Assay Name: Count and measure cell infectivity of microcarriers

Assay ID: Celigo_02_0016

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Experiment: Count and measure cell infectivity of microcarriers

Purpose	In this experiment, we demonstrate the capability of Celigo to measure cell count on microcarrier beads and infected cells positively staining for AlexaFluor488-labeled antibody again the viral protein.
Current Method(s)	Flow Cytometry
Target Cell Type	Epithelial cells cultured on microcarriers
Experiment Plan	Use the Celigo to count DAPI-stained cells on the microcarrier beads, and count the number of microcarriers to get an average cells/microcarrier. Also measure AlexaFluor488-positive cells on the microcarriers (cells are stained against viral protein - for viral infectivity)
Hypothesis	Depending on the infection rate, different number of AlexaFluor488-positive cells will be quantified, and average infectivity will be calculated

Celigo Setup

Plate Type	Greiner 655090 96-well black wall clear bottom
Scan Channels	Bright field + Green + Blue
Resolution	1 µm/pixel
Scan Area	Whole well
Analysis Method	Target 1 + 2 + 3 + 4 Brightfield – Colony for Microcarrier counts
Scan Frequency	Endpoint
Scan Time	~10 min

Assay Protocol and Plate Setup

Goal

In this experiment, we demonstrate the capability of Celigo to measure cell count on microcarrier beads and infected cells positively staining for AlexaFluor488-labeled antibody again the viral protein.

Protocol

Cell preparation

- Obtained microcarrier samples from bioreactors, fixed and stained with DAPI and viral protein of interest with AF488
- After pipetting in the microcarrier beads from spinner flasks into 96-well plates, centrifuged the plate to settle the microcarriers down
- Used the Celigo to scan the microcarrier beads at different focal planes to capture all the nuclei
- In addition, bright field images were captured for the microcarriers, to count the number of microcarriers in the well
- The experiment was repeated by staining with Hoechst and PI to measure the viability of epithelial cells on the microcarriers
    - Notes: Potentially staining the cells with the Caspase 3/7 kit following the attached protocol to measure apoptosis

Data Collection

1. After centrifuging the plate, scaned the plate using the Celigo
2. Setup the scanning parameters for 4 channels, where channel 1 and 2 were DAPI and AF488 for the top of the microcarriers, and channel 3 and 4 were DAPI and AF488 for the bottom of the microcarriers
3. The Celigo was not able to image and analyze the equator of the microcarriers
4. The Celigo was then used to capture bright field images and analyze the number of microcarriers in the well

