m3DinAI-HCS DrugQuest

AI-Enhanced High-Content Screening for Quantifying Drug Cytotoxicity in 3D Cancer Models

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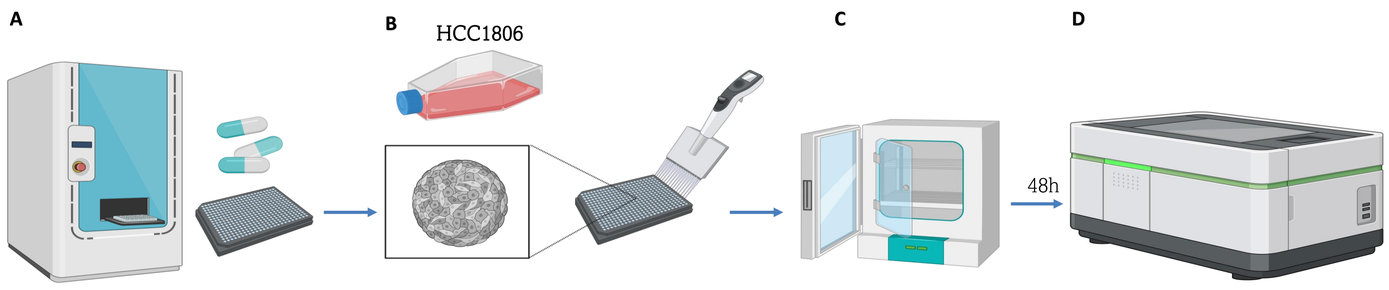
m3DinAI HCS DrugQuest

INTRODUCTION

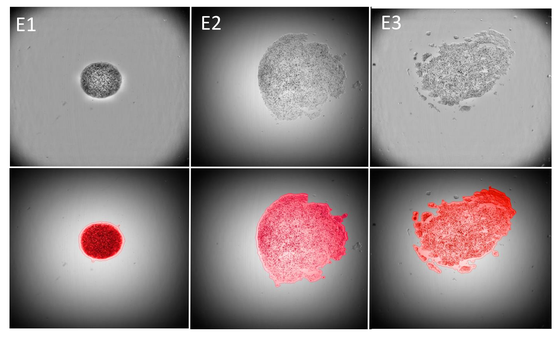
The use of **3D spheroids** that mimic tumors has significantly advanced the field of cancer drug discovery. However, characterizing and quantifying phenotypic alterations in spheroids treated with antitumoral compounds remains a challenge. **m3DinAI HCS (high content screening) DrugQuest** is a machine learning-based tool designed to analyze the cytotoxic effects in 3D spheroids using **high-content imaging (HCI)** data, minimizing manual work and reducing human bias. In this study, m3DinAI has been utilized to characterize the cytotoxic effects of a subset of microbial natural products (NPs) on breast cancer spheroids.

3D PROTOCOL



(**A**) Microbial extracts from MEDINA´s propietary collection are dispensed into 384-well plates (Greiner Bio-One) using the ECHO 550 acoustic dispenser (Beckman Coulter). (**B**) 3D spheroids are formed using Nanoshuttles (Greiner Bio-One) (**C**) during a 48-hour incubation period. (**D**) Images are acquired using the Operetta High Content Imaging System (Revvity).

Segmentation and Feature Extraction



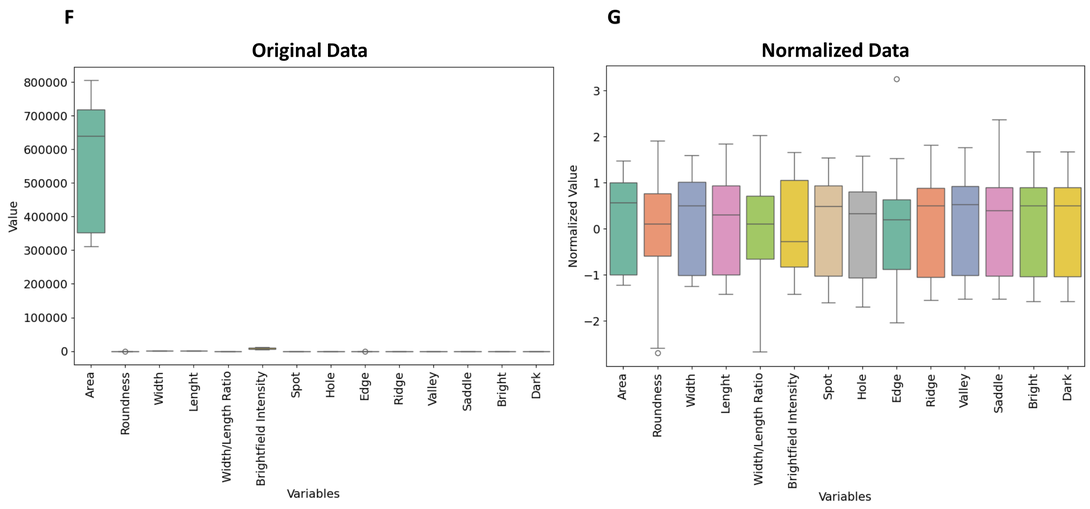
(**E**) A protocol has been developed using the Harmony Software (Revvity) to define “healthy” spheres (E1) vs. spheres altered by treatments, referred to as non-spheres (E2 and E3). The region considered for data acquisition is highlighted in red, from which a total of 14 features were extracted for data analysis.

