A rapid 3D tumor spheroid analysis method using the Celigo Imaging Cytometry

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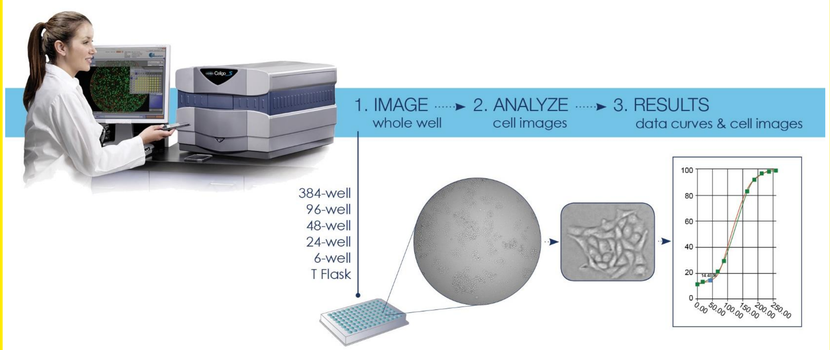
1. ABSTRACT

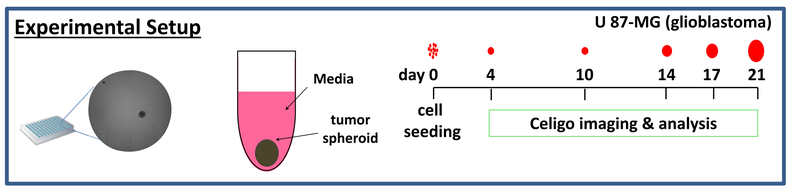
The current 2D methods for cancer drug discovery have had some difficulty in identifying potential drug candidates that can be used for clinical testing. To overcome this challenge, there has been an increase in research of 3D tissue culture, which has facilitated the development of new in-vitro tumor model assays. Traditional 2D and 3D analysis method relied heavily on visual observation using microscopy. However, the method is time-consuming and has high variations. Automated plate-based imaging cytometry 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 novel 3D tumor spheroid analysis method using the Celigo imaging cytometer for spheroid counting, size analysis, tumor migration and invasion, tumor viability, and dose response of drug induced/inhibited tumor growth. The plate-based imaging cytometer utilizes bright-field and three fluorescence channels (Blue, Green, and Red) for multi-channel analyses. By utilizing the F theta lens technology, uniform bright-field image are captured for more accurate counting of the entire well. In addition, Celigo analysis software is used to report numerous parameters allowing detailed spheroid characterization. In addition to direct spheroid counting in the well, the use of specific fluorescent dyes and probes allow the user to define viable and hypoxic areas within spheroids and monitor migration and invasion on or into supporting cells and/or tissues. The results showed that Celigo imaging cytometer can accurately count and measure spheroid sizes in response to drug induction. Furthermore, tumor migration and invasion were clearly observed and quantified in the captured images. By utilizing the 3D spheroid imaging cytometry method, researchers can quickly characterize and quantify tumor spheroids, which can improve the efficiency of identification of potential cancer drug candidates.

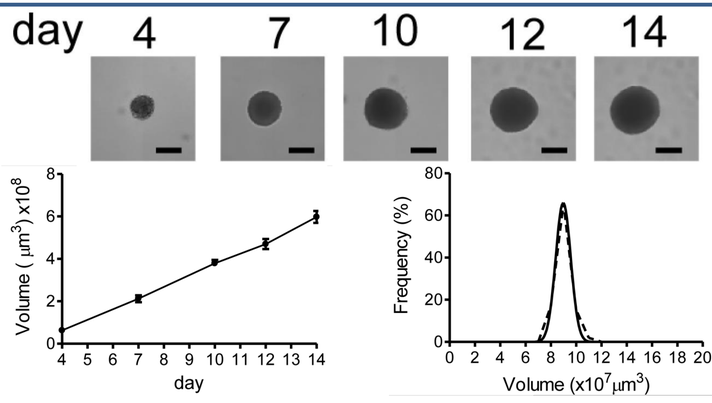
2. CELIGO IMAGING CYTOMETRY FOR DIRECT CELL COUNTING ADCC ASSAY



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 size change, DNA cell cycle analysis, apoptosis, and ADCC cytotoxicity results

