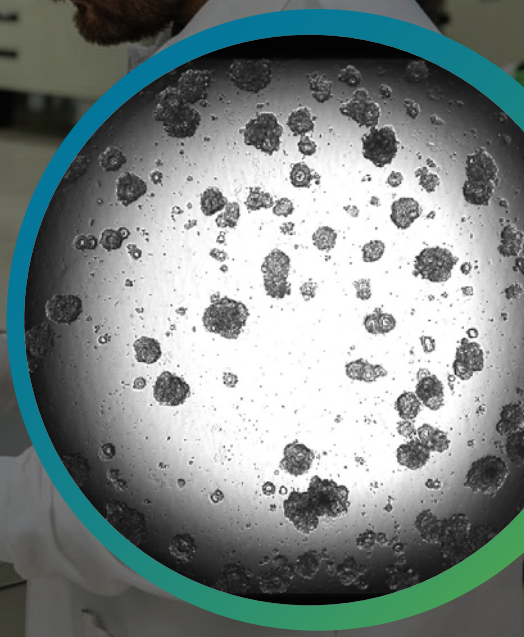
APPLICATION NOTE

Rapid walkaway solution for assessing viability in patient-derived colorectal cancer organoids



Cathy Olsen, PhD | Sr. Application Scientist | Molecular Devices

Prathyushakrishna Macha, PhD | Research Scientist | Molecular Devices

Introduction

Cancer cell lines grown in 2D have long served as experimental surrogates for cancers. In recent years, the 3D culture of cancer cells, often alongside other cell types in formats where they can form multi-layered structures, is enabling new models for cancer research that are considered more biologically relevant. Cancer organoids derived from patient tissue offer researchers a highly relevant disease model system, as these organoids and the patients from which they were derived have been shown to respond similarly to drugs. Recently, these models have become more widely utilized, thanks to standardization and scalability that have made available large numbers of uniformly sized and highly viable organoids.

The characterization of organoid responses to candidate drug treatment is a powerful research tool that provides a wealth of detailed information, but screening numerous compounds requires significant effort and hands-on time. We demonstrate here methods for analyzing organoid viability that allow rapid identification of effective drug candidates and can be combined with more complex downstream image analysis.

Benefits

- Access more biologically relevant information with 3D organoid cultures
- Image live organoids without the need for stains
- Rapidly measure viability with an automated microplate reader luminescence assay

Using patient-derived colorectal cancer organoids, we assessed the differential effects of known anti-cancer compounds on organoid size and morphology using both imaging and a microplate reader-based viability readout. Here we demonstrate the utility of label-free imaging and an ATP-based viability assay for rapidly gauging drug responses, with automation of liquid handling and microplate transport to decrease hands-on time. This streamlined readout may be used to guide identification of potential therapeutics earlier in the screening process, which can then be followed up with more intensive high content imaging or other analyses.

CRC organoid culture and analysis

Seeding and compound treatment

Colorectal cancer patient-derived organoids (PDOs) were thawed quickly, rinsed in media, and suspended in a solution of media + 50% Matrigel (growth factor-reduced). They were then seeded into 384-well white-walled, clear-bottom microplates at 250 organoids per well, in a volume of 10 µL per well. Organoids were incubated in media containing ROCK inhibitor for 48 hours for optimal recovery. Quadruplicate wells were then treated with the compounds 5-fluorouracil, doxorubicin, and romidepsin, each in a four-fold dilution series and with starting concentrations of 20 µM (5-FU), 60 µM (doxorubicin), and 10 µM (romidepsin).

Assays for compound effects

Figure 1 depicts the workflow used to culture organoids, run assays, and analyze results. Immediately prior to compound addition, and again after five days of compound treatment, transmitted light (TL) images were acquired on an ImageXpress® Micro Confocal High-Content Imaging System and analyzed to determine the area covered by organoids using the Custom Module Editor in MetaXpress® High-Content Image Acquisition and Analysis Software. Through a multi-step process, the software identified organoids and applied masks to calculate the total area covered by the organoids in each image.

The viability of organoids after five days of compound treatment was assessed using the CellTiter-Glo® 3D Cell Viability Assay (Promega), with results detected using a SpectraMax® iD5 Multi-Mode Microplate Reader. To each well containing 35 µL of media/compounds and organoids, 35 µL of CellTiter-Glo 3D reagent was added. A Hamilton Microlab® STAR™ Liquid Handler was used to automate the addition of assay reagent to the wells and to shake the microplate vigorously for 5 minutes to ensure organoid lysis. After a 25-minute incubation at room temperature, a PreciseFlex 400 sample handler (Precise Automation) and Genera scheduling software (Retisoft) were used to transfer the microplate from the liquid handler to the SpectraMax iD5 reader. A preconfigured protocol in SoftMax® Software was used to generate data and analyze results automatically.

