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Assessment of Growth and Doubling Time Changes with Increasing Passages Utilizing CellAssist 50®

It has long been established that the results of biomedical research conducted utilizing immortalized cell lines are directly impacted by the health of the cells. Factors including passage number and media conditions directly impact cell health and should be monitored over the course of cell line use. This application note employs the CellAssist 50 to track A549 cell growth at low and high passage numbers and with various media formulations and conditions. Label-free images are captured over a 4-day period to provide full growth curves.

INTRODUCTION

Immortalized cell lines, including the A549 lung cancer line, are critical in biomedical research. The quality of data that is produced in experiments directly relates to cell health and the ability of researchers to consistently execute those experiments. Immortalized cell lines can usually survive many passages, however each passage is associated with the potential for spontaneous genetic drift, thereby creating unintentional changes in experimental conditions. Monitoring cells through passages is therefore critical to good cell culture technique. Cell lines cycle through multiple growth phases following each passage: Lag, Logarithmic Growth, Plateau, and Decline.[1] During the Logarithmic phase, cells have adapted to conditions and are actively growing. This is considered the best time to conduct experiments. During Plateau and Decline, cells have slowed growth and may begin dying from over confluency and lack of nutrients. Changes in the growth cycle of a cell line can be indicative of changes in cell health or genetic drift. An additional measure associated with cell health is the doubling time.

Doubling time refers to the amount of time it takes for the number of cells in a given vessel to double. Understanding this time is useful in planning experiments that require a certain number of cells be harvested or present in the vesel at a specific point in the experiment. For example, in harvesting cells for tumor implantation or completing a transfection protocol. The CellAssist or CellAssist 50 coupled with the CellEval software provides real-time data and calculations to make your monitoring of cell health more efficient and actionable. The CellAssist and CellAssist 50 provide minimal to no disruption to environmental conditions, no need for fluorescent labels, and scalability for easy integration into any experimental protocol or laboratory standard operating procedure.

Changes in the growth cycles of a cell line can be indicative of changes in cell health or genetic drift. Understanding when these changes occur in a given cell line allows researchers to appropriately plan experiments and manage cell lines regularly in use.

DOUBLING TIME

An essential measure for gauging cell health throughout the growth phases, Doubling Time is the amount of time it takes for the number of cells in a given vessel to double. Understanding this time is critical in planning experiments that require a certain number of cells be harvested or present in the vessel at a specific point in the experiment. For example, in harvesting cells for tumor implantation or completing a transfection protocol. The CellAssist or CellAssist 50 coupled with the CellEval software provides real-time data and calculations to make your monitoring of cell health more efficient and actionable. The CellAssist and CellAssist 50 provide minimal to no disruption to environmental conditions, no need for fluorescent labels, and scalability for easy integration into any experimental protocol or laboratory standard operating procedure.

MATERIALS AND METHODS

Cells

A549 (ATCC, CCL-185) were maintained in Dulbecco's Modified Eagle's Medium (DMEM, ATCC, 30-2002) supplemented with 10% Fetal Bovine Serum (FBS, Atlas Biologicals, F-0500-DR). A batch of cells were harvested and frozen at Passage 1 according to ATCC standard procedure. An additional batch of cells was maintained through Passage 20 for the purpose of this experiment. The frozen cells were revived and passaged once, thereby placing them at Passage 3 for use here.

Media

Four media conditions in addition to normal growth media were tested. [1] Normal growth media (DMEM + 10% FBS) [2] Normal growth media that had been stored in a 37 °C water bath for 5 days to represent improper media storage [3] F12-K (ATCC, 30-2004) + 10% FBS [4] RPMI-1640 (ATCC, 30-2001) + 10% FBS.

