Morphological observations using image cytometry for the comparison of trypan blue and fluorescence-based cellular viability

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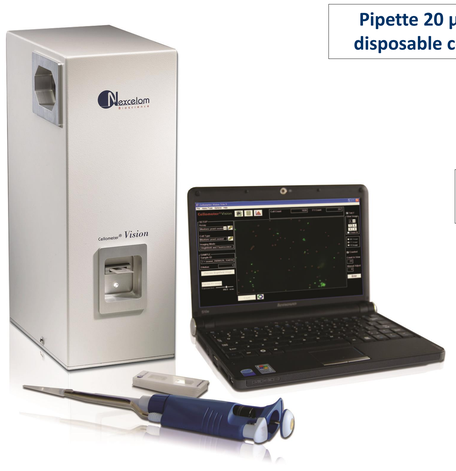
1. ABSTRACT

Determining cell viability is an essential component in many biological experiments ranging from standard cell culture to immunology and oncology research. The traditional method for cell viability measurement is the trypan blue exclusion assay, which utilizes a hemacytometer to manually enumerate trypan blue stained cells. However, trypan blue exclusion assays can be time-consuming, tedious, and have user-dependent variation. Studies have also shown that long incubation with trypan blue can induce cytotoxicity and artificially produce lower viability measurements. With the increase in the availability of fluorescence detection systems and fluorescent stains, numerous fluorescence-based viability measurement methods have been adopted, such as acridine orange (AO) and propidium iodide (PI), Calcein AM, DAPI, 7AAD, and CFDA. There have been numerous comparisons between trypan blue exclusion assays and fluorescence-based viability methods that have shown that trypan blue yields higher viability measurements than fluorescent staining in cell culture time course study. In this work, we compared cellular viability measured using trypan blue and AOPI. Similar results were obtained showing trypan blue produced higher viability measurements when viability decreased below 70%. Utilizing image-based cytometry, morphological changes of trypan blue stained cells were observed, which may explain the differences between the two methods. Results showed that as cells begin to die in a cell culture, dying cells displayed a large blue haze when stained with trypan blue that may be missed during manual cell counts. In addition, multiple concentrations of trypan blue were tested that yielded similar results. At low trypan blue concentrations, cells were not properly stained, but at high trypan blue concentrations, many cells exhibited the large blue haze, which may generate artificially higher viability measurements. In conclusion, using image-based cytometry, we were able to observe morphological changes in the cells, which may have contributed to the differences between trypan blue and fluorescent staining. In addition, it allowed further characterization of the trypan blue exclusion assay, which showed that the assay can potentially only function properly in a certain range of cell viabilities. Further studies will be performed to observe morphological changes of trypan blue stained cells at various incubation times.