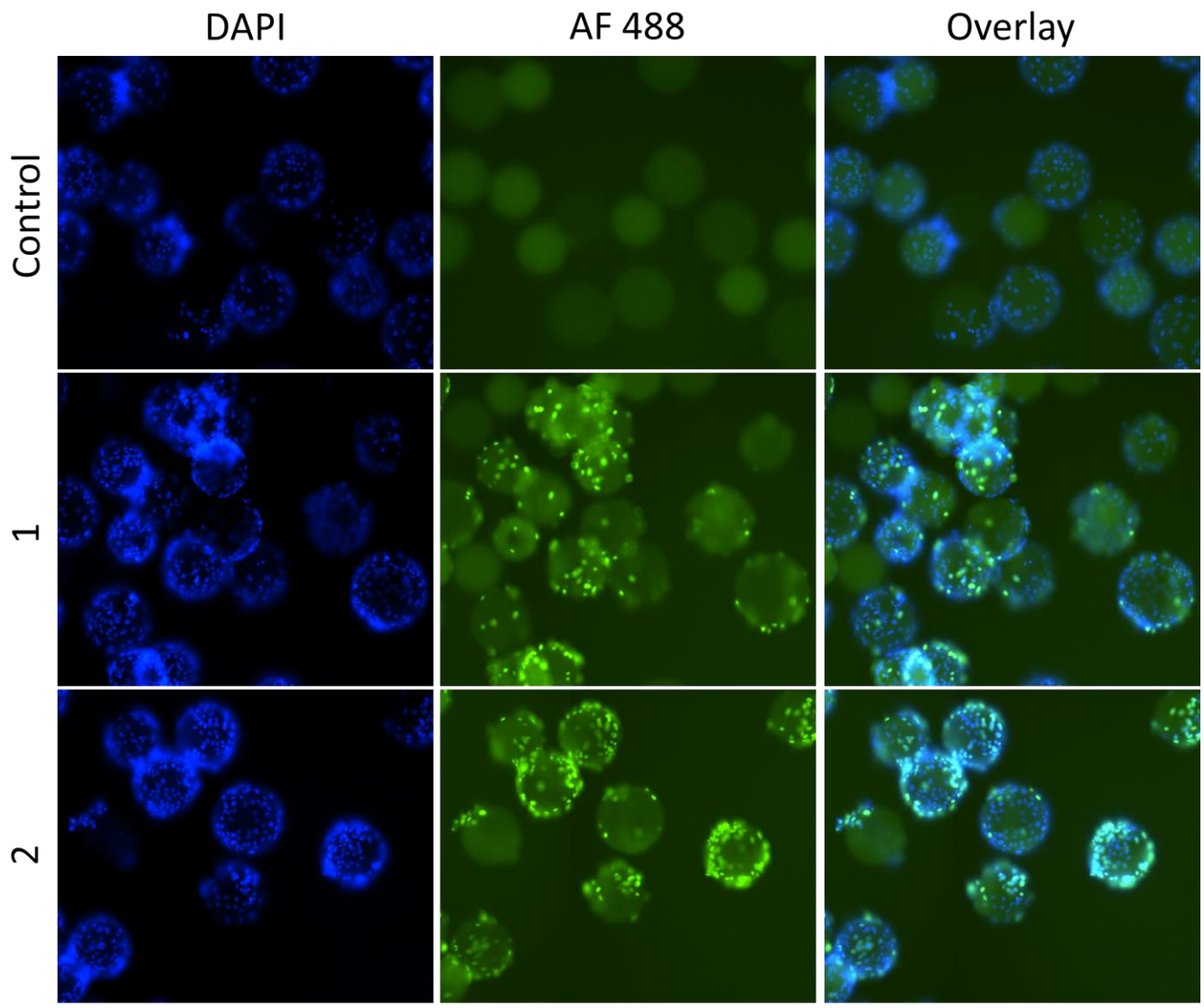
Data Analysis

- The images from each DAPI and A488 fluorescent channels were counted
- The total number of cells stained with DAPI were counted
- The total number of AF488-positive cells were counted
- The AF488 # / DAPI # was calculated to determine the Infectivity %
- The Af488 # / BF # was calculated to determine the average infected cells/microcarrier

