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# BEST PRACTICES FOR CELL CULTURE CONFLUENCY CALCULATION WITH INCELLIS®

Cells are used as a working tool everyday in all R&D labs and quality checks must be performed in order not to compromise downstream experiments. In cell culture applications, confluence assessment is an important parameter that is commonly used to estimate the proportion of adherent cells. Most confluency measurements are estimations of the area covered by the cells on a growth surface. The common method to determine cell culture confluency is visual estimation but it is not consistent or accurate enough for scientific experiments. As an example, most of transfection reagents came with a specific confluence at the time of transfection.

InCellis® from Bertin Technologies greatly facilitates the calculation of the cell confluency and improves the results' accuracy, thanks to the specific and dedicated application "Cell Confluency".

## IMPROVE YOUR CELL CULTURE CONFLUENCY ESTIMATION USING THE EMBEDDED APPLICATION ON INCELLIS®

## SUMMARY




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CNIM GROUP

# AUTOMATIC CALCULATION OF HELA CELL CULTURE CONFLUENCY

Bertin Technologies & Team MDVA, IRIM/UMR 9004, Research Institute in Infectiology of Montpellier France

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## / CONTEXT

Cell confluency is a key parameter for all cell biologists as it is the beginning of all other cell culture experiment such as transfection, cell-based assays and cell culture quality control.

To obtain the maximum efficiency of transfection and avoid using expensive reagents for nothing, the cell confluency needs to be calculate with accuracy.

Today, the majority of cell biologists estimate the cell confluency in culture flasks in a subjective way, looking at the amount of space covered by cells.

Protocols using hematocytometers or specific dying (Trypan blue) can be used but it requires an extra step.

InCellis® from Bertin Technologies is used to observe the cells and calculate the cell confluency directly on the bench thanks to the specific and dedicated application “Cell Confluency”.

## / MATERIALS

- InCellis® Cell Imager for Brightfield, Phase contrast and Fluorescent applications 004393-003-RD0001-A
- InCellis® - 20X FL/Ph LWD objective: for petri dish, flask
- HeLa Cell Line (supplied by Team MDVA)

## / PROTOCOL

HeLa Cell line was plated and then incubated at 37°C.

After incubation, InCellis® has been used to control cell culture confluency every 24h.

An image of the cell culture was taken with InCellis® in phase contrast on day 1, day 2 and day 3 to check the confluency.

InCellis® embedded application allows the calculation of a 58% confluency, directly on the bench.

The percentage of confluency is the expected result to continue the experiment.

## / RESULTS

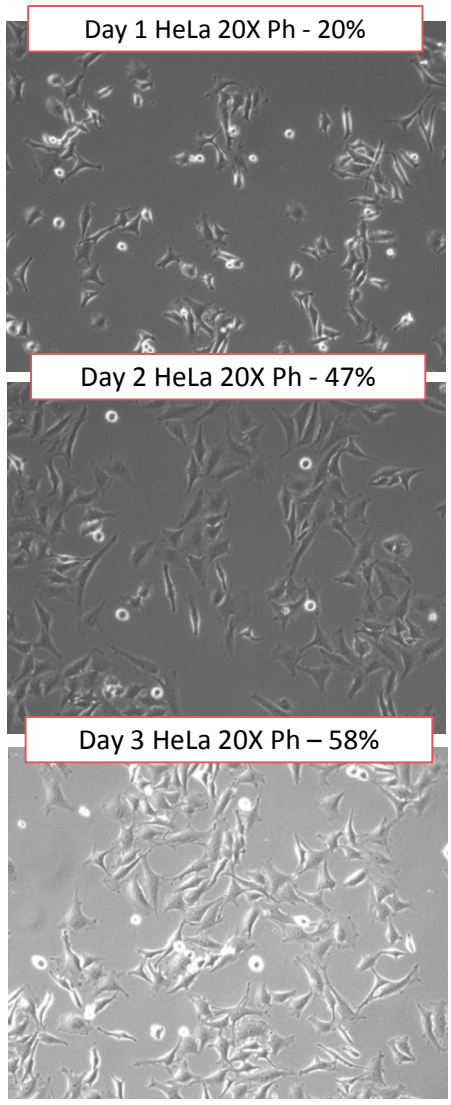


Fig1: image of HeLa Cell at day 1, day 2 and day 3. The confluence is 58%. Cell line kindly provided by Delphine Muriaux, Team MDVA, IRIM/UMR 9004, Research Institute in Infectiology of Montpellier France

## / CONCLUSION

InCellis® allows to easily control the confluency of any living cell line on the bench without compromising the cell culture and the experiment.