2. CELLOMETER® IMAGE CYTOMETRY

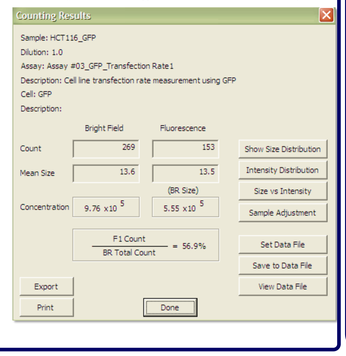


Pipette 20 µL of sample into disposable counting chamber

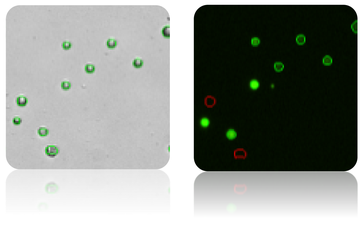
Insert chamber in Cellometer

Count

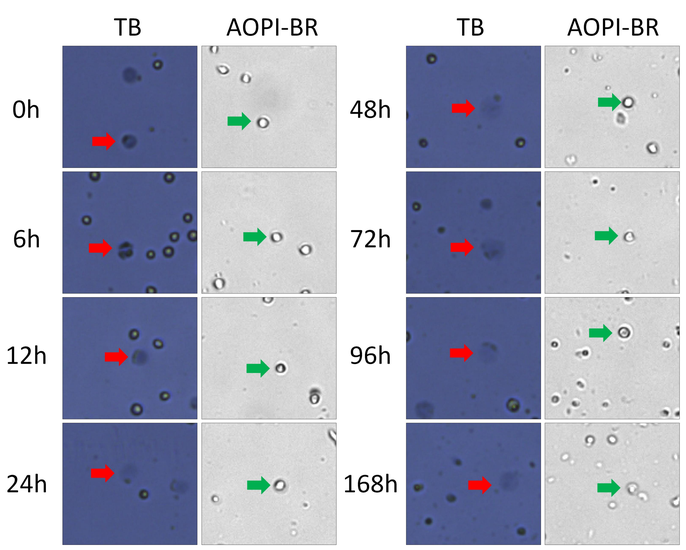
Output data generated instantly

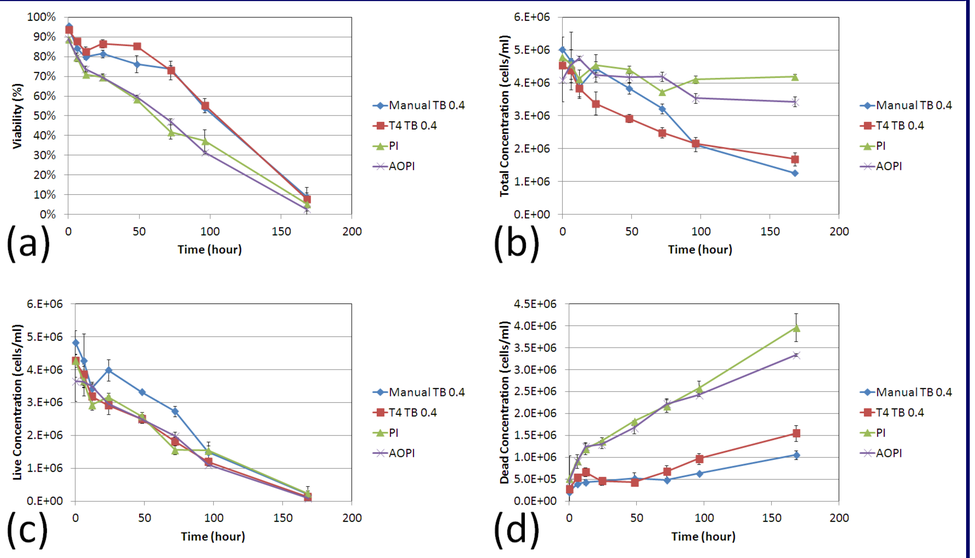


Bright-field (BR) and fluorescent (FL) images



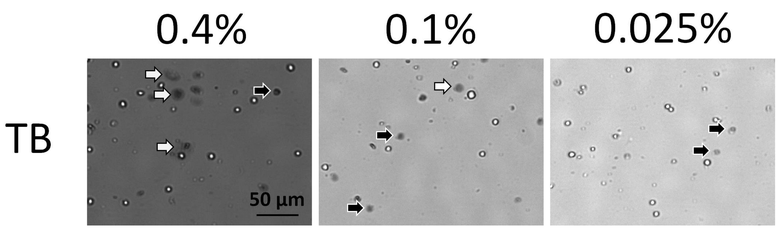
3. TIME-COURSE NATURALLY DYING JURKAT CELLS COMPARISON

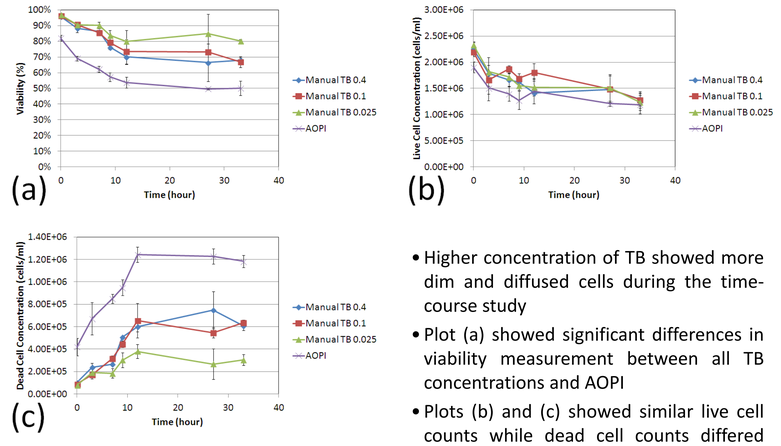




- Trypan blue, PI, and AOPI were measured using a hemacytometer and Cellometer image cytometer
- The trypan blue (TB) and bright-field (BR) images showed the morphology changes of TB-stained Jurkat cells from 0 – 168 hours, when left outside the incubator
  - The morphology of cells stained with TB changed from dark and tight to dim and diffused as time progressed
  - It can create difficulty in manual counting using light microscopy, where some of these dim diffused can be missed, creating artificially high viability measurements
- Plot (a) showed the viability measured with TB was higher than PI or AOPI. Plot (b) showed that total cell count changed in time, but was not affected by live cell (Plot (c)). Plot (d) showed a significant difference between TB and AOPI/PI
- The reduction in dead cell counts can have an effect to over estimate viability measurement by TB, thus typically TB has a viability accuracy range from 70 – 90+ %

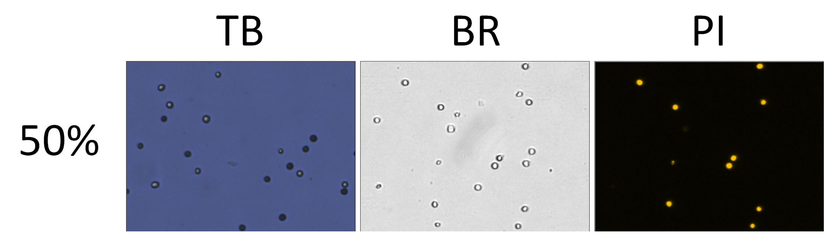
