Bioprocess analysis with the LUNA-FX7™

INTRODUCTION

The biomanufacturing industry is experiencing rapid growth due to improvements in cell therapies, such as CAR-T cell therapy, and increasing bioactive production[1]. It is expected to grow at least 12 to 17% annually over the next decade[2]. In response to these demands, biomanufacturers have been expanding the production capacity while maintaining quality and regulatory compliance. Furthermore, it is even more critical to monitor cell growth and health accurately while managing multiple cell batches. To provide biomanufacturers with the flexibility, accuracy, and power to accommodate these analysis demands, we recently developed the LUNA-FX7™ Automated Cell Counter.

The LUNA-FX7™ Automated Cell Counter provides a new bioprocess monitoring feature as well as a Quality Control mode with unique validation slides that can be utilized for quality outcomes. The Bioprocess feature enables scientists to monitor, record, and analyze an individual batch of bioprocessing activities. Once counting is performed under the Bioprocess mode, the LUNA-FX7™ automatically generates growth rates, cell doubling time, and trend charts. Furthermore, entire cell counting data may easily be saved and transferred via a USB flash drive, Wi-Fi, or Ethernet. Notably, the 21 CFR Part 11 compliant "CountWire™" software packages allow more advanced remote access and data management for multiple LUNA-FX7™ devices connected to the same network. By providing a convenient means to trace individual batches and monitor various operations securely, the LUNA-FX7™ can facilitate a more efficient workflow. Here, we demonstrate how the LUNA-FX7™ monitors the cell growth of three suspension cell lines using the Bioprocess option. Each cell line was applied to the three counting modes, Fluorescence Cell Counting mode, Bright Field Total Cell Counting mode, and Bright Field Cell Counting & Viability mode.

MATERIALS AND METHODS

Cell preparation and counting of batch run simulation

Three suspension cell lines, HL60, K562, and U937, were used in this assessment. All cell lines were grown in RPMI 1640 culture media supplemented with 10% fetal bovine serum (100 units/mL). Batch runs were simulated by seeding 10 mL of initial concentrations at 6 x 10^4 cells/mL in 100 mm cell culture dishes and grown for seven days. During batch runs, counts were performed twice a day for all seven days utilizing the Bioprocess feature. For each count, 100 µl of cells were sampled. Each sample was counted via three different cell counting modes: Fluorescence Cell Counting mode, Bright Field Total Cell Counting mode, and Bright Field Cell Counting & Viability mode. For brightfield viable cell counting, a 1:1 mix of 0.4% Trypan blue stain (Cat# B13101) was used. For fluorescent cell counting, a 2:18 mix of Acridine Orange/Propidium Iodide (AO/PI) (Cat# F23001) was used.

Bioprocess operations

The LUNA-FX7™, the most advanced model in the LUNA™ Family comes equipped with the Bioprocess software plus an expanded 1 TB hard drive. First, a unique protocol name for an individual batch is assigned to start bioprocess monitoring. In this manner, multiple batches are easily monitored and stored in the profiles of status. For this demonstration, the three protocols for bioprocess monitoring were named as the cell lines - HL60, K562, and U937* [1],

*[1] *As the three cell lines have shown almost the same trends, the detailed bioprocess data of HL60 is representatively selected and presented in this application note.*

respectively. Actual protocol parameters may be customized to specific cell lines or remain unchanged. Here, the default protocol parameters were used for all cell lines. Prior to counting, the assigned protocol for a specific batch is loaded. For each counting, load the target protocol for the bioprocess, count the cells, and save the results with a check on the Bioprocess saving option. The LUNA-FX7™ automatically calculates the growth rate and the doubling time, generating updated growth curves for individual batches based on the counting in the last two intervals (Figure 1). The on-screen chart is flexible; the y-axis can be toggled to total cell concentration, viable cell concentration, and viability; simultaneously, the x-axis is changeable to time, date, and month (More details in the User Manual).

