



ACCURATE AND 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

/ 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.

/ 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.

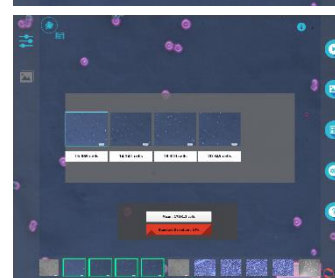


Fig 4 : A mean of 25736 cells were detected in 20µl of stained cell solution

Cell concentration can be calculated as follow:

$$\text{Cell concentration/ml} = 2 \times \text{total cell number} / 20 * 1000$$

Cell concentration of 2.5×10^6 cells/ml was obtained in this study.