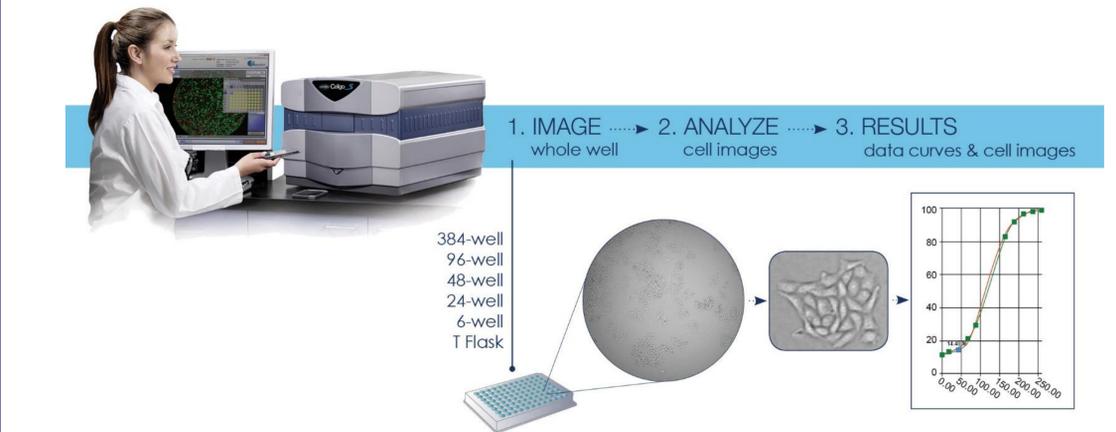


1. ABSTRACT

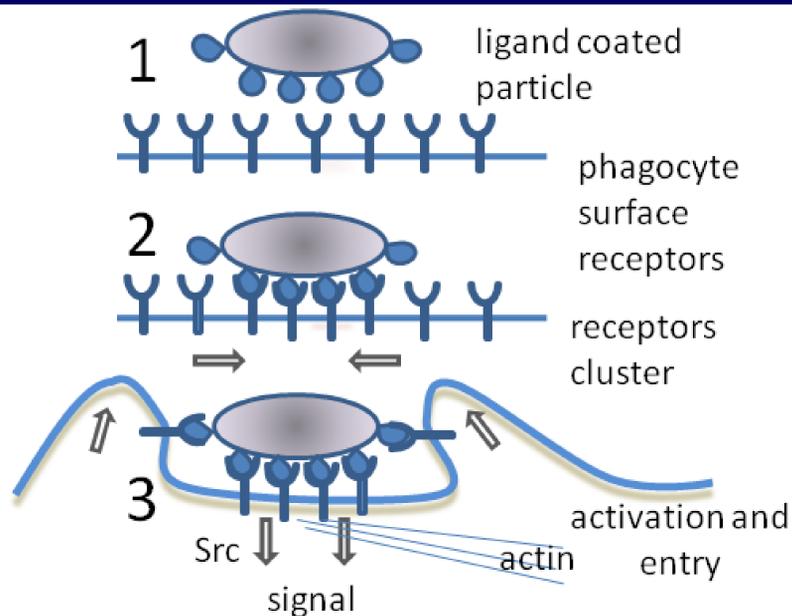
Phagocytosis is an essential process of the immune system to eliminate cellular debris and pathogens. It is a specific form of endocytosis involving vesicular internalization. Phagocytic cells such as macrophages can be attracted to pathogens or cellular debris and engulf the material to be trapped in an internal vesicle called phagosome. The phagosome would fuse with the lysosome to form phagolysosome, where enzymes and toxic peroxides can digest the pathogen or cellular debris at a low pH value. Traditionally, phagocytosis can be qualitatively examined via standard optical microscopy to observe the engulfment of bacteria or cellular debris. In addition, phagocytosis can also be quantitatively measured by incubating phagocytes with particles bound with serum or IgG. After incubation, the excess particles are washed away, leaving only phagocytes with engulfed particles. These cells are lysed to release the engulfed material, which can be examined using microplate reader or western blot can also be utilized to quantify the level of phagocytosis. However, these methods are time-consuming and requires experienced researchers to obtain meaningful results. By using the Celigo Imaging Cytometer, phagocytosis can be measured using a pH sensitive label called pHrodo. The labels will only fluoresce when they are trapped in the phagolysosome where the pH value low. Therefore, by measuring the amount of fluorescence or percentage of cells fluorescing, one can use the Celigo to determine the fluorescent intensities of the target cell populations and measure the level of phagocytosis. In this work, THP-1 cells were stimulated with LPS into macrophages and incubated for 24 hours. After incubation, the cells were mixed with particles labeled with pHrodo, and with 2 different drug treatments (with and without induction of phagocytosis). The cells were allowed to incubate with the 2 drugs and particles for 24 hours. The results showed clear difference between the 2 drug treatments, where the difference in fluorescent intensities were measured. The ability of Celigo to rapidly perform plate-based phagocytosis assay has the potential of improving research efficiency, especially for adherent cells such as macrophages where plate-based cytometer does not require trypsinization for cell population analysis.