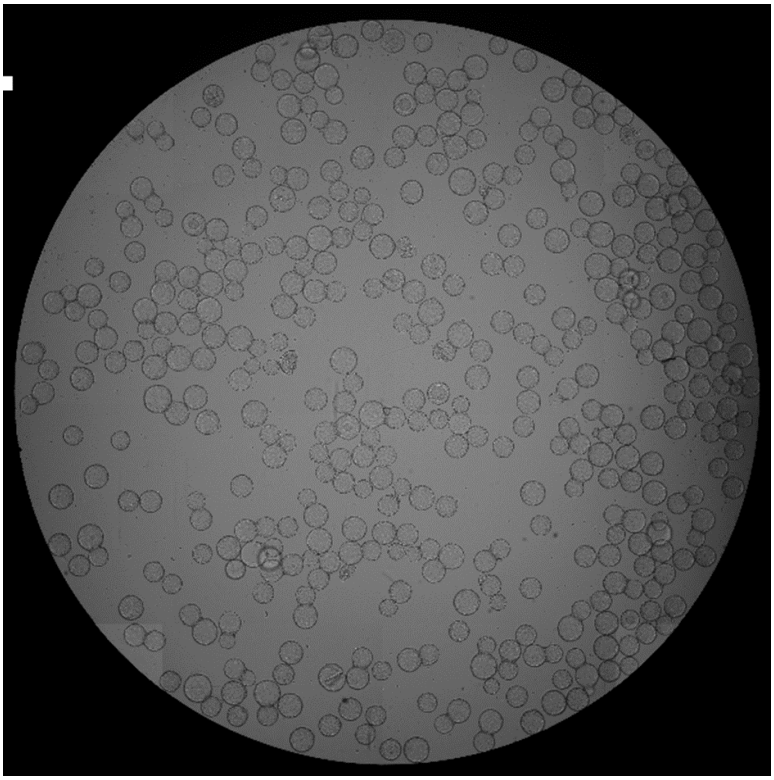
Results

1. Celigo-captured bright field and fluorescent images of DAPI and Alexa Fluor 488

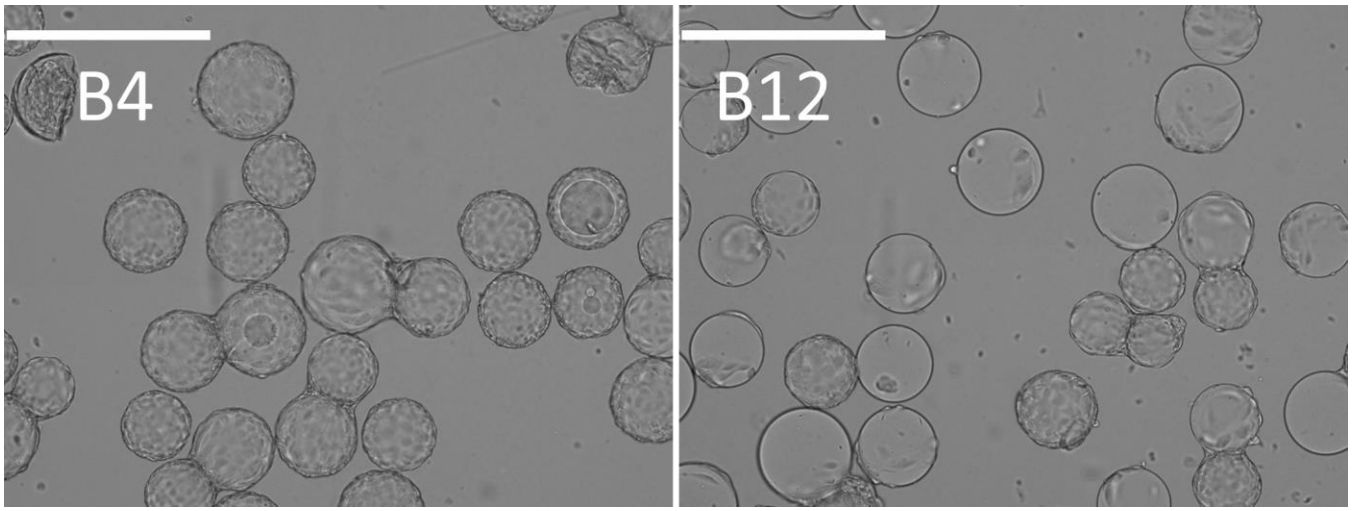
- The Celigo was able to measure % infectivity by counting total number of DAPI and Alexa Fluor 488 positive cells
- By dividing Alexa Fluor by DAPI, the % infectivity was calculated