InCellis® Smart Cell Imaging System provides a useful application to automatically calculate the confluency in a few seconds. It ensures a rapid and efficient quality control of the viability of your cell line before use in transfection , cell-based assays or any other cellular analysis.

ICLS-915-DU003

# IN VITRO SCRATCH ASSAY: A CONVENIENT METHOD FOR ANALYSIS OF CELL MIGRATION

Bertin Technologies, Montigny-Le-Bretonneux, France

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## / CONTEXT

The “scratch” assay is the most widely used as it is a simple, well established, and inexpensive method to measure cell migration in vitro.

The basic steps involve creating a "scratch" in a cell monolayer, capturing the images at the beginning and at regular intervals during cell migration to close the scratch, and comparing the images to quantify the migration rate of the cells. It is often applied to study cell migration, drug effects on cells, angiogenesis, cell-cell interactions, metastasis, wound healing…

To ideally follow the scratch test, a live cell imaging is often use, but only few labs own a live cell imaging system. The InCellis®, with the cell confluency application, offers the best alternative to follow the results of a scratch test, as the cell confluency % will increase within the cell growth and can be correlate to the cell migration rate.

## / MATERIALS

- InCellis® Cell Imager for Brightfield, Phase contrast and Fluorescent applications 004393-003-RD0001-A
- InCellis® - 10X FL LWD objective: for petri dish, flask
- HeLa Cell Line (supplied by Karolinska Institute - Sweden)
- 1 ml pipette tip

## / PROTOCOL

The cells were seeds on a 6 well plate, in DMEM, to obtain a monolayer of cells with 100% confluency. The media is changed every two days.

Without changing the media, a scratch was done manually with a new 1 ml pipette tip across the center of the well.

Then, a drug will be applied and the growth and cell migration will be compared between the control cell culture and the treated cells.

## / RESULTS

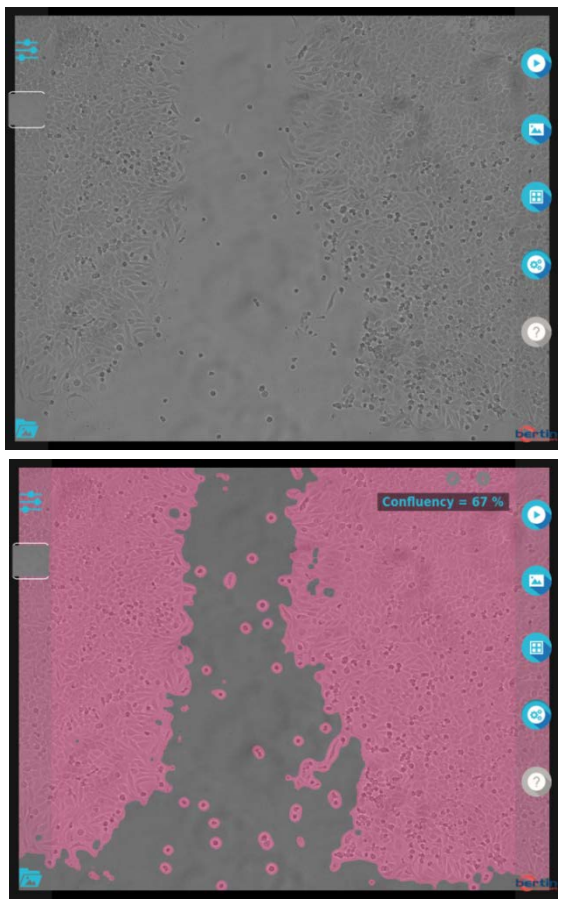


Figure 1. image of HeLa Cell at 100 % cell confluency after scratching.
The cell confluency application is used to estimate the confluency and follow the migration of the cells every 6 hours

## / CONCLUSION

The InCellis® allows to document easily and within a few minutes a scratch assays. Using the cell confluency application provided with the InCellis®, the scratch assay results can be performed on the bench without labeling cells or compromising the cell culture and the experiment.

