There have been 1,600 FDA approved drugs since their inception in 1938. Currently it costs approximately $833 million dollars to bring a drug candidate to the market, and often times the potential candidate fails at the beginning of the clinical phase. Therefore, it is important to develop drug screening methods that are more clinically relevant or predictable. The current 2D methods for cancer drug discovery have had some difficulty in identifying potential drug candidates that can be used for clinical testing. To overcome this challenge, there has been an increase in research of 3D tissue culture that facilitated the development of new in-vitro tumor model assays. Traditional 3D spheroid analysis method relied heavily on visual observation using standard microscopy, which is time-consuming and has high operator variations. In the recent years, high-throughput image-based cytometers, such as Celigo, have demonstrated the ability to perform bright-field and fluorescence cell-based assays. Celigo imaging cytometer can be employed to rapidly analyze and characterize 3D tumor spheroids, which can be used to generate both quantitative and qualitative results. In this work, we demonstrate a high-throughput 3D tumor spheroid screening method using the Celigo imaging cytometer to screen the effects of 14 drug compounds (HH/NACAT) on U87MG spheroid size, matrigel invasion, and tumor spheroid viability. First, a dose response experiment is performed to screen the growth inhibitory effects of the drug compounds. In addition to direct spheroid size analysis, dose inhibitory responses of tumor invasion into the matrigel are also examined. Finally, the use of specific fluorescent dyes such as Calcein AM, PI, and Caspase 3/7 were used to screen drug induced cytotoxicity on the tumor spheroids. The results showed that Celigo imaging cytometer can quickly generate accurate growth inhibitory data to identify potential drug candidates. Furthermore, tumor invasion were clearly observed and quantified in the captured images, as well as fluorescent analysis of tumor spheroid viability. By utilizing the 3D spheroid screening method, researchers can rapidly characterize and quantify drug effects on tumor spheroids in a high-throughput format, which can improve the efficiency of identifying more qualified cancer drug candidates.