2. CELIGO IMAGING CYTOMETRY FOR PHAGOCYTOSIS ANALYSIS



1. Celigo Imaging Cytometer is a plate-based cytometer that can scan the entire well of standard microplates and captures bright-field and fluorescent images
2. The captured images are analyzed with the Celigo software to measure size, morphology, cell count, confluence, and fluorescent intensity
3. The measured parameters are used to generate cell proliferation kinetic data, GFP/RFP expression, tumor spheroid size change, DNA cell cycle analysis, apoptosis, and ADCC cytotoxicity results

3. DETECTION OF PHAGOCYTOSIS USING CELIGO



1. Target cells (THP-1) are seeded at 2×10^4 cells/well in a 24-well plate (Greiner 662160)
2. Cells were stimulated with LPS for differentiation into macrophages
3. Cells were incubated for 24 hours
4. Two compounds were added to the wells, compound A induces phagocytosis, and compound B is the control
5. The cell are then mixed with particles labeled with pHrodo, the pH sensitive dye can fluoresce in acidic environment (endosome and lysosome)
6. The cells are then incubated for 24 hours
7. After incubation, the plate is scanned with Celigo and analyzed to measure % internalization

6. SUMMARY AND CONCLUSION

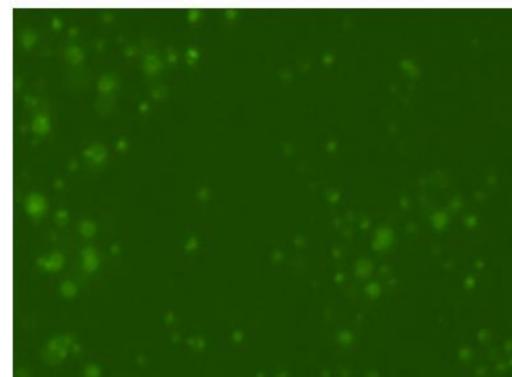
In conclusion, the Celigo Imaging Cytometer can be used to measure % phagocytosis via fluorescence detection of Dextran pHrodo dye. The ability to capture bright-field and fluorescent images in a multi-well microplate automatically allows for high-throughput cell-based assays. In addition, analyzing adherent cells directly on the plate eliminates the need for trypsinization and removes the disruption to the cells.

