# ACCURATE & RAPID VIABLE CELL COUNTING IN AN AUTOMATED MANNER USING INCELLIS®

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## / CONTEXT

The trypan blue dye is a commonly used staining agent to distinguish death tissues and cells from live cells. The method is well known and is widely used by a majority of research laboratories that conduct cell biology-based assays. Live cell counting is routinely performed manually using a microscope and a hemacytometer. However, accuracy and reliability of manual approach is limited, in addition to being a time consuming process.

Here, we report on how the use of a Total Cell Counting App available on InCellis®, rapidly and accurately number of dead and live cells in an in-vitro cell culture can be calculated in a novel automated approach.

## / MATERIALS

- **Imaging System:** InCellis® Cell Imager for Brightfield, Phase contrast and Fluorescent applications
- **Objectives:** 20× FL/Ph LWD objective: for petri dish and flask (Olympus)
- **Cell Line:** S2 Cell Line (supplied by EA 4589 team)
- **Staining:** 2× filtered trypan blue solution
- Single use cell counting chamber

## / PROTOCOL

- The S2 cell line was plated in Petri dish, and then incubated at 37°C in recommended cell culture media for 72 hours.
- After an incubation period, cells were trypsinized for 5 minutes
- 500µl of suspended cells were mixed with 1 to 1 ration filtered 0.8% trypan blue agent (final concentration 0.4%). 20µl of the stained cell solution was loaded on a single use cell counting chamber for cell counting
- Four images of the cell suspension were captured using a 20× magnification objective in phase contrast under the color mode setting
- The Total Cell Counting App threshold was set at 6 to count viable and dead cells. Whereas threshold was increased to 14 to count viable cells only

## / RESULTS

*Fig 1: S2 cells stained with trypan blue.*
*Live cells excluded from staining appear in light blue (green circle), whereas dead cells absorbed trypan blue and appeared in dark blue color (red circle).*

*Fig 2: Dead and viable cells detected (highlighted in pink). Threshold set at 6.*

*Fig 3: Only viable cells detected using InCellis®. Threshold set at 14.*

*Fig 4: A mean of 25736 cells were detected in 20µl of stained cell solution*

Cell concentration can be calculated as follow:

Cell concentration/ml = 2 × total cell number / 20 * 1000

Cell concentration of 2.5 × 10e6 cells/ml was obtained in this study.

## / CONCLUSION

Total Cell Counting App available on InCellis® Smart Cell Imaging System, provides an automated calculation of viable and dead cells in seconds. The method ensures a rapid and efficient quality control of cell lines prior cell plating.

The system allows to follow any cell culture experiment at different stages: an accurate cell counting before cell seeding; direct observation of cell grow and cell morphology; to determine cell transfection rate; capture high resolution images of immunofluorescence assays; perform image analysis.

InCellis® is a true All-In-One solution for cell biologists.

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