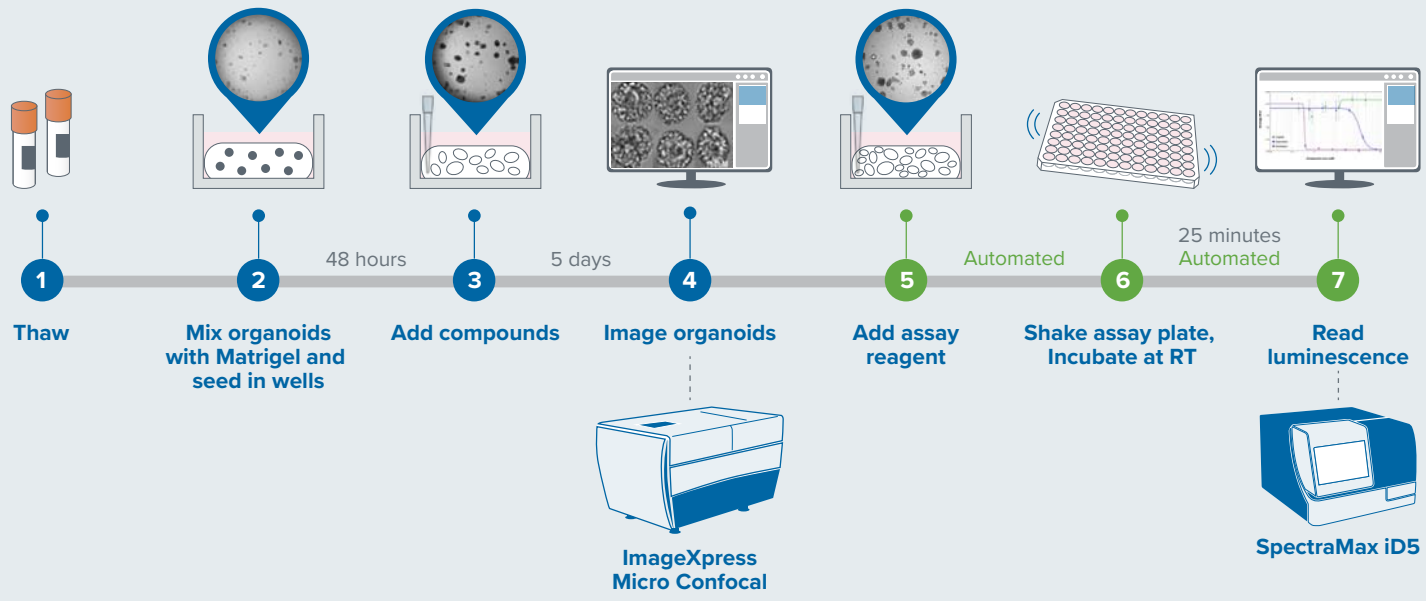


Figure 1. *Overview of the experimental process from organoid seeding (day 0) and compound addition (day 2) to results by imaging and automation of a luminescent microplate reader viability assay (day 7).*

Results

Analysis of TL images revealed differences in the area covered by CRC organoids in images of control and compound-treated wells (Figure 2). The average area of compound-treated organoids vs. compound concentration was plotted using a 4-parameter curve fit in SoftMax Pro Software in order to assess responses to treatment (Figure 3). Images in Figure 4 illustrate the size differences observed between untreated and compound-treated organoids.

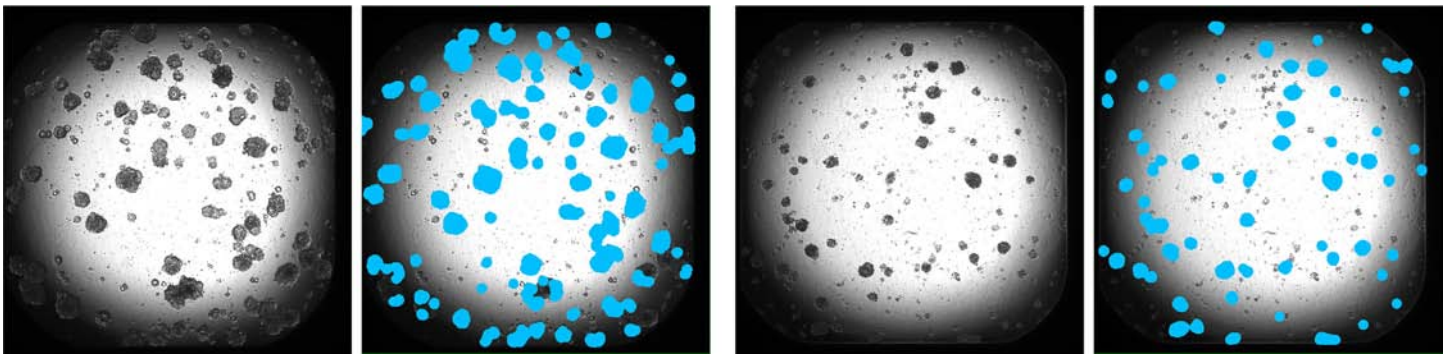


Figure 2. *The Custom Module Editor was used to analyze TL images, identifying organoids and applying masks used to calculate the area covered by organoids in each image. Left two images, untreated (control) organoids; right two images, organoids treated with 60 µM doxorubicin.*