References

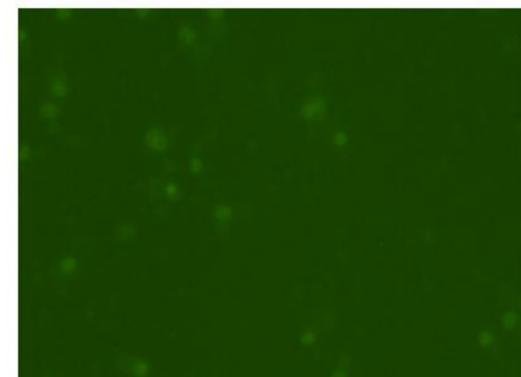
- Ambrose, Charles T. (2006) The Osler slide, a demonstration of phagocytosis from 1876: Reports of phagocytosis before Metchnikoff's 1880 paper. *Cellular Immunology* 240(1): 1-4
- Gould, Gwyn W., Lippincott-Schwartz, Jennifer. (2009) New roles for endosomes: from vesicular carriers to multi-purpose platforms, *Nature Reviews Molecular Cell Biology*, 10, 287-292

4. FLUORESCENT IMAGES OF PHAGOCYTOSED PARTICLES

Compound A (Phagocytosis)

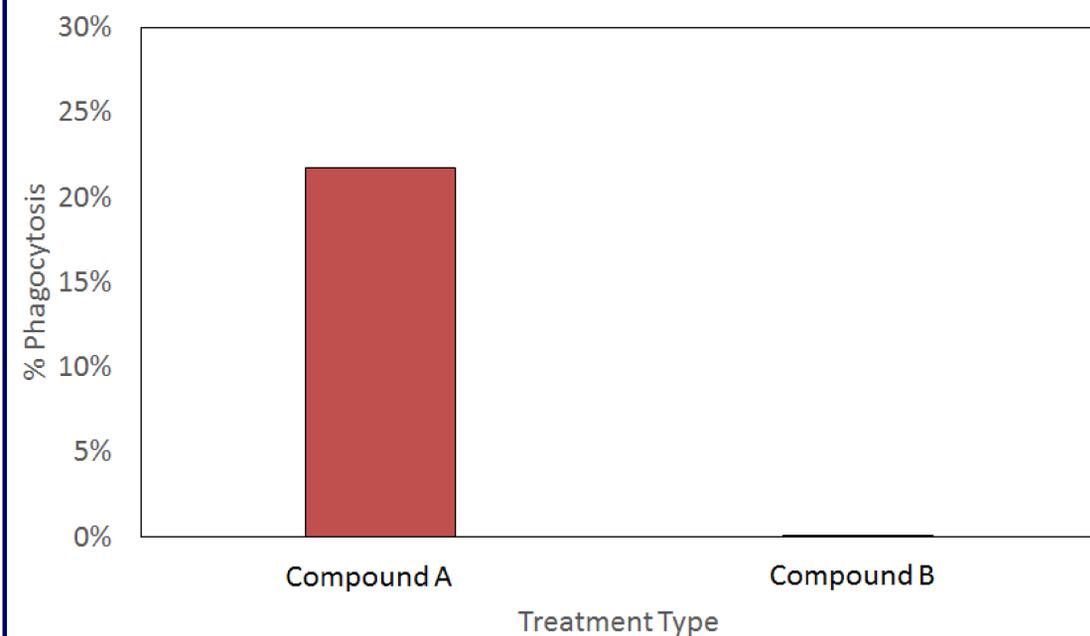


Compound B (No Phagocytosis)



- Fluorescent images showed high % of cells with Dextran pHrodo green fluorescence when treated with Compound A
- Fluorescent images also showed low % of cells with Dextran pHrodo green fluorescence when treated with Compound B, which is the control
- The green fluorescence occurs when particles labeled with Dextran pHrodo are engulfed into the phagosome and fused with the lysosome
- The total number of cells are counted using the Celigo captured bright-field images, while the fluorescent images are counted to determine the number of cells showing internalization
- The % phagocytosis is then calculated by # fluorescent cells/total

5. PHAGOCYTOSIS POPULATION RESULTS COMPARISON



- Cells treated with Compound A showed high % of phagocytosis in comparison to the control Compound B, which show no increase in fluorescent intensity
- Bacteria or proteins can also be labeled with pHrodo to detect phagocytosis capability of immune cells