CellAssist 50

The CellAssist® 50 combines the convenience of an automated incubator with the power of the CellAssist benchtop imager, providing a fully automated solution. This combination enhances reproducibility by tackling some of the industry's most pressing challenges in cell culture and cell-based experiments: time-consuming and costly, high variability, and poor data quality. With enclosed transportation between the incubator and the imaging system, there are minimal changes in the environmental conditions (CO_2, Temperature, humidity). Brightfield imaging eliminates the use of disruptive probes and phototoxicity, and enables real-time monitoring. Convenient scheduling in a multi-user environment coupled with rapid imaging and analysis allows for multiple experiments to run simultaneously with confidence. And CellAssist® 50 runs 24/7, day in and day out with limited human interaction.

CellEval Analysis Software

CellEval analysis software utilizes Thrive Bioscience's breakthrough proprietary analysis suite, Thrive IQ, to calculate quantitative cell features of every cell in every well. This drives accurate segmentation of cells **(Figure 1)** and data including counts, area, confluency, and over 20 morphological and phenotypic measures. Graphical visualizations linked to images allows for user inspection of data and exclusion of measures as needed within CellAssist, and all data and graphs can be easily exported for downstream processing or further analysis using 3rd party tools.

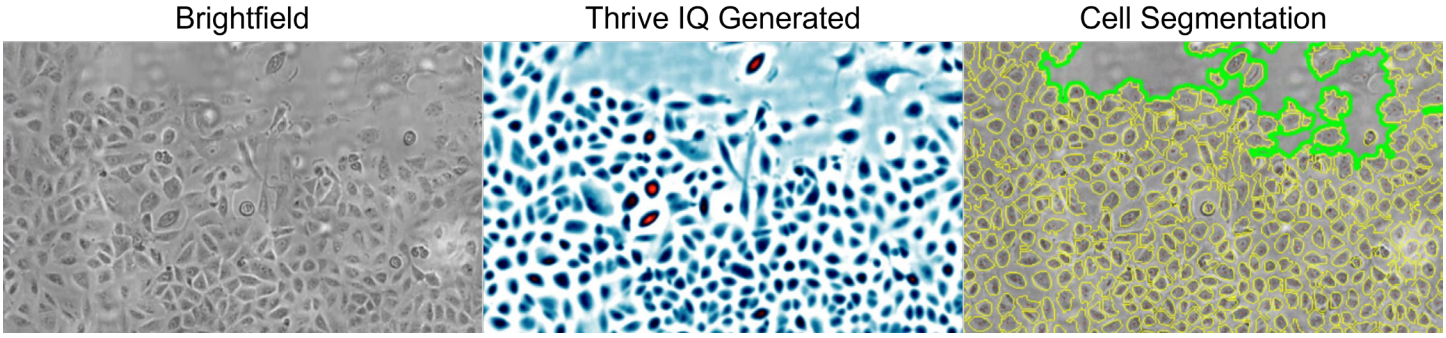

Figure 1 - *Example of a brightfield image transformed via Thrive IQ, which is then utilized to yield highly accurate cell segmentations.*

Cell Preparation and Plating

Cells were harvested utilizing TrypLE Express (ThermoFisher, 12604013) and plated in 96-well plates (Corning Costar, 3596) at a concentration of 1500 cells/well in 300 µl of media. Each media condition was tested on two separate plates and 12 wells in each plate.

Imaging and Monitoring

Test plates were placed in the CellAssist® 50 incubator set to 37 °C, 5% CO2, and 95% humidity. Plates were scheduled to be imaged every 6 h for 4 days beginning 12 h after the plates were placed in the incubator. Plates were imaged at 4x utilizing the standard area setting which covers ~80% of the well. Twenty Z slices were imaged at a distance of 30 µm between each slice and exposure time of 2050 µs. **Figure 2** shows the user interface at the scan configuration page for this experiment.

Data Analysis

Growth curves were generated in CellEval software and represent the average of the 12 wells per condition in that plate. Where values are indicated in text, well-by-well data was exported to CSV. In Excel, the average cell count per time point of each condition separated was calculated using all 24 replicates and reported as the average ± standard error of the mean. Doubling time calculations are performed in the CellEval software with a log base 2 calculation assuming a constant doubling rate. For this reason, to achieve an accurate doubling time, data outside of the linear growth curve is excluded.