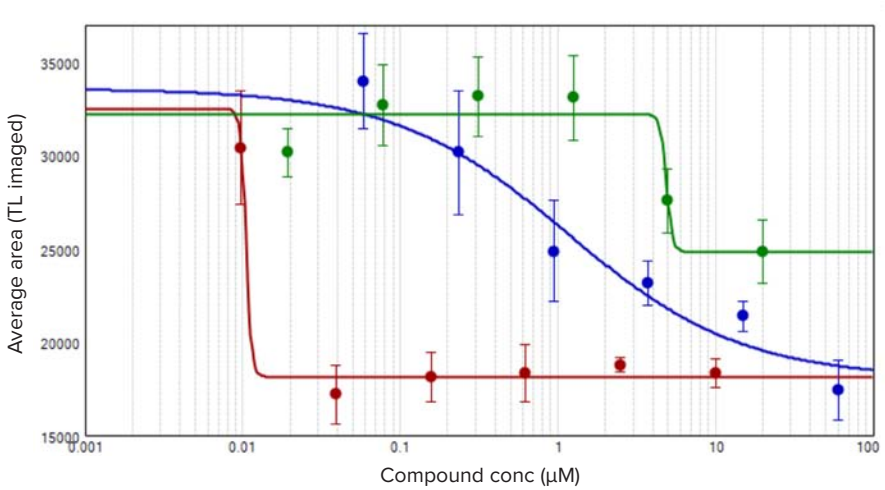


Figure 3. *Concentration-response curves for 5-day compound-treated organoids imaged using transmitted light (TL). Average calculated area was plotted vs. compound concentration using a 4-parameter curve fit in SoftMax Pro software: blue, doxorubicin; red, romidepsin; green, 5-fluorouracil.*

Figure 4. *TL images of untreated (control) organoids and organoids treated with the indicated compounds (doxorubicin 60 µM, romidepsin 10 µM).*

To measure relative numbers of viable cells in compound-treated organoids, the CellTiter-Glo 3D Cell Viability Assay was employed. Based on ATP detection, this assay involves lysis of the organoids and can be performed as a standalone method, or after imaging (TL or otherwise) is completed. It can be easily automated as it requires only the addition of a single reagent, followed by shaking and incubation at room temperature, prior to detection of luminescence on a SpectraMax microplate reader. In this case, signal from 5-day treated organoids was measured using the SpectraMax iD5 reader, and relative light units (RLU) were plotted vs. compound concentration using a 4-parameter curve fit in SoftMax Pro software (Figure 5).

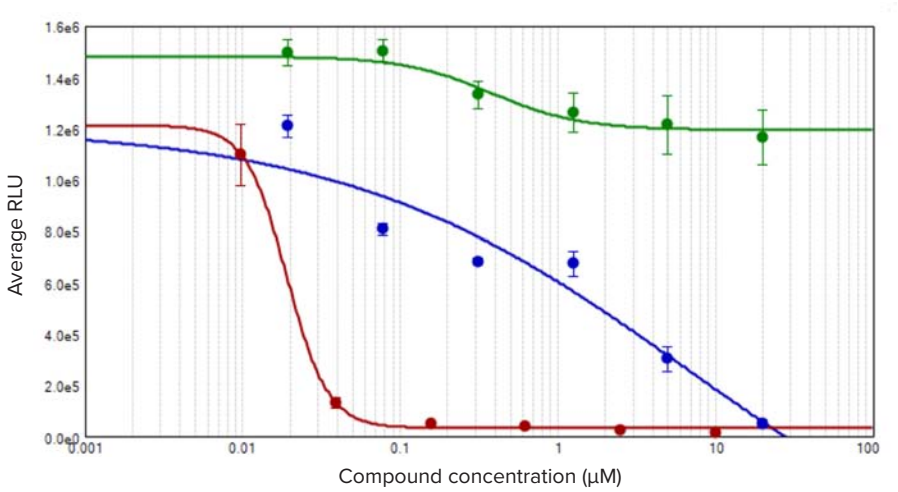


Figure 5. *5-day compound-treated organoids assessed for viability with the automated cell viability assay: blue, doxorubicin; red, romidepsin; green, 5-fluorouracil. An add-mix-read format, in combination with automation of reagent handling, assay detection, and analysis produced results in about 30 minutes.*

Conclusion

Patient-derived organoids give researchers the opportunity to explore the possibilities of personalized treatment. This exploration often involves time-consuming imaging and image analysis that provide a wealth of information but may be less practical for initial screening of large numbers of compounds. The use of label-free imaging and automated plate reader-based viability analysis can serve as preliminary approaches to identifying potential drugs with measurable effects on treated organoids. Selected compounds can then be followed up with more demanding studies involving high-content imaging utilizing a variety of fluorescent labels and analysis techniques.

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