A high-throughput 3D tumor spheroid screening method for drug discovery using imaging cytometry

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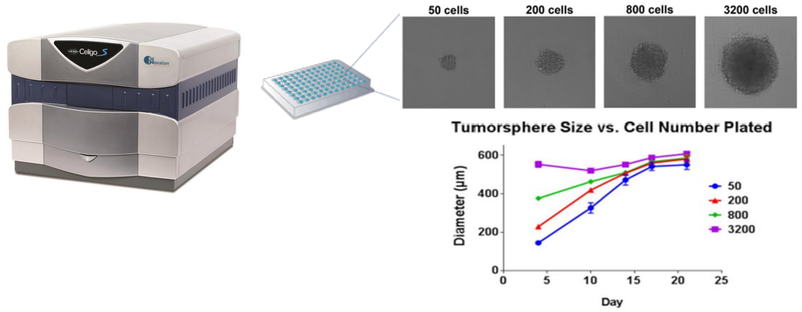
1. ABSTRACT

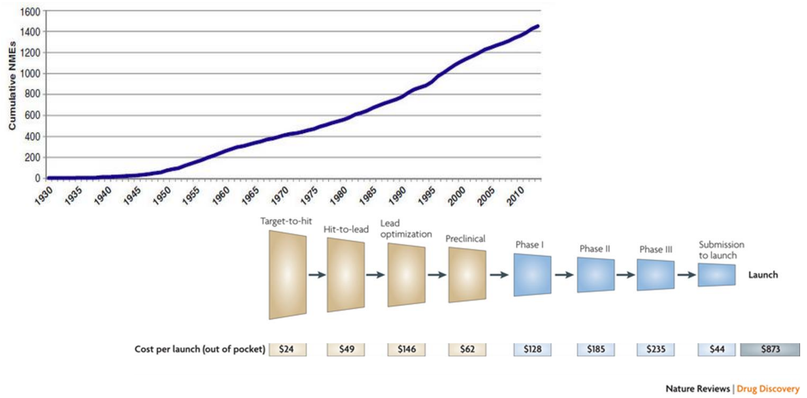
There have been 1600 FDA approved drugs since their inception in 1938. Currently it costs approximately 883 million dollars to bring a drug candidate to the market, and often times the potential candidate fails at the beginning of the clinical phase. Therefore, it is important to develop drug screening 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 (NIH/NCAT) 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.

2. CELIGO IMAGE CYTOMETRY FOR TUMOR SPHEROID ANALYSIS



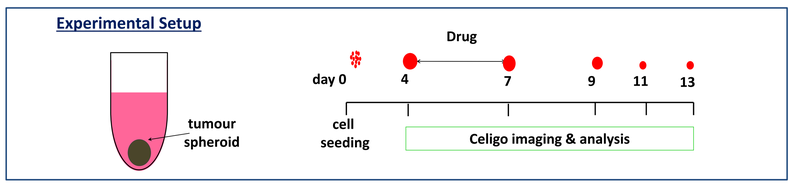
1. Celigo Imaging Cytometer is a plate-based cytometer that can scan the entire well of standard microplates and captures bright-field and fluorescent images
2. The captured images are analyzed with the Celigo software to measure size, morphology, cell count, confluence, and fluorescent intensity
3. The measured parameters are used to generate cell proliferation kinetic data, GFP/RFP expression, tumor spheroid growth and inhibition, DNA cell cycle analysis, apoptosis, and ADCC results

3. WHY BOTHER WITH 3D TUMOR MODELS?



- FDA approved drugs released to the market have been increasing rapidly in the recent years
- Cost per launch of out of pocket for a drug also increased to $873 million dollars
- Improve cancer models can help reduce the total cost per launch of out of pocket

