# Cell culture confluency estimation, transfection efficiency and total cell counting applications on the InCellis® Smart Cell Imaging System

Laima Antanaviciute[1], Olivier Varet[2] and Sophie Dubacq[2]

[1]Bertin Corp, 2096 Gaither Road, Suite 230, Rockville, MD 20850, USA; [2]Bertin Technologies, 10 bis, avenue Ampère, 78180 Montigny-Le-Bretonneux, France
*laima.antanaviciute@bertin-corp.com

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## / ABSTRACT

Cell biology studies of cell proliferation, invasive behaviour of cells, and cell line expression require a rapid and robust cell line confluency estimation, transfection efficiency investigation, and accurate total cell counting. Incorrect observations might lead not only to inaccurate results but also to incorrect conclusions and recommendations. In this study, breast cancer cell line (MCF-7) was used to accurately estimate cell line confluency and total cell counting in an automated manner using embedded applications on the InCellis® Smart Cell Imaging System (Bertin Technologies). To validate the automated transfection efficiency application, HeLa cell culture lines and GFP label were used. The confluency and cell counting applications supply robust results with a stain-free method in comparison to the standard method. The transfection application provided a rapid and user-friendly method for estimation of transfected cells before use in further analysis. The automated applications provide consistent results and significantly reduce the hands-on time to define the perfect time to start cell-based assays and normalize results.

## / METHODS

**Cell Confluency Estimation**

- MCF7 (breast cancer) cell lines were plated on the 85mm perti dish and incubated at 37°C in DMEM cell culture media for 2 days
- 4 different images were taken at exactly the same time of day over a three-day period in phase contrast mode using 10x (images are not shown) and 20x objectives
- The cell confluency application embedded on the InCellis® was used to estimated cell culture confluency

**Total Number of Cell Counting**

- The same (MCF7) cell lines were used for total cell counting experiment
- Cell lines were plated on petri dishes in two cell densities (1/5 & 1/10), and were incubated at 37°C in DMEM for 2 days
- 8 different images were taken on day-2 in phase contrast mode using 10x and 20x objectives
- The total cell counting application embedded on the InCellis® was used to determine the total number of cells in culture
- For the comparison analysis between automated (InCellis®) and manual (Malassez) cell counting methods, cell lines were tripsinized, collected in cell culture media and an aliquoted cell suspensions were loaded on a Malassez cell. The number of cells was counted within 100 small squares following manufactures recommendations

**Cell transfection efficiency**

- HeLa Cell line was transfected with a GFP tagged protein and incubated in DMEM cell culture media at 37°C for 48h.
- 4 2-channel images of the cell culture were taken with InCellis® in phase contrast and with GFP F.L.M. to visualize transfected cells

## / CONCLUSION

All three (Confluency, Cell counting and Transfection) applications are robust approach for a rapid investigation of quality control of cell lines before use in other analysis

Applications provides accurate and consistent results when compared to the standard visual and manual methods

A non-invasive (stain and trypsinisation-free) method for confluency and a total cell counting allows the normalisation of cell-based assays by eliminating reagent usage and significantly reducing hands-on time

Acknowledgement:

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## / RESULTS

MCF7 cell line confluency estimation using an automated **Cell Confluency** application on InCellis®

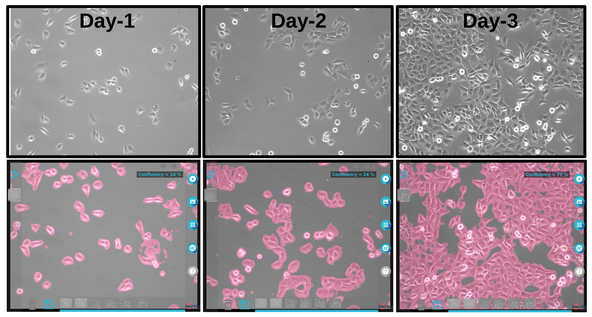



**Figure 1.** Images captured of the MCF7 cell lines over a thee-day period using 20x objective. The confluency calculation is presented in percentage, whereas detected cells are highlighted in pink by the application.