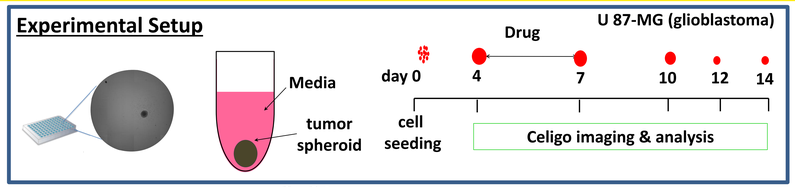
3. TIME-COURSE MEASUREMENT OF TUMORSPHEROID SIZE

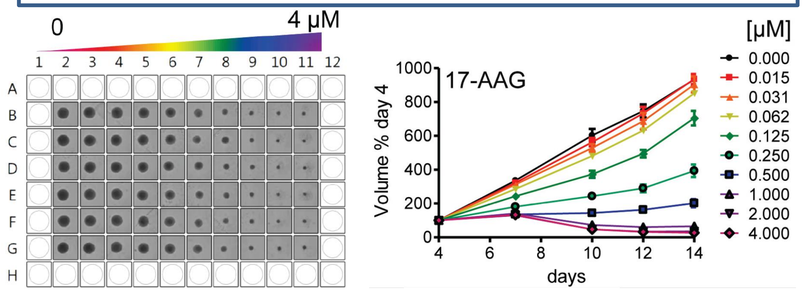


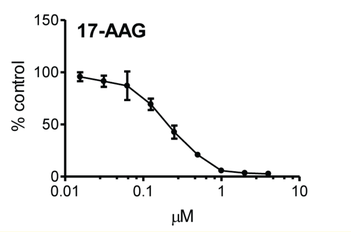


- The U 87-MG (Glioblastoma) tumorspheroid was grown with 1000 cells up to 4 days before imaging analysis
- From 4 days on, the size of the tumorspheroid linearly increased over a period of 14 days, with low variation
- Celigo was able to rapidly analyze tumorspheroid and monitor size changes over time

4. TUMORSPHEROID GROWTH INHIBITION ASSAY

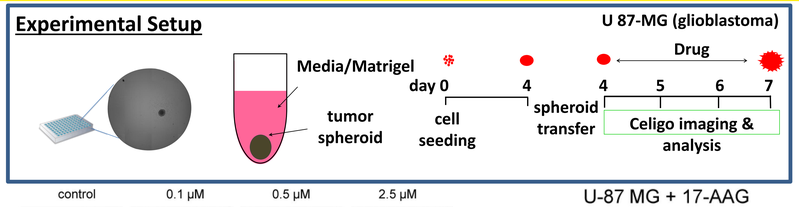


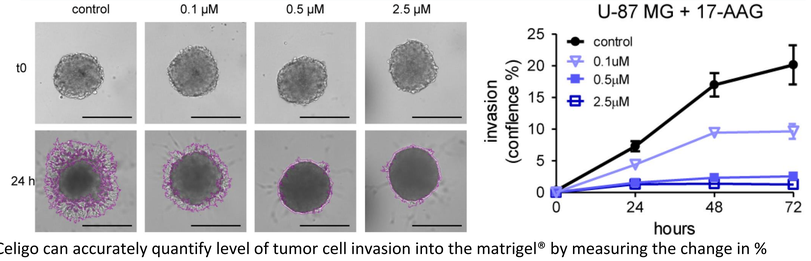




- The U 87-MG (Glioblastoma) formed tumorspheroid was initially grown up to 4 days before imaging analysis
- From 4 – 7 days on, 2 drugs were used to inhibit tumor growth using 17-AAG and PI-103
- It was clear in the images and data that as drug concentration increased, the size decreased indicating growth inhibition
- Celigo was able to rapidly analyze tumorspheroid and monitor drug induced growth inhibition over time

