Cell culture confluency estimation using embedded application on the InCellis® Smart Cell Imaging System

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/ ABSTRACT

In cell biology related studies, accuracy and efficiency in cell culture quality checks are crucial in order to avoid any potential complications in the downstream analysis. Usually, different cell parameters such as cell counting and cell size measurement are assessed and estimated visually in a subjective way. However, visual assessments are unreliable, time consuming and often yield inaccurate results which lead to incorrect conclusions and incorrect recommendations. This also adds to the end cost of the analysis with regards to consumables such as costly reagents. In this study, cell confluency application was used to accurately estimate the proportion of adherent cells in an automated manner using a cell culture confluency application available on the InCellis® Smart Cell Imaging System (Bertin Technologies).

/ METHODS

MCF7 (breast cancer) cell lines were plated on the petri dish and incubated at 37°C for in appropriate cell culture media (DMEM).

The cell confluency application installed on the InCellis® was then used to estimate cell culture confluency every 24 hours for three consecutive days.

A series of images of the cell line were taken in phase contrast mode using 10x and 20x objectives on day-1, day-2 and day-3 at the same time in order to check the accuracy of cell confluency. Each day, in every magnification, 4 different fields of view were used for the study.

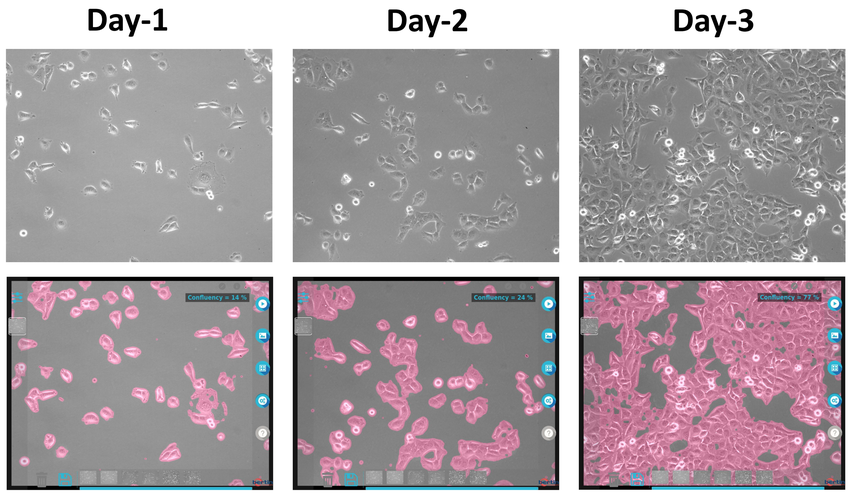


Figure 1. Confluency of the MCF7 cell lines across 3-days using 20x objectives and cell confluency application installed on the InCellis®

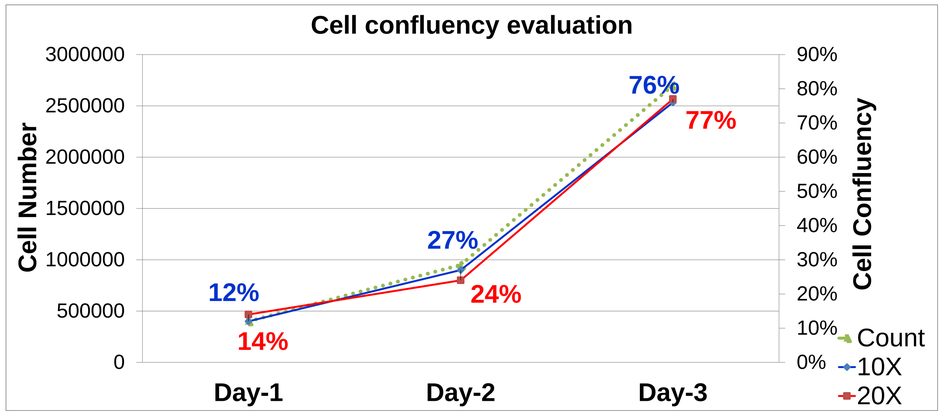
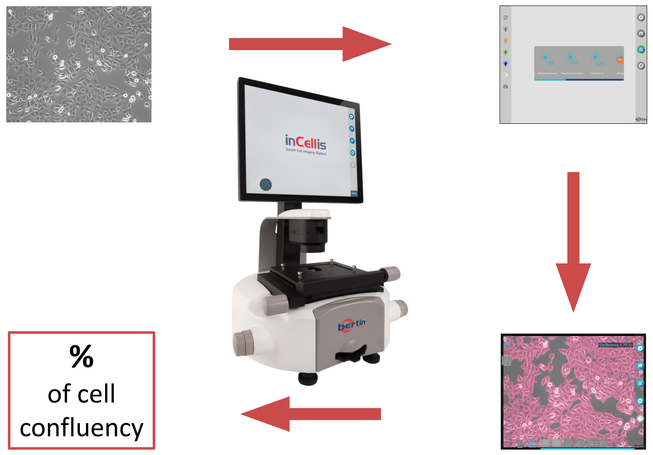


Figure 2. Evolution of the cell confluency over three days using the InCellis® Smart Cell Imaging System (Bertin Technologies). The cell line confluency ranged from 12% on Day-1 to 76% on Day-3 when using 10x objective, and from 14% to 77% on Day-1 and Day-3 respectively when using 20x objective.

/ RESULTS



As soon as the confluency is less than 70%, there is a lot of heterogeneity in the visual eyes estimation depending on the operators.

With the confluency App, the comparison of the cell culture confluency between each field of view for the same cell line on the same day give a good standard deviation (less than 10%).

We defined that 4 fields of view is the best to generate a robust value of confluency with 10X or 20X magnification.

As a control we followed the cell number by a manual cell counting performed with Malassez cell and InCellis Total Cell counting App. Both methods are correlated and show an increase of total cell number as expected.

/ CONCLUSION

The cell confluency application provides robust results with a stain free method to follow the cell proliferation.

The application ensures a rapid and efficient quality control of cell lines before use in other analysis such as transfection, drug assay, signal transduction study, etc…

The automated estimation of the cell confluency provides not only consistent results but also significantly reduces the hands-on time for all cell-based assays

Acknowledgement:

We would like to thank the LGBC (Laboratoire de génétique et biologie cellulaire, Université de Versailles Saint Quentin en Yvelines) for kindly providing images of the MCF7 breast cancer cell lines for confluency estimation using InCellis® Smart Cell Imaging System (Bertin Technologies).

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