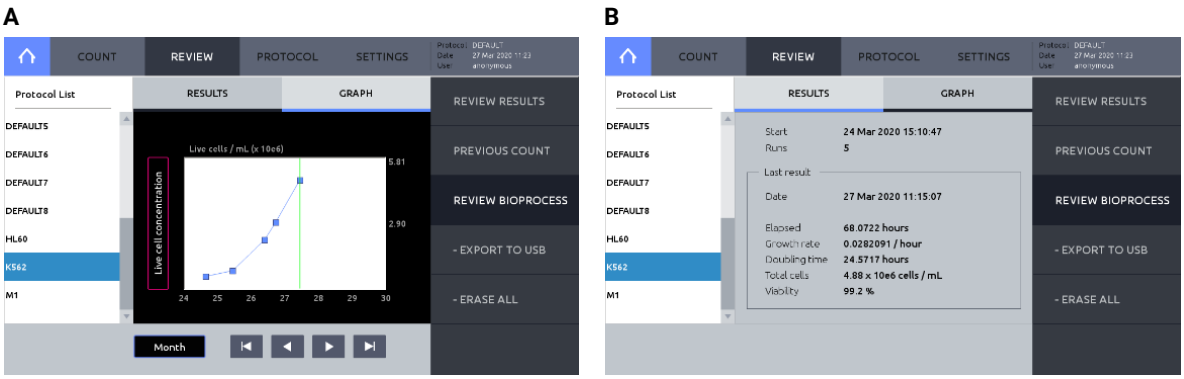


***Figure 1.** On-screen growth chart and results of the K562 cell in the Bioprocess Review. (A) The growth chart is showing the cumulative results of cell concentration over date. (B) Summarized results show the start date, the number of measurements, current growth rate, doubling time, current concentration, and viability.*

Data visualization and extrapolation

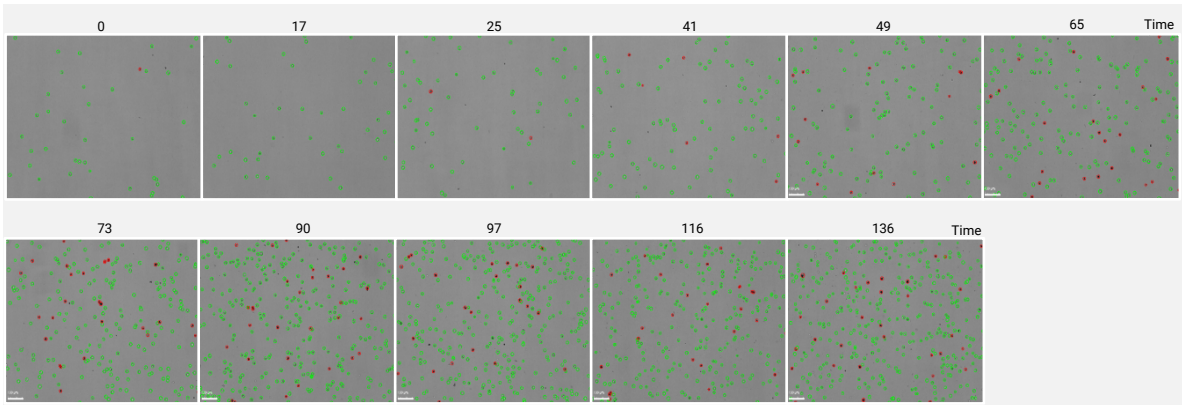
The LUNA-FX7™ automatically records and calculates bioprocess indicators such as growth rate, viability, and doubling time. Thus, the report is generated including the trend charts of the data and tables of cell counting status (Figure 2). Further, the cell status also includes cumulative tracking at each time point, along with immediate or overall cell growth rates. The data can be exported as a CSV file for more compiled analysis if needed (Table 1).

