>> Development of a Real-Time Assay for Tracking the Proliferation of iPSCs and iPSC-derived Organoids

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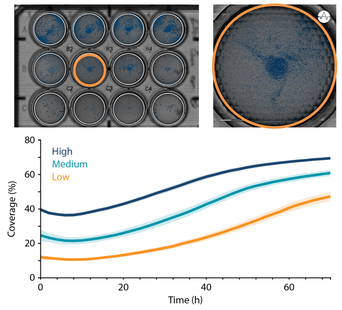
Omni: Dynamic Cell Tracking

Real-time Cell Analysis with Whole-Vessel Imaging

The flexibility and accessibility of induced pluripotent stem cell technology has allowed complex human biology to be reproduced in vitro at high throughput scales. Indeed, rapid advances in stem cell technology have led to widespread adoption for the development of in vitro models of human physiology to be used in screening applications in drug discovery and safety. Furthermore, advanced cell preparations, such as organoids, are under investigation with aims toward establishing mature human phenotypes in vitro. For the development and validation of relevant in vitro iPSC-derived organoid models, it is critical to develop a label-free assay to track organoid growth and expansion.

Axion **BioSystems'** Omni platform offers live-cell imaging within an incubator for real-time tracking of cell proliferation, colony formation, and organoid size. Here, we used the Omni to monitor iPSC expansion for passaging and maintenance, and then present assay results from patient-derived organoids.



The Omni Product Family

>> Assay your cells in brightfield and fluorescence – From label-free cell monitoring to fluorescence-based assays, the Omni adds dynamic visual results to any experiment.

>> Track every moment, straight from your incubator – The Omni operates within an incubator, automatically capturing images as your cells grow in their optimal environment.

>> See every cell – The Omni moves the camera, not the cells, capturing detailed brightfield images of the entire culture without disturbing the cells.

>> Monitor and analyze your cells remotely – The software allows you to monitor your cells and perform data analysis from your desktop.

>> Get started quickly – With an easy-to-install, maintenance-free device that does not require calibration, a short training is all it takes to start using the Omni.

Features	*Omni BR*	*Omni Pro 12*	*Omni FL*
Whole-well brightfield	✓	✓	✓
Automated acquisition	✓	✓	✓
Incubator compatible	✓	✓	✓
Fluorescence (Red)		✓	✓
Fluorescence (Green)		✓	✓
Number of plates	1	12	1
Plate handling	Manual	Automated	Manual

AI-Driven Imaging Software for Powerful, yet Simple, Analysis

Axion's software modules for the Omni platform enable simple assay setup, real-time cellular visualization, and fast analysis. Discover the module best suited for your research and transform your complex data into clear results.

Cell Confluence

Scratch Assay

Fluorescent Object Count

Clonogenic Assay

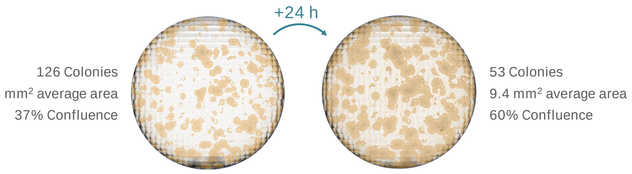
Organoid Analysis

Real-time Monitoring for iPSC Expansion

Colony Forming Algorithm Tracks Colony Number and Size

The Omni brightfield, full-vessel scan is ideal for tracking the formation and growth of colonies over time. The full-vessel scan ensures that each colony is counted as the cultures mature over time. The label-free, brightfield image monitors colony formation without impacting the biology. The colony formation algorithm tracks the following endpoints:

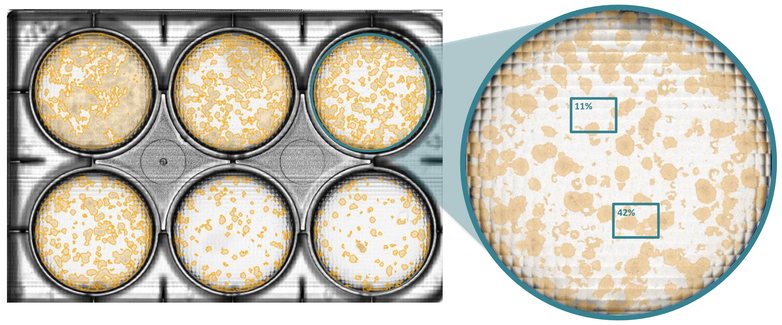
- Number of Colonies – the algorithm automatically detects colonies larger than a user-defined threshold, allowing user flexibility in defining which colonies to track.
- Average Colony Size – the area is tracked for each individual colony and reported as an average across each well or analysis region.
- Coverage – taken together, the average colony size and number of colonies can be used to compute coverage.



Whole Vessel Imaging Provides Unbiased Data

Manual inspection and estimation of culture confluence can be a time consuming and inaccurate process. Only a small fraction of a given well may be seen at once with a 2x objective, requiring the scientist to navigate across the well and mentally combine confluence estimates. The Omni full-vessel scan eliminates tedious scrolling on the microscope and provides an accurate measure of the number of colonies, colony size, and ultimately confluence.

