Introduction
Visual assessment and estimation of cell culture confluency is commonly used in cell biology related studies, but unreliable, time consuming and often yielding inaccurate results leading to incorrect conclusions. This application note reports a novel approach for an automatic, accurate and rapid cell confluency estimation of the proportion of adherent cells using a cell culture confluency application available on the InCellis® Smart Cell Imaging System (Bertin Technologies).

Materials and Methods
Human breast adenocarcinoma cell lines (MCF-7) were plated on petri dishes containing cell culture media (DMEM) following the 37°C incubation.
Cell line confluency was assessed and estimated using the InCellis® confluency application every 24 hours for three consecutive days.
Two phase contrast objectives (10x and 20x) were used to capture four images at different fields of view of the cell lines on day-1, day-2 and day-3 at the same time, to check the accuracy of cell confluency.

Results
The results of this study showed that the confluency application is a rapid approach for accurate estimation of cell confluency (Figure 1). Confluency of the MCF-7 cell lines ranged between 12% on Day-1 to 76% on Day-3 using 10x phase contrast objective, whereas between 14% to 77% on Day-1 and Day-3 respectively using 20x phase contrast objective (Figure 2). Four field of views offered a sufficient number of images to generate robust value of confluency. A good standard deviation (>10%) was observed when comparing the images of the same cell line on the same day.

Conclusions
The cell confluency application provides robust results with a stain free method to follow cell proliferation. It ensures a rapid and efficient quality control of cell lines before use in other analysis (transfection, drug assay, signal transduction study...). The automated estimation of the cell confluency provides consistent results and significantly reduces the hands-on time for all cell-based assays.

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

Figure 2. Evolution of cell confluency over three days using the InCellis®.