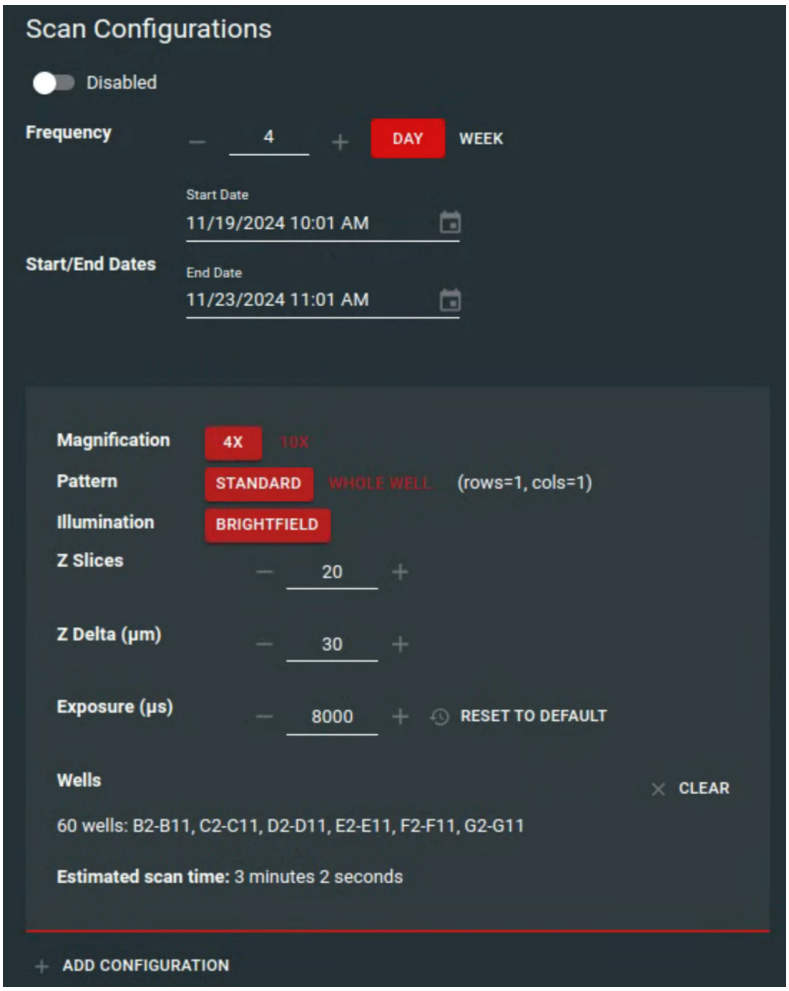

Figure 2 - *The user interface of the scan configuration page for this experiment. Users can adjust the frequency of imaging on a per day or per week basis and indicate the start and end date and time of imaging; imaging does not have to be when cells are placed in the incubator. Based on user-selected imaging conditions (magnification, pattern, Z slices and exposure time) and the number of wells selected, an estimated scan time is indicated.*

