Title: **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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Abstract:

Fundacion MEDINA (MEDI) is one of the partner sides of EU-OPENSCREEN (EU-OS), which is a not-for-profit European Research Infrastructure Consortium (ERIC) for chemical biology and early drug discovery. EU-OS operates an open-access database and owns compound collections, among which is the European Chemical Biology Library (ECBL), consisting of over 100K compounds. One of the goals of the EU-OS is to characterize each of the compounds before they are utilized by the scientific community, and one of the most powerful tools to configurate this bioprofiling is the Cell Painting. Morphological profiling using the Cell Painting assay has emerged as a highly relevant method for drug discovery research. This method involves a multi-stain approach combined with confocal high content images which contain information about morphological changes in cellular compartments triggered by stressors like small chemical compounds. Four EU-OS partner sites (FMP, IMTM, MEDI, and USC) have successfully established and validated an interlaboratory Cell Painting assay to screen ~2,5K compounds of the EU-OS bioactive compound library in HepG2 cells.

We have established and successfully implemented the image acquisition and data analysis workflow for rapid analysis and extraction of cell morphological profiles. The results confirmed the quality of the generated data, which is comparable to other established opensource Cell Painting datasets. An interlaboratory analysis of replicates of compound treatments validates the robustness and reproducibility of the generated data. By mapping the resulting morphological features across cellular compartments to the activity and toxicity of the basic compound targets, we were able to perform a comprehensive characterization of the datasets as well as to demonstrate their potential for determining mechanisms of action. The raw images and morphological features will be made publicly available, adding value to existing morphological profiling datasets, and further facilitating a precise phenotypic characterization of compounds.

These data, and the data generated by applying this methodology to the full ECBL library, will provide a rich source of powerful computational approaches that promise to unlock the hidden potential of many small molecules and thereby accelerate early drug discovery.