Cell Painting, a High-Content Image-based Assay for Morphological Profiling using Multiplexed Fluorescent Dyes, applied to EU-OPENSCREEN Compound Library

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EU-OPENSCREEN (EU-OS)

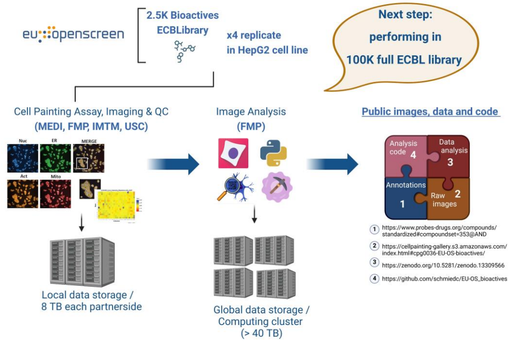
Fundacion MEDINA (MEDI) is one of the partnersides of EU-OS, which is a not-for-profit European Research Infrastructure Consortium (ERIC) for chemical biology and early drug discovery. EU-OS aims to make available to the scientific community a full characterization of the European Chemical Biology Library (ECBL), incuding a bioprofiling of its 100k compounds via Cell Painting.

Cell Painting

Morphological profiling by a multi-stain approach combined with confocal high content images that contain information about subcellular changes triggered by stressors like small chemical compounds

AIM

Four EU-OS partner sites (FMP, IMTM, MEDI, and USC) participated in an interlaboratory Cell Painting assay to screen ~2,5K bioactive compounds of ECBL in HepG2 cells.



Cell Painting Assay workflow

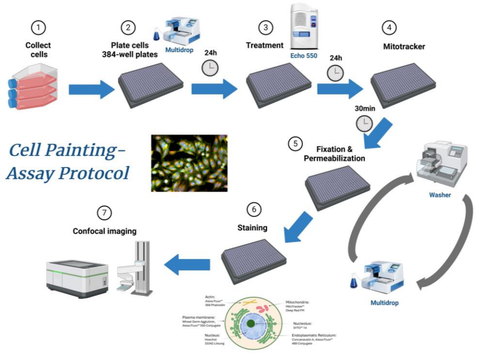


Image Acquisition and Profile Generation

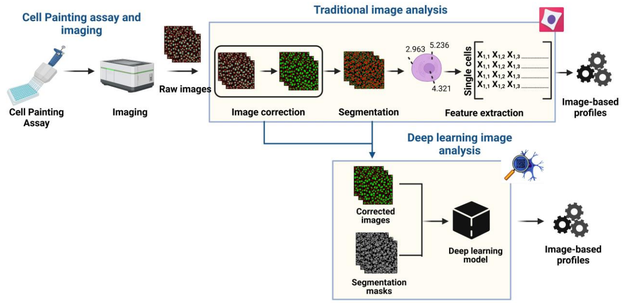
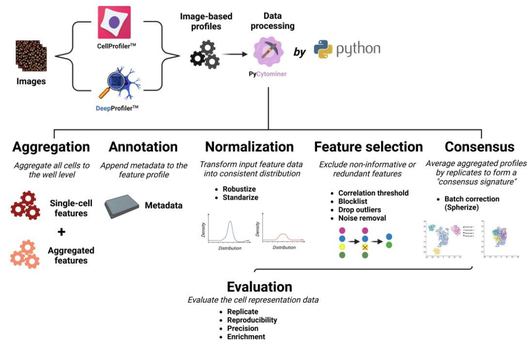
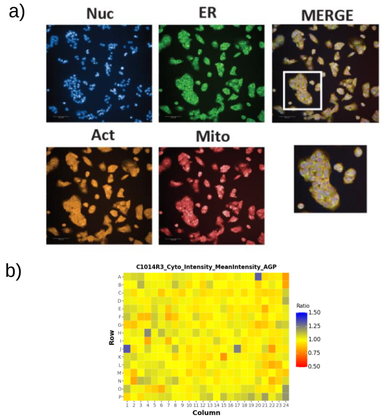


Image-based profiler and Data Processing



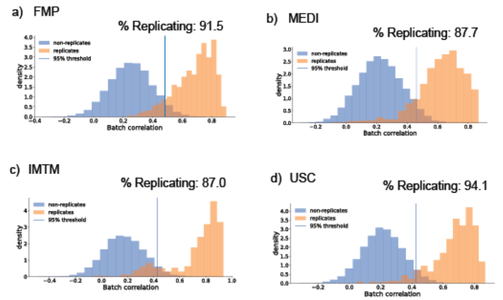
Cell painting data analysis is a combination of Traditional image analysis and Deep Learning analysis (by CellProfiler & DeepProfilerr) with image-based profiler by Pycytominer (by Phyton)

Confocal Images & Data QC



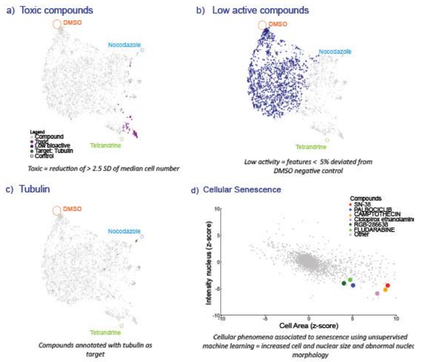
a) Confocal images of Nuclei (Hoechst 33342), Endoplasmic Reticulum (ConcanavalinA Alexa488 and SYTO14), Act (Wheat germ agglutinin Alexa 555 and Phalloidin Alexa 568) and Mito (Mitotracker Deep Red) acquired with Operetta (Revvity) b) Data QC: Representative feature of a selected plate.

Reproducibility of Dataset Profiles Interlaboratory



a) Percentage of replication based on 861 highly bioactive and non-toxic compounds in HepG2 cells in FMP. b) Percent replication based on 846 highly bioactive and non-toxic compounds in HepG2 cells in MEDI. c) Percent replication based on 676 highly bioactive and non-toxic compounds in HepG2 cells in IMTM. d) Percent replication based on 608 highly bioactive and non-toxic compounds in HepG2 cells in USC.

Compound characterization by profiles



Morphological feature space of 4 datasets. (a-c) using UMAP (Uniform Manifold Approximation and Projection) after feature reduction and filtering of non-reproducible compounds. Controls (DMSO, Tetrandrine and Nocodazole) are labelled. Color labeling for toxic (a), low active (b), and compounds annotated for tubulin as target (c). (d) Assessment of specific parameters indicative of cellular senescence.

CONCLUSIONS

- By mapping the resulting morphological features across cellular compartments to the activity and toxicity of the basic compound targets, we performed a comprehensive characterization of the dataset, demonstrating their potential to determine mechanisms of action (compound associated to cellular senescence) and targets (such as tubulin).
- The data generated and its application to the whole ECBL library will provide a source of powerful computational approaches to unlock the potential of small molecules, and thereby accelerate early drug discovery.

Affiliations

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