4. HIGH-THROUGHPUT DRUG SCREENING PROTOCOL FOR SPHEROID GROWTH



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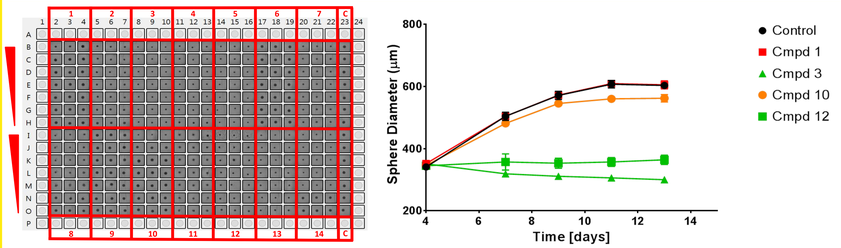
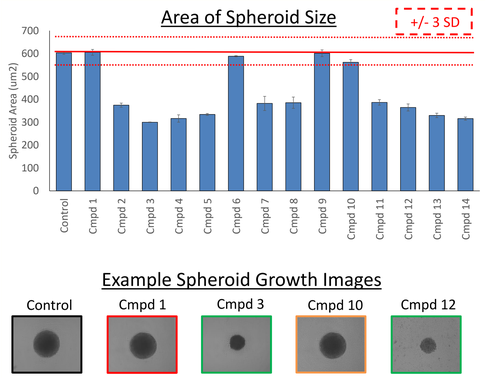
5. KINETIC MEASUREMENT OF SPHEROID GROWTH INHIBITION USING BRIGHT-FIELD



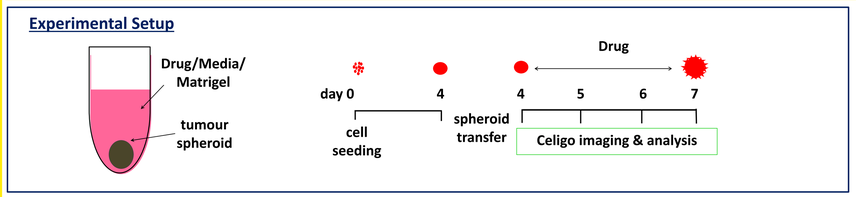
- 14 compounds and a control were imaged and spheroid diameters were monitored from 4-13 days in bright field

6. DRUG SCREENING OF SPHEROID GROWTH INHIBITION USING BRIGHT-FIELD



- 14 compounds and a control was screened at 7 concentrations to measure the effect of tumor spheroid growth inhibition
- Images were acquired and analyzed from day 4-13
- The results on day 13 showed that different spheroid diameters were measured when treated with different drug compounds, which are shown in the example images
- 3 x Standard Deviation was used as a threshold to quantify the number of hit compounds
- 10 drug compounds inhibited spheroid growth shown in the bar graph

7. HIGH-THROUGHPUT DRUG SCREENING PROTOCOL FOR SPHEROID INVASION



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D		2.5	2.5	2.5	2.5	2.5	2.5	2.5																
E		1.25	1.25	1.25	1.25	1.25	1.25	1.25																
F		0.625	0.625	0.625	0.625	0.625	0.625	0.625																
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K		2.5	2.5	2.5	2.5	2.5	2.5	2.5																
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M		0.625	0.625	0.625	0.625	0.625	0.625	0.625																
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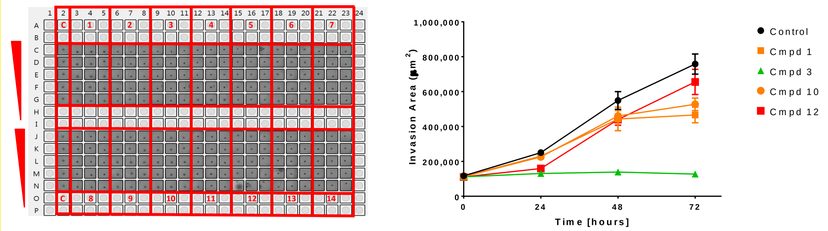
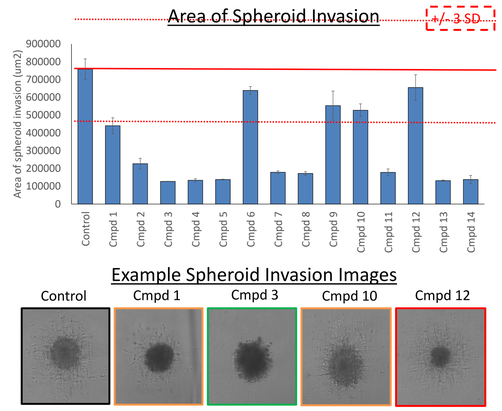
8. KINETIC MEASUREMENT OF SPHEROID INVASION INHIBITION USING BRIGHT-FIELD