4. TRYPAN BLUE CONCENTRATION DEPENDENCE COMPARISON

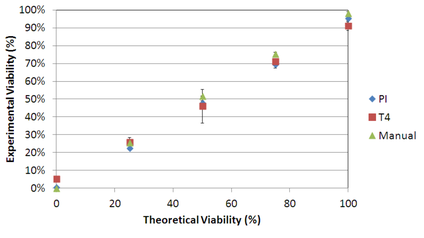




- Higher concentration of TB showed more dim and diffused cells during the time-course study
- Plot (a) showed significant differences in viability measurement between all TB concentrations and AOPI
- Plots (b) and (c) showed similar live cell counts while dead cell counts differed between the samples

5. CONTROLLED HEAT-KILLED VIABILITY COMPARISON





- By heat-killing method, TB-stained cells all displayed dark and tight feature, which is very different than naturally dying cells
- Live and dead cells are mixed to artificially create different % viability
- Results showed highly comparable viability measurement between PI and manual TB counting methods
- Heat-killed cells have well-preserved membrane structure, thus TB is well-contained, which can create higher contrast between live and dead cells

6. CONCLUSION

Over the years, there have been numerous comparisons between the two viability detection methods, but the reports have not stated reasons linked to the morphological changes caused by trypan blue. By using image-based cytometry, we were able capture and examine images of trypan blue-stained Jurkat cells, which allowed visual confirmation of morphological differences between high and low viability samples. Future studies will be conducted to examine the effect of trypan blue incubation time on cell morphology, as well as time-course video recording of cells stained with trypan blue using image cytometry.