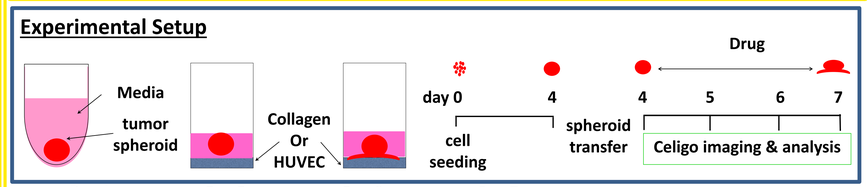
5. TUMORSPHEROID INVASION ASSAY

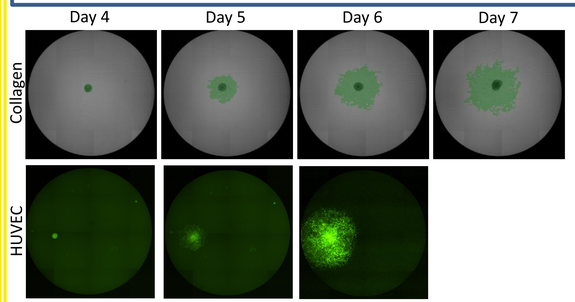


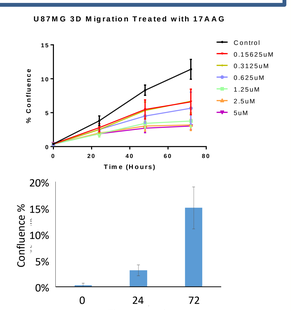


- Celigo can accurately quantify level of tumor cell invasion into the matrigel® by measuring the change in % confluence of the cell area in respect to 17-AAG concentrations
- The images showed as the 17-AAG concentration increased, it inhibited tumor invasion in to the matrigel®

6. TUMORSPHEROID MIGRATION ASSAY ON COLLAGEN OR HUVEC

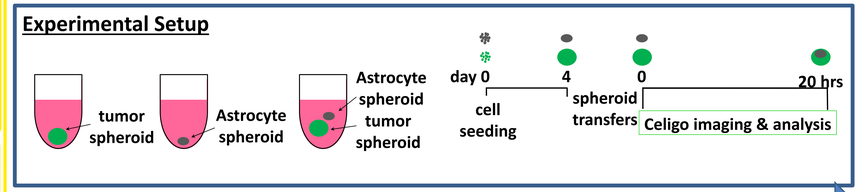


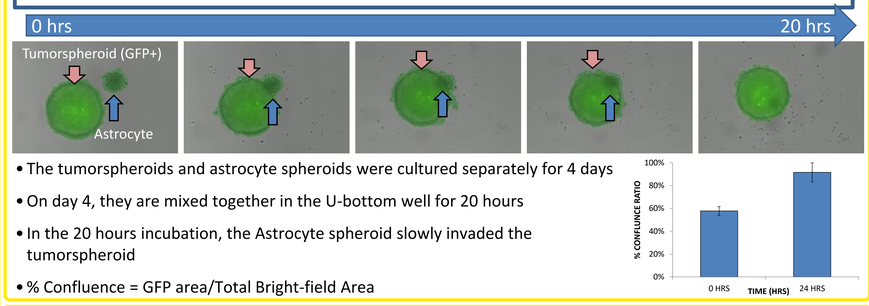




- The tumorspheroid was formed and transferred on a layer of collagen or HUVEC
- After transfer, 17-AAG was added and induced migration of tumor cells on to the surface of the collagen

7. TUMORSPHEROID TISSUE INVASION ASSAY





- The tumorspheroids and astrocyte spheroids were cultured separately for 4 days
- On day 4, they are mixed together in the U-bottom well for 20 hours
- In the 20 hours incubation, the Astrocyte spheroid slowly invaded the tumorspheroid
- % Confluence = GFP area/Total Bright-field Area

8. SUMMARY AND CONCLUSION

- Celigo Imaging Cytometer is a versatile and powerful tool for 3D tumorspheroid analysis
- Assays such as measuring optimal seeding density for forming tumorspheroids and viability measurement have been demonstrated
- Advanced assays such size measurement, growth inhibition, invasion into matrigel®, migration on to collagen and HUVEC, and tissue invasion that have been performed by manual microscope observation can now be easily quantified using the automated imaging cytometry method