m3DinAI | QC

HCS DrugQuest

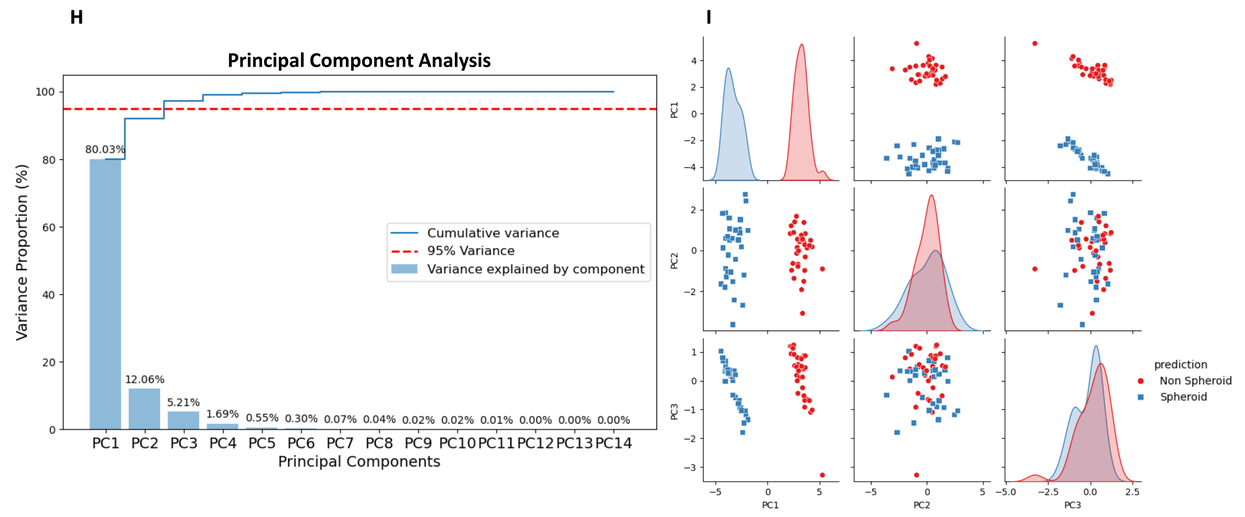
In the initial phase of **m3DinAI**, a quality control (**QC**) is conducted using unsupervised machine learning (ML) to ensure that no inconsistent data is present in the negative controls treated with 0.5% DMSO (spheres) or the positive controls treated with 4mM methyl methanesulfonate (MMS) (non-spheres). This step is crucial for ensuring an accurate classification in the subsequent phase using supervised ML (Decision Tree).

Normalization



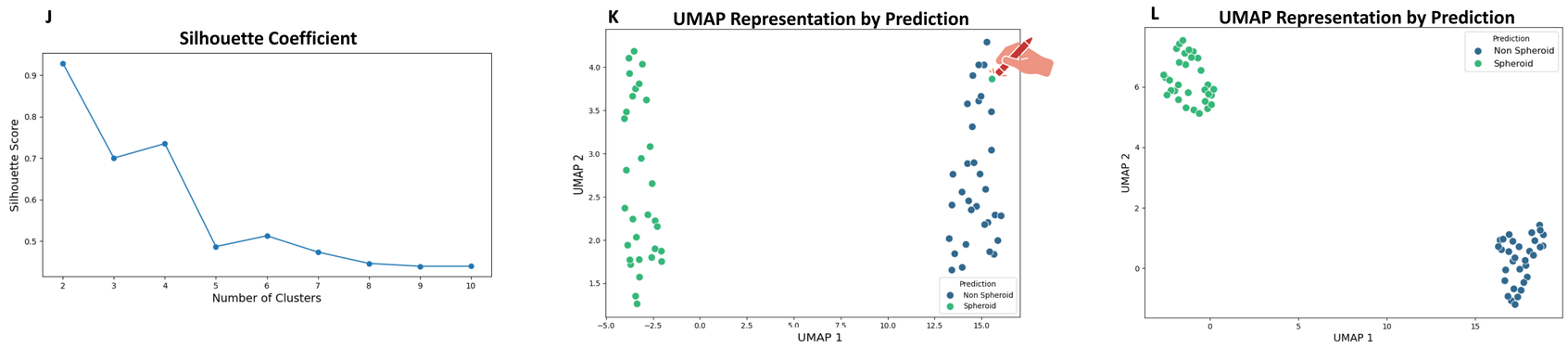
(**F**) The data is standardized to a mean of 0 and a standard deviation of 1, ensuring that all features are on the same scale so that variations in the data reflect actual patterns rather than differences in measurement units. (**G**) This prevents any single variable from disproportionately influencing the model, thereby it improves the stability and accuracy of subsequent analyses.

