PharmaFlow™ Technical Report

Laura Breen, James Flynn, Adam Bergin, Evangelia Flampouri and Michael Butler

National Institute for Bioprocessing Research and Training (NIBRT), Dublin

Summary

The dynamic imaging system described in this report, Canty PharmaFlow™, can accurately and reproducibly measure density and viability of mammalian cells. Furthermore, the classification system employed by the accompanying software provides more detailed information than standard methods. This allows the potential for a user to intervene in a culture at an early stage in order to prolong the health of the culture or to terminate the culture in order to reduce the release of host cell proteins into the media.

Background

In pharmaceutical bioprocessing, cell viability is routinely monitored by the integrity of the outer membrane using dye exclusion, typically trypan blue.

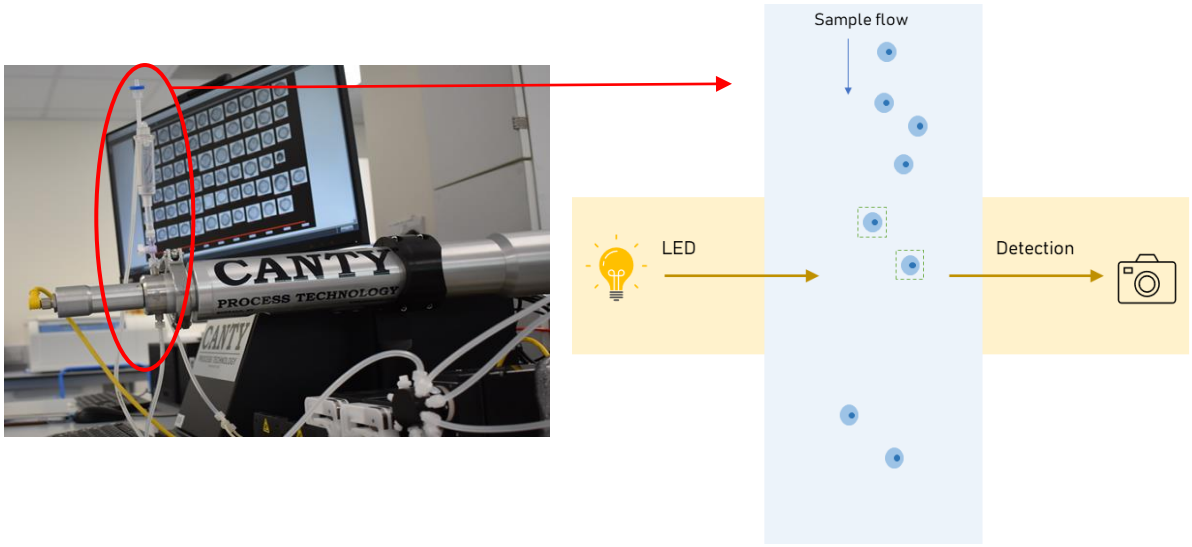

Figure 1: Canty Pharmaflow system. A sample of cells is introduced to the system where they pass through a flow cell between an LED light source and a camera and objective lens. The detected images are sent to the imaging software for analysis.

In this report we used the Canty PharmaFlow™, a non-invasive high resolution optical system (described in Figure 1 above) to analyse Chinese Hamster Ovary (CHO) cells and compared the results to traditional trypan blue measurements. The classification system used by the accompanying CantyVision™ software and potential for more detailed analysis of mammalian cell populations are also discussed.

Cell Counting

Chinese Hamster Ovary cells (CHO-EG2) were cultured in either bench-top Applikon bioreactors or 125 mL shaker flasks. Samples were taken periodically and analysed using an automated cell counter and trypan blue or the Canty PharmaFlow™ and total cell density, percentage viability and viable cell density were calculated (Figure 2).

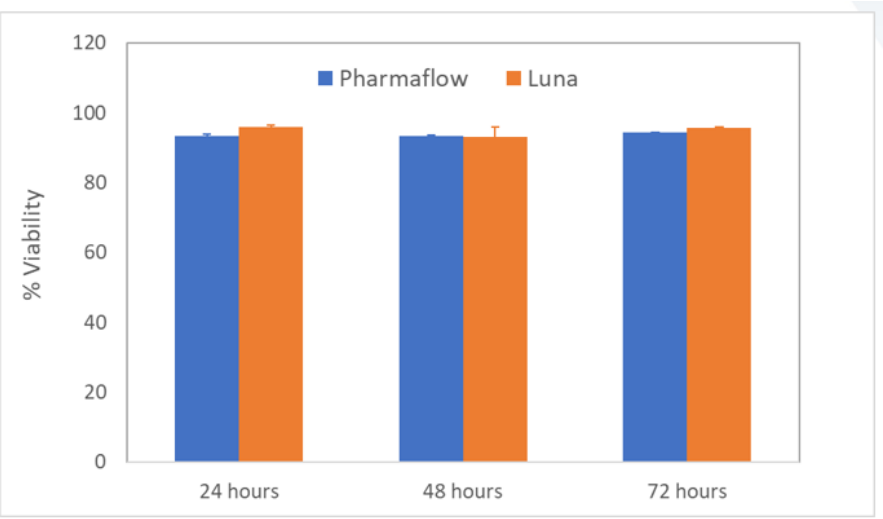

Figure 2: Cell viability determination. This graph shows that the PharmaFlow can measure the cell viability of mammalian cells in line with the gold standard method.

