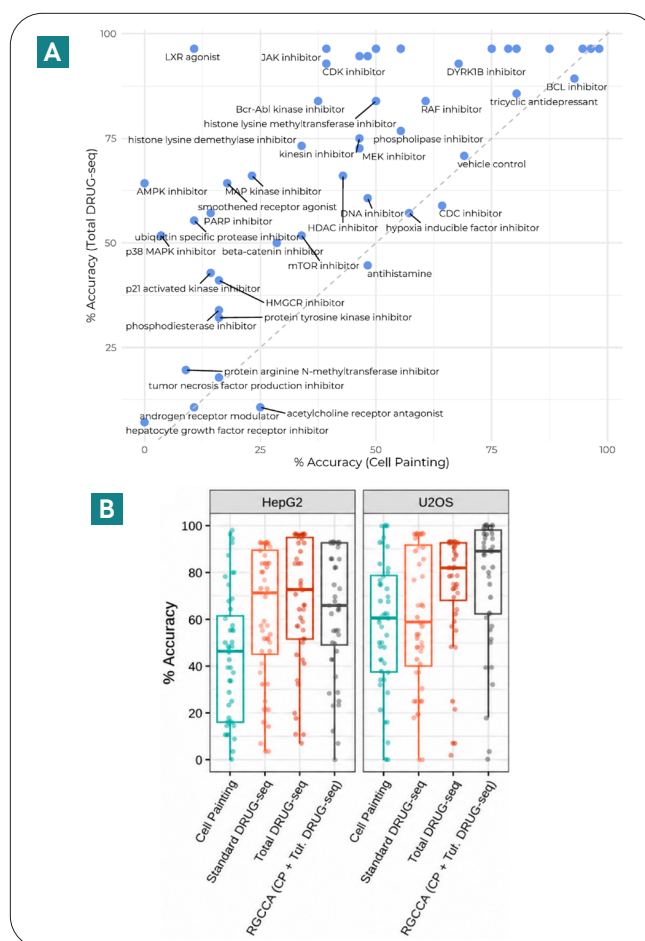


Figure 4: (A) MoA classification accuracy across profiling modalities in HepG2 cells. Each point represents an individual MoA, comparing Cell Painting and MERCURIUS™ Total DRUG-seq accuracy averaged across seven machine-learning models; points above the diagonal indicate higher accuracy with Total DRUG-seq. (B) Boxplots of per-MoA classification accuracy (%) in HepG2 and U2OS for Cell Painting, MERCURIUS™ DRUG-seq, MERCURIUS™ Total DRUG-seq, and RGCCA-based integration of Cell Painting and MERCURIUS™ Total DRUG-seq. Points represent individual MoAs, with accuracies averaged across seven machine-learning models; boxplots summarize their distributions, and higher values indicate better MoA classification.

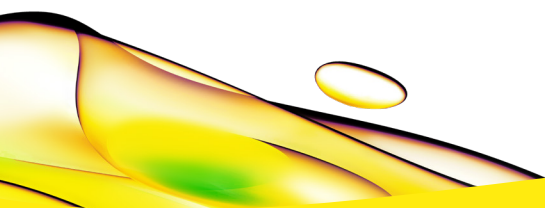
Conclusion

This study demonstrates that transcriptomic and morphological profiling provide complementary views of compound-induced biology across the JUMP-MoA panel. In the present analysis, transcriptomic profiling generally provided stronger MoA clustering than cell painting alone, while integration of MERCURIUS™ Total DRUG-seq and cell painting improved MoA clustering in both U2OS and HepG2 cells. Supervised classification similarly favoured transcriptomic profiles, although the benefit of multiomics integration was cell-type dependent, improving classification in U2OS but not in HepG2.

These results support the use of MERCURIUS™ DRUG-seq and MERCURIUS™ Total DRUG-seq as scalable molecular complements to high-content imaging. Cell Painting provides a rich phenotypic representation of cellular responses, while transcriptomic profiling provides gene- and pathway-level molecular readouts that facilitate mechanistic interpretation. Combining these modalities can improve mechanism-of-action resolution and provide a more comprehensive view of compound activity, although the added value of integration depends on the cellular model and analytical endpoint.

References

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