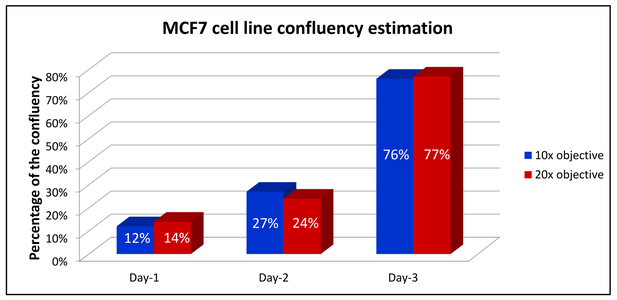
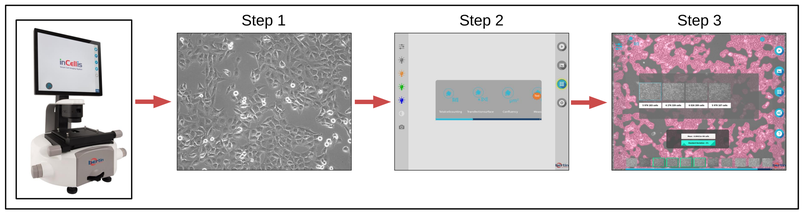



**Figure 2.** Cell confluency over a three-day period using InCellis®. The cell line confluency ranged from 12% on Day-1 to 76% on Day-3 using 10x objective (in blue), and from 14% to 77% using 20x objective (in red).

A total MCF7 cell line number counting using an automated **Total Cell Counting** application on InCellis®

**Workflow:** Step 1) At least 4 images of cell culture are captures in phase contrast at different field of views. Step 2) Cell Counting application is selected on the InCellis® to count the number of cells in the cell culture. Step 3) Cell Counting APP displays results across 4 images and generates mean value of cell number in the cell culture.



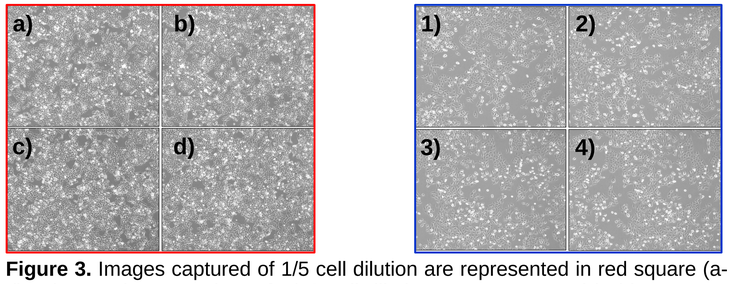




**Figure 3.** Images captured of 1/5 cell dilution are represented in red square (a-d), whereas images taken of 1/10 cell dilution are represented in blue square (1-4) using 20x objective. A total of 6.3M cells (standard deviation of 3% for 1/5 cell dilution and 3.5% for 1/10 cell dilution) were obtained using InCellis®.

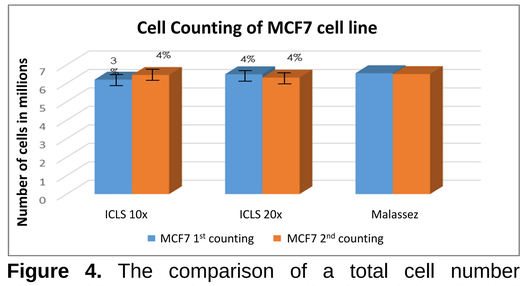



**Figure 4.** The comparison of a total cell number observed between the MCF7 cell culture (1/10 dilution) calculated with an automated Total Cell Counting application (InCellis, 10x and 20x magnification) and a manual cell counting (Malassez cell).

Transfection efficiency estimation using HeLa cell lines and **Transfection Efficiency** application on InCellis®

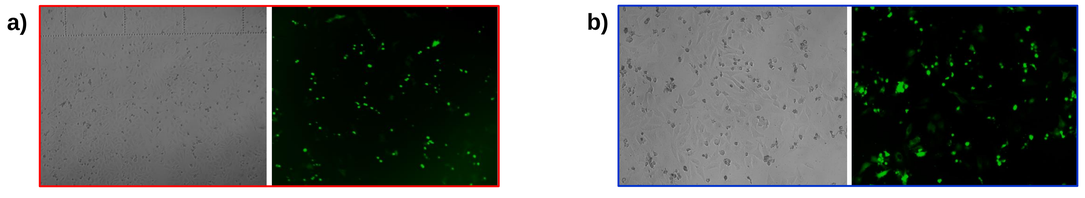



**Figure 5.** Transfection efficiency calculation of 2 different HeLa cell cultures. a) 28% transfection efficiency for the first cell culture, 10x magnification; b) 41% transfection efficiency for the second cell culture, 20x magnification

CONTACT
Email: cellimaging@bertin-instruments.com
www.bertin-instruments.com

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