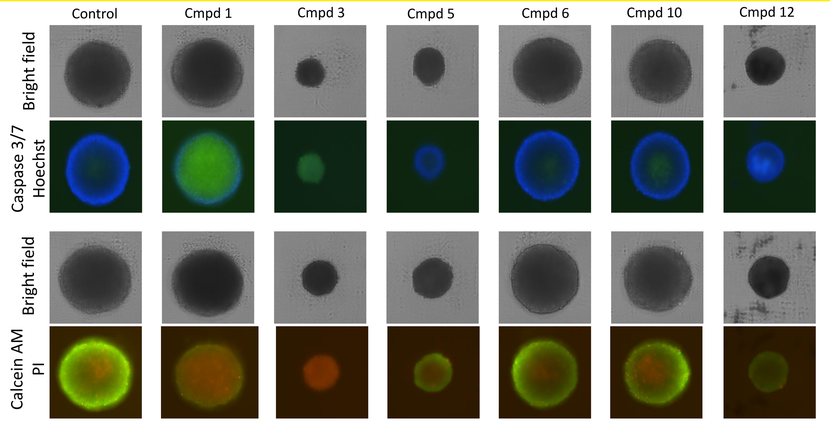
- 14 compounds and a control were imaged and invasion area were monitored from 4-7 days in bright field

9. DRUG SCREENING OF SPHEROID INVASION INHIBITION USING BRIGHT-FIELD



- 14 compounds and a control was screened at 5 concentrations to measure the effect of tumor spheroid invasion inhibition into Matrigel
- Images were acquired and analyzed from day 4-7
- The results on day 7 showed that different invasion area were measured when treated with different drug compounds, which are shown in the example images
- 3 x Standard Deviation was used as a threshold to quantify the number of hit compounds
- 9 drug compounds inhibited spheroid invasion shown in the bar graph

10. SCREENING FOR VIABILITY AND APOPTOSIS USING CALCEIN AM/PI AND CASPASE 3/7



11. 3D TUMOR SPHEROID DRUG SCREENING RESULTS

Cmpd 1	Sunitinib malate
Cmpd 2	PACLITAXEL
Cmpd 3	Romidepsin
Cmpd 4	Panobinostat (LBH589)
Cmpd 5	GSK-2126458
Cmpd 6	Cisplatin
Cmpd 7	Bortezomib
Cmpd 8	Carfilzomib
Cmpd 9	CISPLATIN
Cmpd 10	Sorafenib
Cmpd 11	Trametinib
Cmpd 12	BEZ-235
Cmpd 13	Staurosporin
Cmpd 14	17-AAG

	Growth	Invasion	Cell Death (PI)	Cell Death (Apoptosis)
Cmpd 1				
Cmpd 2				
Cmpd 3				
Cmpd 4				
Cmpd 5				
Cmpd 6				
Cmpd 7				
Cmpd 8				
Cmpd 9				
Cmpd 10				
Cmpd 11				
Cmpd 12				
Cmpd 13				
Cmpd 14				

HIT
NO HIT

12. SUMMARY AND CONCLUSION

- Celigo Imaging Cytometer is a versatile and powerful tool for 3D tumor spheroid analysis
- This method increases the throughput of the tumor spheroid analysis in 384-well microplates, which can highly increase the efficiency of testing potential cancer drug candidates
- Screening of tumor spheroid growth, invasion into matrigel, viability, and apoptosis have been demonstrated
- The Celigo screened 14 drug compounds, which showed growth and invasion inhibition for 10 compounds
- This method can also be applied to 2D assays, so that both 3D and 2D assays can be performed simultaneously to determine which cancer model is the most optimal for different cancer cells
- The authors would like to thank Steve Titus from NCATS High Content Imaging & Discovery for donating the 14 cancer drug compounds