- The whole well image showed all the microcarriers in the well and were counted

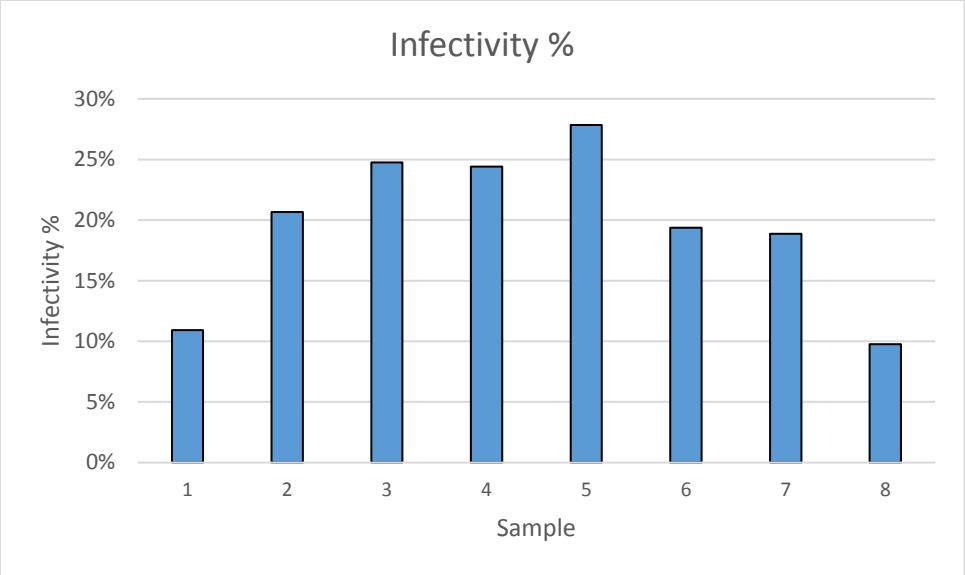


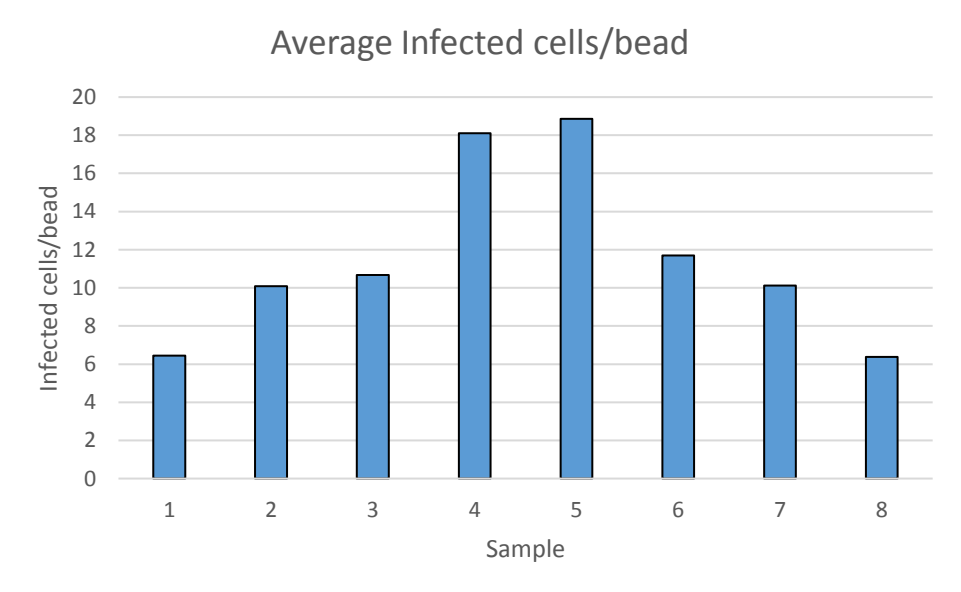
- When zoomed in, the coverage of cells can be clearly observed in two different samples, B4 and B12



2. Infectivity percentage results

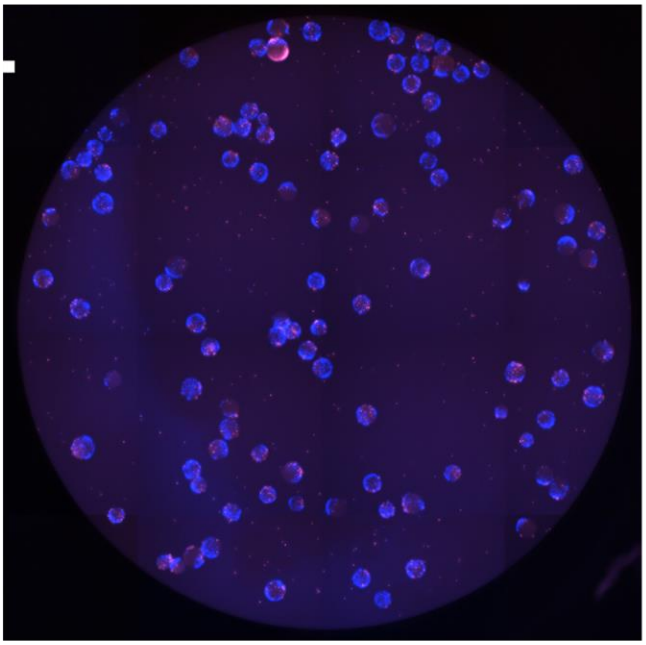
- After measuring DAPI and Alexa Fluor 488-positive cells, as well as the number of microcarriers per sample, the % infectivity and average infected cells/bead were calculated
- The results showed that different samples have different rate of infection