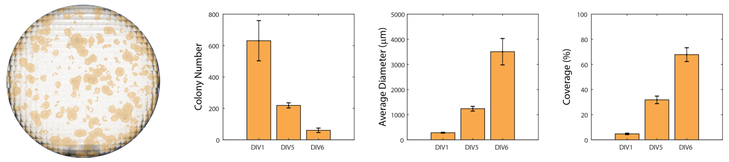
A post-thaw density sweep was performed with iPSCs (SCTi003-A line, STEMCELL Technologies) in a 6-well culture plate (A1 – highest density; B3 – lowest density). The colony forming algorithm detects and quantifies the iPSC colonies across the plate. The full vessel scan provides a comprehensive view of confluence and colony size.



Monitoring of iPSC Expansion Informs Passaging and Culture Maintenance

iPSC expansion and maintenance require vigilant culture inspection to evaluate colony size and overall confluence. Indeed, if colonies become too large, spontaneous differentiation can occur. The Omni brightfield scan provides an automated, and quantitative, assessment of iPSC cultures over time.

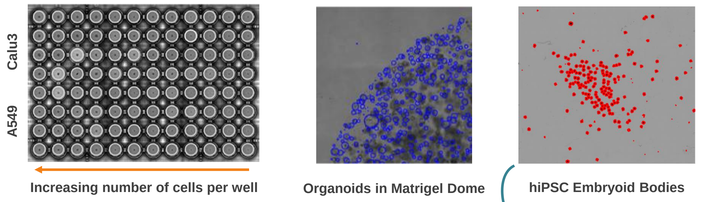
The Omni was used to monitor colony formation and expansion for iPSCs (SCTi003-A line, STEMCELL Technologies) in a 6-well culture plate. The colony forming algorithm produced colony number and average area. The diameter and coverage were computed separately from these endpoints.



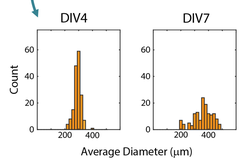
Assays with iPSC-Derived Organoids

Organoid Analysis Module Tracks Organoid Number and Size

The Omni brightfield scan provides flexibility tracking organoid growth across various culture vessels and organoid formation protocols. For example, continuous, real-time imaging can track the formation of 3D models within ultra-low attachment U-bottom 96-well plates (left). In this case, a single 3D model forms within each well of the plate. Alternately, the Matrigel dome approach (middle) can be used to produce many individual organoids, of varying size and shape, within each well of a multi-well plate. Here, the organoid analysis module can track the number, size, and shape of 3D models within the field of view. Finally, hiPSC embryoid bodies (right) can be tracked after moving the 3D models from an AggreWell plate (STEMCELL Technologies) to another vessel for growth and maturation.



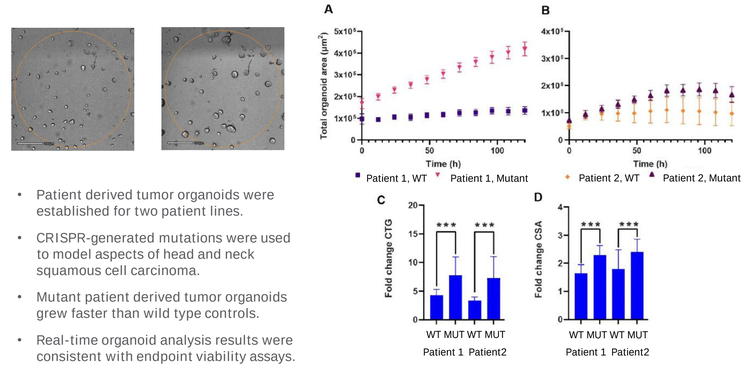
- hiPSC embryoid bodies were initially formed in an AggreWell plate, and then transferred to another vessel.
- The organoid analysis model tracked number and size of the embryoid bodies, which grew significantly from DIV4 to DIV7 in culture on the orbital shaker.



Patient Derived Tumor Organoids for Disease Modeling

Data provided by Roan Gobbits, Oncode Institute, Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences (KNAW)

For iPSC-derived organoids, patient-derived tumor organoids (PDTOs) were developed to model head and neck squamous cell carcinoma (HBSCC), with CRISPR editing to create specific mutations. Cross-sectional area was used to quantify the growth and expansion of wild-type and mutant organoids, with mutant organoids growing faster than wild-type in both patient models (fold change over 5 days: Patient 1 Mutant = 2.3 +/-0.3, Patient 1 WT = 1.6 +/-0.3, Patient 2 Mutant = 2.4 +/- 0.5, Patient 2 WT = 1.8 +/- 0.6). A CellTiter-Glo Assay was performed on the same models yielding similar results. These results support the continued development and use of real-time, label-free imaging assays to efficiently track the growth and expansion of iPSCs and iPSC-derived organoid models.



- Patient derived tumor organoids were established for two patient lines.
- CRISPR-generated mutations were used to model aspects of head and neck squamous cell carcinoma.
- Mutant patient derived tumor organoids grew faster than wild type controls.
- Real-time organoid analysis results were consistent with endpoint viability assays.

Conclusions

- The Omni brightfield, whole-vessel scan allows for real-time cell analysis of iPSCs and iPSC-derived models.
- The colony forming algorithm was used to quantify the number and size of iPSC colonies during the expansion and maintenance culture phase. The whole-vessel scan provides reliable, unbiased measurement of colony size and overall confluence for decision-making in passing iPSCs.
- The organoid analysis algorithm was used to quantify growth of patient-derived tumor organoids developed for modeling head and neck squamous cell carcinoma. The CRISPR-generated mutant exhibited faster tumor growth, as compared to the control, for each patient line.