***Table 1.** The report in Fluorescence Cell Counting Mode. The example CSV report from the HL60 batch run. The report contains date, time, concentration and viability. The data of escape time, growth rate, and doubling time are calculated from the last count (Blue shadows) and the run's total time (Green shadows).*

Date & Time	Total cell conc. (/mL)	Live cell conc. (/mL)	Total cell #	Live cell #	Viability (%)	Elapsed time (last) (hrs)	Growth rate* (last) (/hr)	Doubling time** (last) (/hr)	Elapsed time (total) (hrs)	Growth rate* (total) (/hr)	Doubling time** (total) (hrs)
3/31/2020 5:34	6.75E+05	6.55E+05	742	720	97						
3/31/2020 23:09	9.53E+05	9.28E+05	1048	1020	97.3	17.01	1.96	35.34	17.59	1.96	35.34
4/1/2020 7:41	1.24E+06	1.23E+06	1360	1350	99.3	8.01	3.08	22.47	26.12	2.33	29.77
4/1/2020 21:59	1.84E+06	1.82E+06	2028	2005	98.9	14.01	2.76	25.11	40.42	2.48	27.94
4/2/2020 5:39	2.23E+06	2.21E+06	2447	2433	99.4	7.01	2.51	27.64	48.09	2.49	27.89
4/2/2020 22:55	3.61E+06	3.59E+06	3972	3951	99.5	17.01	2.79	24.84	65.35	2.57	27.02
4/3/2020 5:58	3.80E+06	3.79E+06	4176	4168	99.8	7.00	0.73	95.28	72.40	2.39	29.04
4/3/2020 23:46	5.33E+06	5.29E+06	5863	5820	99.3	17.01	1.90	36.46	90.20	2.29	30.26
4/4/2020 6:45	5.43E+06	5.37E+06	5967	5905	99	6.00	0.27	260.40	97.19	2.15	32.31
4/5/2020 1:16	5.43E+06	5.18E+06	5970	5690	95.3	18.01	0.00	0.00	115.71	1.80	38.47

*Growth rate per hour = (LN V2 - LN V1) / (t2 - t1)
**Doubling time in hours = LN (2) / Growth rate per hour
LN = Natural log,
t1, t2 = Unit of hour,
V1 = Cell concentration in cells/mL at elapsed time t1 in hours,
V2 = Cell concentration in cells/mL at elapsed time t2 in hours

A



B

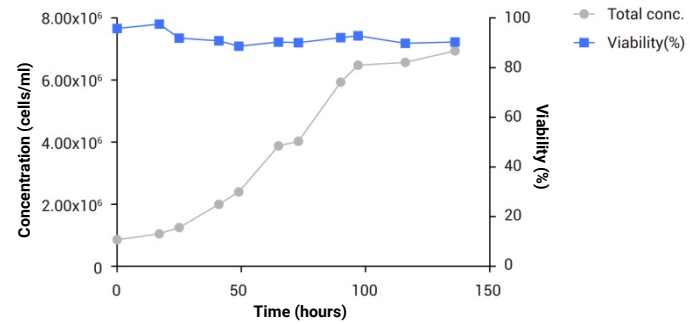


Figure 2. Bioprocessing data output. *Images and charts from fluorescent counting of HL60 cells during a 136-hour batch run. (A) Tagged images at each sampling of the batch run. (B) The plots of cell concentration and viability curves over date were accordingly generated in the LUNA-FX7™.*

LUNA-FX7™ Automated Cell Counter

CONCLUSION

The Bioprocess option in the LUNA-FX7™ reduces unnecessary effort by automating the recording and analyzing status indicators such as cell growth and viability, simultaneously performed three cell culture batches of 3 different counting modes. Remarkably, the automated calculations of doubling times, growth curves, and viability status provide near-real information to monitor and forecast bioprocess production timelines reliably and accurately. When combined with the CountWire™ software package, the Bioprocess feature allows team members to monitor multiple culture batches in real-time across multiple facilities.

REFERENCES

[1] Joubert S, Dodelet V, Béliard R, Durocher Y. La bioproduction des anticorps monoclonaux [Biomanufacturing of monoclonal antibodies]. Med Sci (Paris). 2019;35(12):1153-1159. doi:10.1051/medsci/2019219

[2] Aijaz A, Li M, Smith D, et al. Biomanufacturing for clinically advanced cell therapies. Nat Biomed Eng. 2018;2(6):362-376. doi:10.1038/s41551-018-0246-6