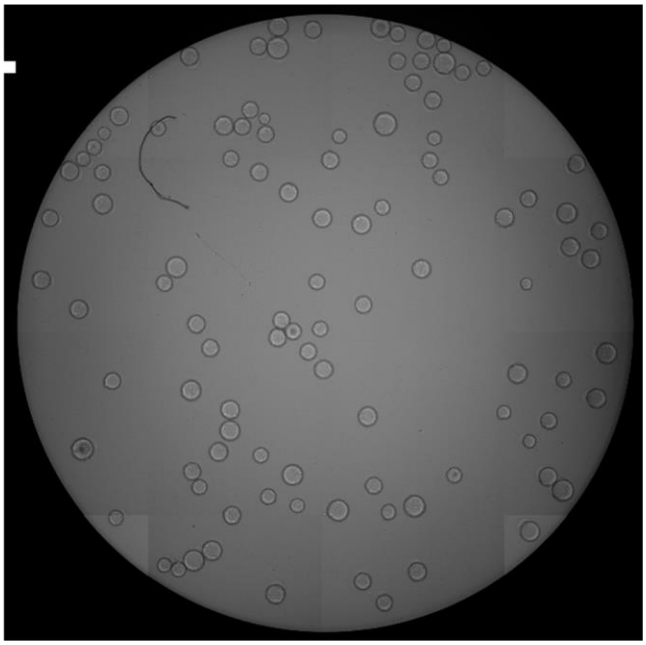


3. Celigo-captured bright field and fluorescent images of cells and microcarriers for Hoechst and PI
   - The Celigo was used to capture bright field images of the microcarriers for counting
   - The Celigo was also used to capture Hoechst and PI stained cells growing on the microcarriers in order to measure the total cell counts per well, and per microcarrier
   - Individual Hoechst and PI-positive cells were counted directly in the 96-well plate, as well as the microcarriers, shown in the images below.