RESULTS AND DISCUSSION

Monitoring Cell Growth

Cell lines were imaged every 6 h for a total of 4 days to allow for the generation of full growth phase curves **(Figure 3)**. For both P3 and P20 A549 cells, serum free media cells grew the least . This is expected as FBS is provided as a supplement to cell culture media to provide essential albumins and growth factors.[1] Interestingly, it appears that over the course of 4 days, P20 cells continue to grow in serum free media, albeit at a very slow rate, while P3 cells plateau. Similarly, the worst performing, serum-supplemented media is F-12K. Compared to both DMEM and RPMI, F-12K has a lower glucose concentration and more inorganic salts leading to higher concentrations of sodium, magnesium, copper, and zinc. While additional differences exist among the three media formulations, though it is likely the decreased glucose concentration contributes most significantly to F-12K's poor performance as others have shown that A549 cells utilize more glucose than other cell lines.[2,3] Interestingly, ATCC recommended base media for A549 cells is F-12K, suggesting growth rates of cells may be artificially increased in this experiment.[4] At P20, cells grown in RPMI reach a greater total number of cells at endpoint than any of the other culture conditions, and did so more quickly. While at P3, RPMI more closely mimics DMEM standard growth media and improperly stored media. This suggests, at a higher passage number, the additional amino acids, vitamins, and HEPES buffer present in RPMI versus DMEM plays a larger role in maintaining cell health. Poor media storage conditions (DMEM + 10% FBS stored at 37 °C for 5 days) did not adversely affect cell growth immediately. On average, more cells were not counted in normal growth media until time 66 h at P3 (8062 ± 115 compared to 8011 ± 281) and time 30 h at P20 (1471 ± 38 compared to 1469 ± 38). Again suggesting that higher concentrations of nutrients and supplements, which are degraded by prolonged storage at 37 °C, more drastically affect cell growth and health at higher passage numbers.

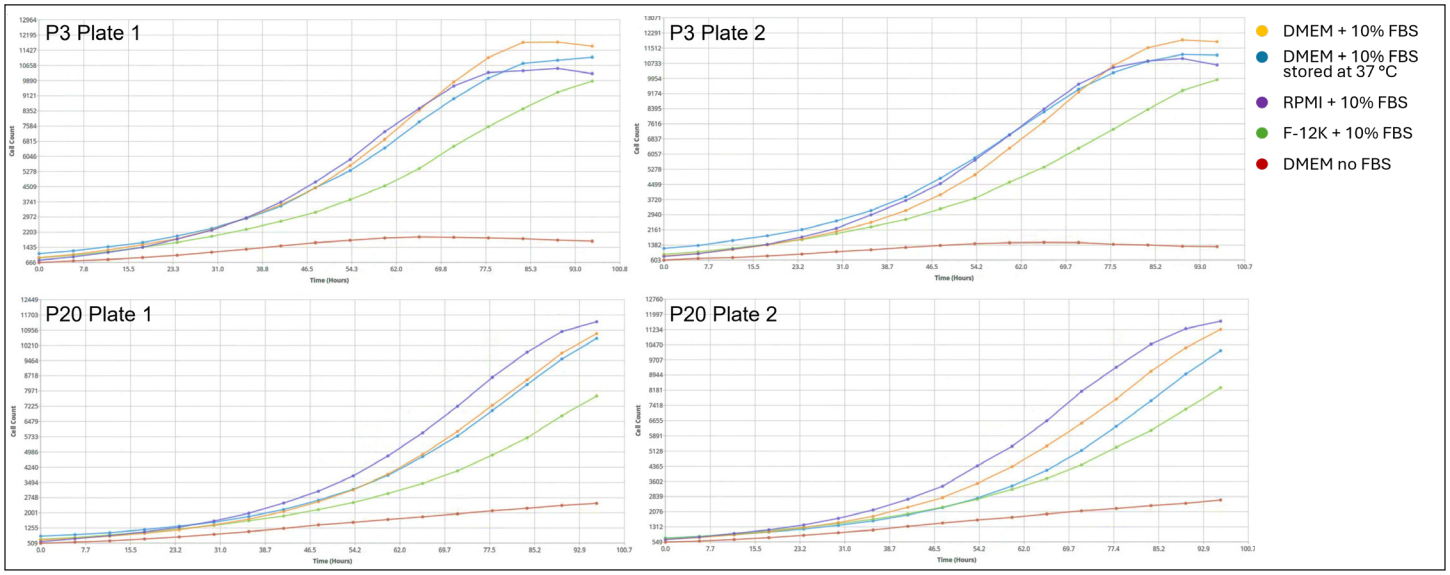

Figure 3 - *Growth curves of the 4 plates included in this experiment. All five media conditions were tested on each plate with 12 replicates per plate for a total of 24 replicates per condition per A549 passage number. Graphs displayed here are directly from the CellEval software and represent the average of the 12 replicates per condition on the indicated plate.*