The Cell Imaging system InCellis ® offers the best alternative to expensive instrument or time consuming experiment with an easy and reproducible quantitative measurement.

ICLS-915-DU005

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# SPECIFIC PROTOCOL FOR CELL CONFLUENCY CALCULATION WITH INCELLIS®

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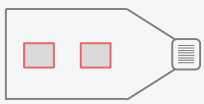
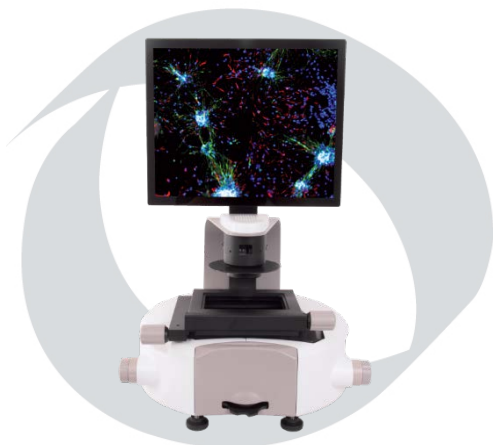
## / CELL CONFLUENCY WITH A 10X OBJECTIVE

- SAMPLE TYPE — Living cells in a flask
- OBJECTIVE REF — UPLFLN10X/2
- PHASE WHEEL — Black position
- NUMBER OR IMAGE* avoiding edge effect — 2 fields of view in the middle



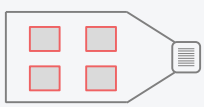
- INSTRUMENT — InCellis®

*minimum of images to take to get consistent result



## / CELL CONFLUENCY WITH A 20X OBJECTIVE

- SAMPLE TYPE — Living cells in a flask
- OBJECTIVE REF — LCACHN-PH20X/0.4
- PHASE WHEEL — 20X
- NUMBER OR IMAGE* corner avoiding edge effect — 4 fields of view on each



- INSTRUMENT — InCellis®

* minimum of images to take to get consistent result

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- **Find application notes** presenting validated protocols & application
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- **Share with the InCellis® community**

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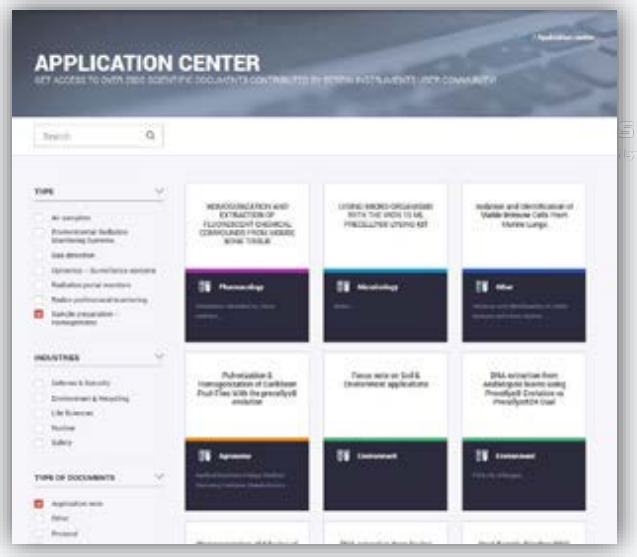


InCellis® is a unique cell imager developed to generate publication-quality images of cells, on tissue slide or in cell culture:

- **High sensitivity** in fluorescence
- **Embedded cell culture applications** for accurate results
- **Smart interface** to save and share results!


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