Figure 1. Graphical Abstract. (A) Data Acquisition. We used Incucyte and microscope platforms to generate spheroid images for the training and evaluation of deep learning model. **(B) Deep Learning (DL) Pipeline**. Two models were trained using Incucyte and microscope image datasets. These models were then evaluated on validation and test datasets. **(C) SpheroScan** consists of two submodules: Prediction and Visualization. The Prediction module applies the trained deep learning models to mask the input spheroid images, producing a CSV file with the area and intensity of each detected spheroid as output. The Visualization module enables the user to analyse the output from the Prediction module by providing various plots and statistical analyses.