Determining Doubling Time

Cell count during the logarithmic growth phase was utilized to calculate the doubling time in hours.The standard doubling time calculation, and that utilized by CellEval, is a log base 2 calculation. Therefore, a gate is applied to consider only the exponential growth phase of cells in the doubling time calculation **(Figure 4)**. It is important to note that this allows users to be more accurate in calculations and therefore in further experimental planning. The doubling rate of cells would falsely look lower should the entirety of the data be included in the calculation. This would also happen if users were relying on an assay with only endpoint data being assessed at the end of the 4 days.

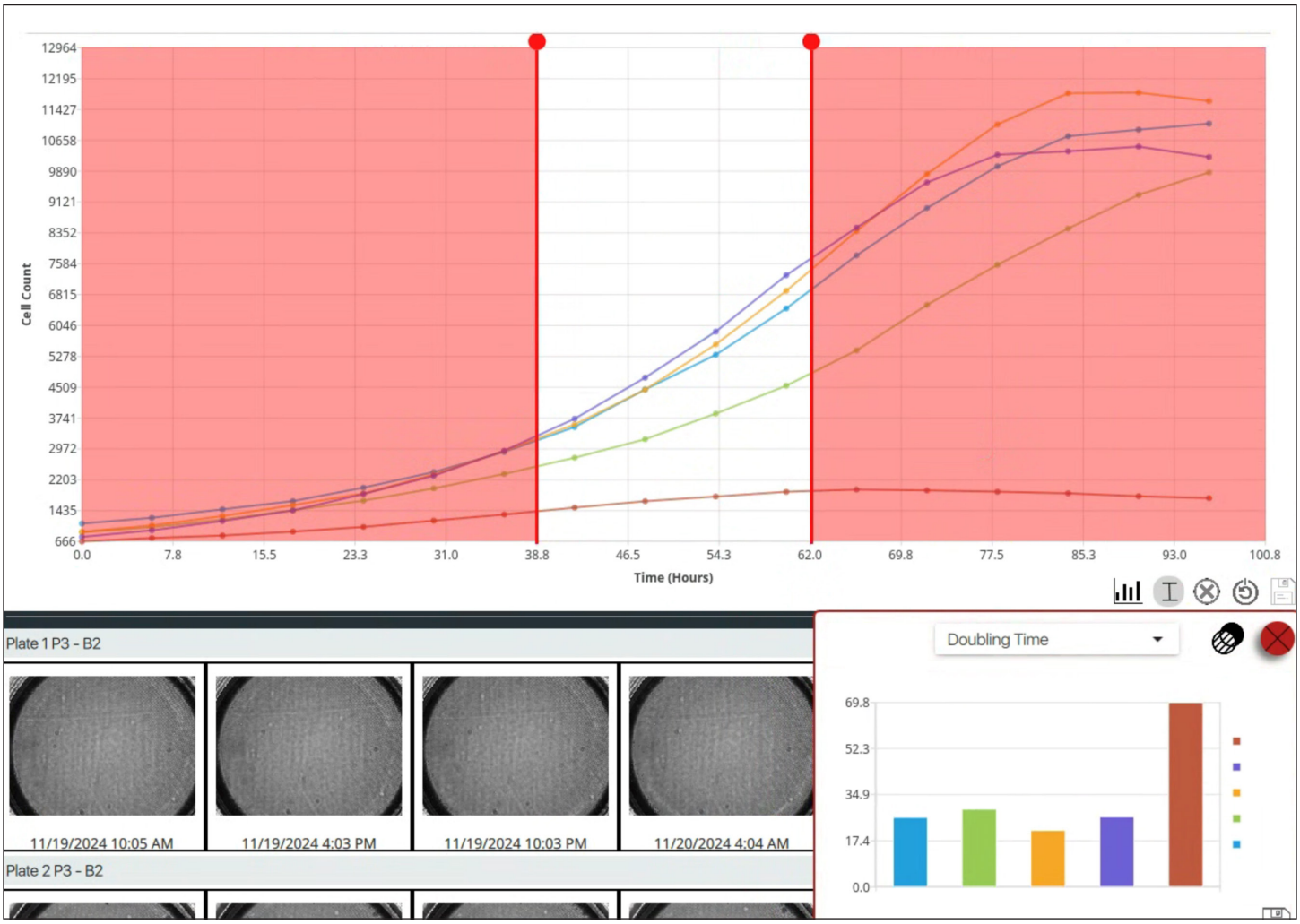

Figure 4 *- Growth curve of within user interface of P3 Plate 1 showing the gated region of the data used to calculate the doubling time. Data highlighted in red is excluded from the calculation. A pop-out graph appears showing the doubling time of each condition on the plate.*

The doubling times for all 5 media conditions in each group, P3 and P20, of A549 cells are provided in **Table 1**. In both low and high passage number cells, normal growth media has the lowest doubling time, 29.1 h and 22.3 h, respectively. Regardless of the media condition, P20 cells have a lower doubling time than P3 cells, demonstrating that A549 cells grow faster the longer they are maintained in culture. This could indicate some change in mitotic processes, loss of dependence on factors specific to DMEM supplemented with 10% FBS, or gain of resistance to less ideal growth conditions. Understanding this change between P3 and P20 could be particularly important in planning experiments that examine changes in proliferation as a result of compound treatment because high passage cells may persist where low passage cells would not.

	Doubling Time (h)	
Media Condition	P3	P20
DMEM + 10% FBS	29.1	22.3
DMEM + 10% FBS stored at 37 °C	40.6	27.6
RPMI + 10% FBS	43.3	25.2
F-12K + 10% FBS	34.2	28.3
DMEM no FBS	552.5	53.1

Table 1

CellAssist

CONCLUSIONS