Dimensionality Reduction



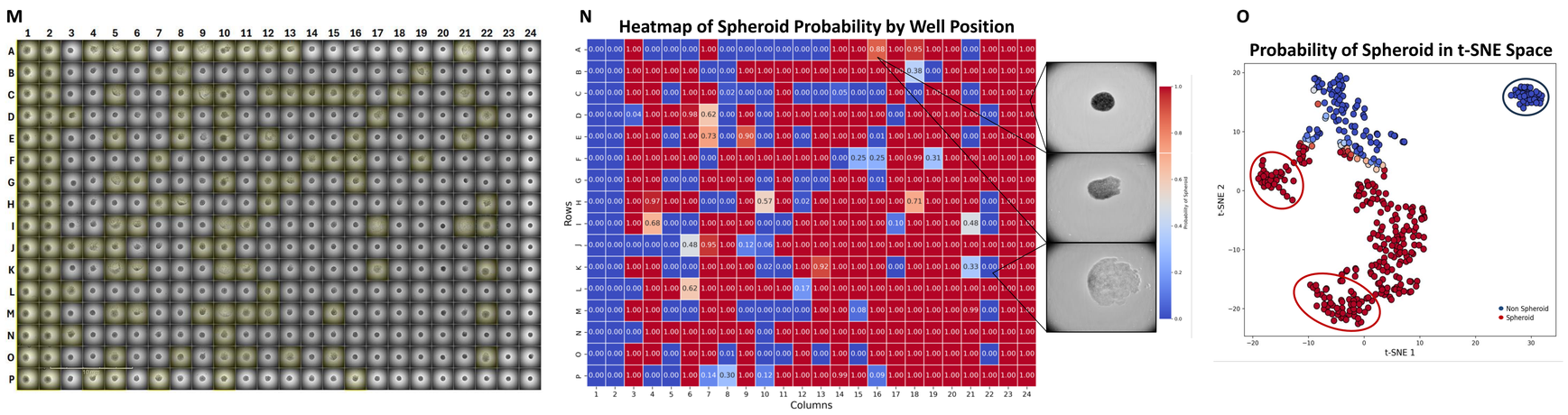
(**H**) To explain the variance within each dataset, a **Principal Component Analysis (PCA)** is performed. PCA reduces dimensionality by transforming original features into orthogonal components, focusing on the components that account for 95% of the variance. (**I**) A pair plot shows that PC1 and PC2 alone explain the model effectively and separate the data of interest.

Clustering



(**J**) A **silhouette coefficient** is initially used to determine the optimal number of clusters so that clustering quality can be assessed. (**K**) UMAP, a non-linear dimensionality reduction method, projects clustered data in two dimensions, preserving proximity relationships. This visualization helps to confirm if natural groupings align with existing labels and identify outliers for removal. (**L**) After removing poorly clustered data points, clustering improves significantly, allowing for the correct classification of the decision tree.

m3DinAI | Python



m3DinAI performs supervised classification using a Decision Tree model trained with control data (sphere and non-sphere) to predict whether a sphere is affected by the treatment. (**M**) The image displays a 384-well plate (Greiner Bio-One), with wells identified as non-spherical highlighted in yellow by m3DinAI. (**N**) Next, t-SNE is applied to reduce the dimensionality of the data, a heatmap is generated to reflect the probability of each well being spherical or non-spherical, facilitating easier visual analysis. (**O**) Finally, these data are represented graphically in the spatial domain.

RESULTS

The application of the m3DinAI platform in the high-content imaging (HCI) analysis of **2,400 microorganism-derived extracts** from the Fundación MEDINA library has yielded a total of **68 hits** with significant activity compared to controls.

CONCLUSIONS

- The **m3DinAI** tool allows for the identification of microbial extracts with potential antitumoral activity in breast cancer.
- The integration of **m3DinAI** has notably accelerated both HCI analysis and the identification of cytotoxic activity in **3D cultures,** which highlights the transformative role of **machine learning** in expediting drug screening processes, ultimately enhancing the identification of novel therapeutics.

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