Classification of Cell State in Real Time

For each sample introduced to the PharmaFlow™ thousands of individual cells are analysed by the software, depending on the initial cell density of the sample. As described earlier the CantyVision™ software accurately assesses the cell density and viability, but it also allows

the user to visually observe morphological changes. Figure 3 below shows examples of the classified cells as viable at different timepoints during culture.

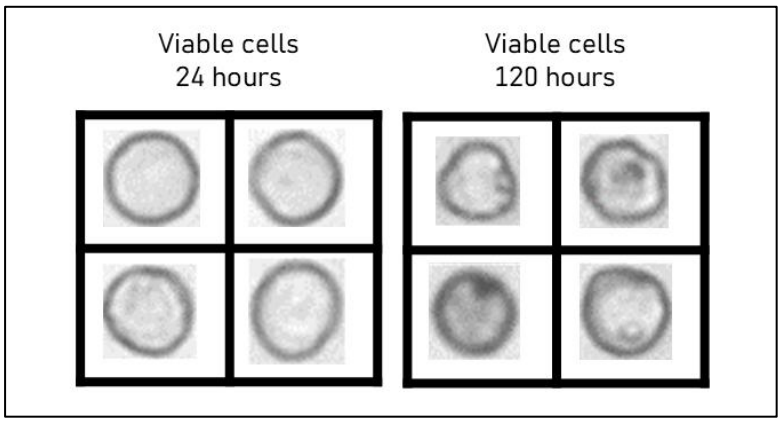

Figure 3: CantyVision™ software cell classification. Morphological changes can be observed in cells at 120 hours of culture even though the cells are still viable.

Early Detection of Poor Cell Health

As mammalian cells grow the health of the cell population will decline over time. We observed the formation of "holes" or vacuoles in the CHO cells after approximately 4 days in culture which were visible of the PharmaFlow™ imaging system. Further investigation revealed that these vacuoles are likely to be related to autophagy induction in the cells (Figure 4). The ability to detect and quantify these cells could potentially allow an intervention at earlier detection of declining cell health by reintroduction of nutrients.

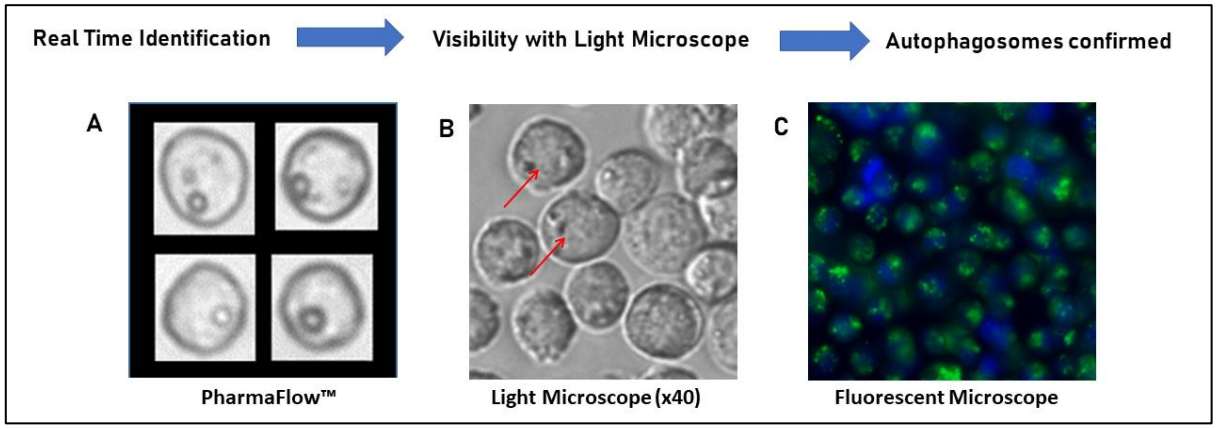

Figure 4: Morphological changes observed in the CHO cells using (A) PharmaFlow™, (B) conventional microscope and (C) fluorescent imaging system.

Conclusion

- The Canty PharmaFlow™ records digital images of cells that pass through its flow cell.
- The CantyVision™ software analyses the digital images by 40 morphological parameters
- Multivariate statistical analysis allows correlation of the morphological data with measurements of viable cell density by trypan blue using a Luna or Vi-Cell counter.
- The digital analysis of the images also allows determination of the metabolic status of the cells that includes apoptosis and autophagy.