The Thrive Bioscience CellAssist, paired with the CellEval software powered by Thrive IQ, provides an automated, label-free, and highly reproducible method for monitoring cell proliferation in real time. By enabling quantitative assessment of cell growth phases and doubling times, the CellAssist enhances the accuracy of experimental planning and ensures consistency across experiments.

Key advantages of the CellAssist for proliferation assays include:

- **Label-free Imaging** - Eliminate unneeded fluorescent dyes, minimize phototoxicity, and preserve native cell behavior.
- **Flexible Throughput** - Support a range of experimental throughputs, from small-scale single plate scans to large-scale workflows.
- **Fully Automated** - Reduce manual tasks while ensuring consistent, reproducible data acquisition.
- **Environmental Stability** - Experience minimal environmental impact on your cells with the environmental enclosure.
- **Advanced Quantitative Insights** - Capture robust morphological and phenotypic quantitative data, providing more detailed growth phase analysis.
- **Accurate Doubling Times** - Enables precise calculations of proliferation rates without relying on endpoint
- **Ease of Data Management** - Easily export your results for integration into your other lab work flow tools.

These features position the CellAssist as an optimal solution for researchers aiming to improve the reliability of their proliferation assays, cell health monitoring, and long-term cell culture studies. The ability to track changes in cell growth dynamics over multiple passages ensures that researchers can detect variations early, optimize culture conditions, and greater experimental reproducibility.

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CellAssist 50

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Thrive Bioscience, located in the Boston area, offers customers a family of instruments and software that provide imaging, analytics, and automation for reproducible cell culture. Our products empower biologists by combining advanced software, microscopy, and robotics, to acquire, organize, and analyze images of all their cells.

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