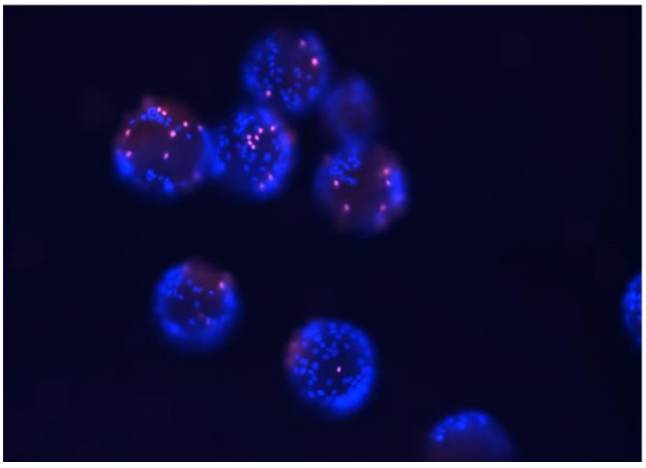
Whole Well FL



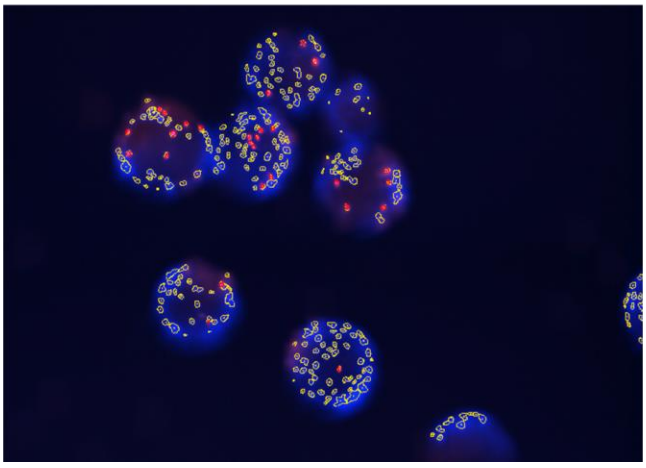
Whole Well BF

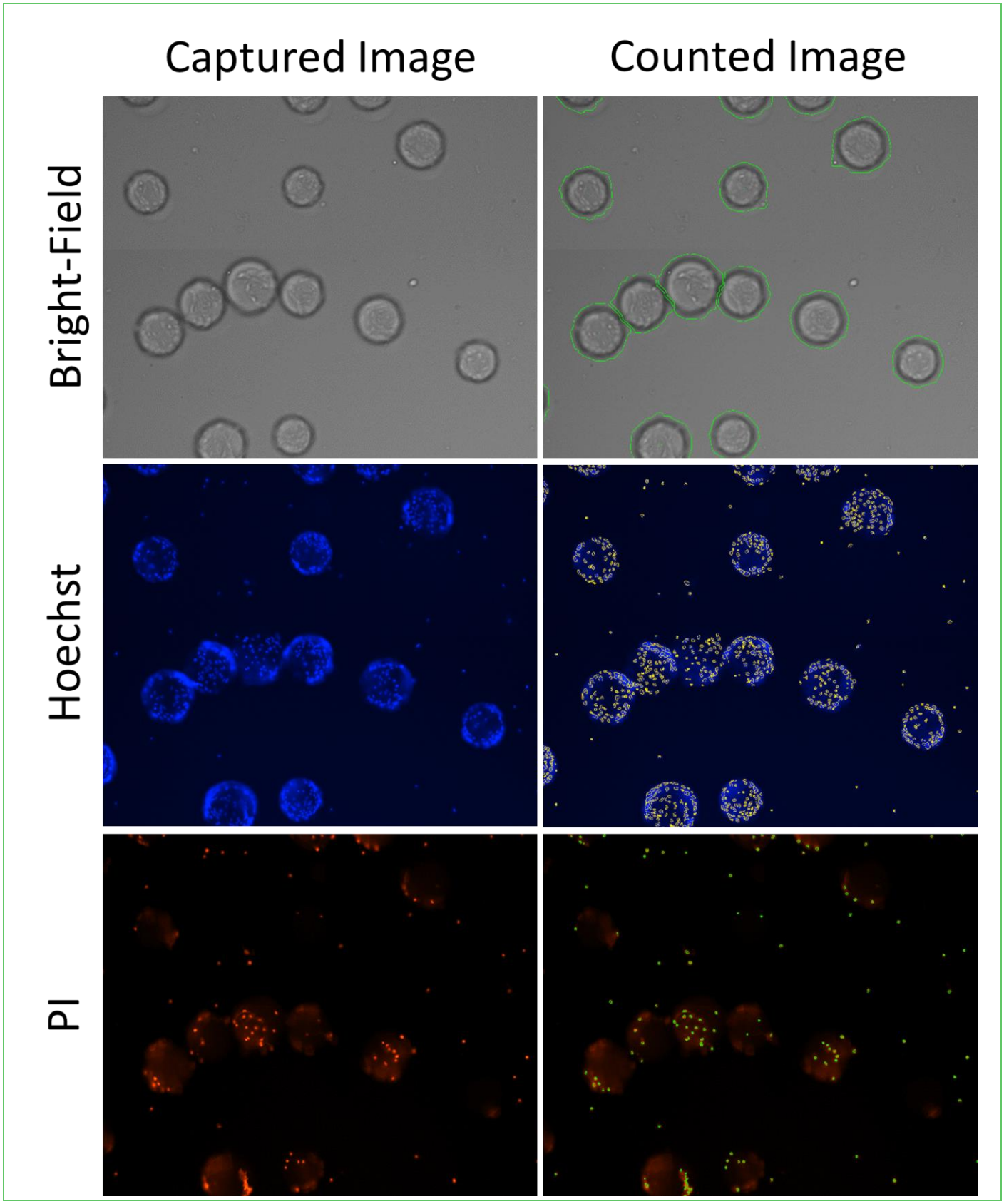


Zoom FL Image

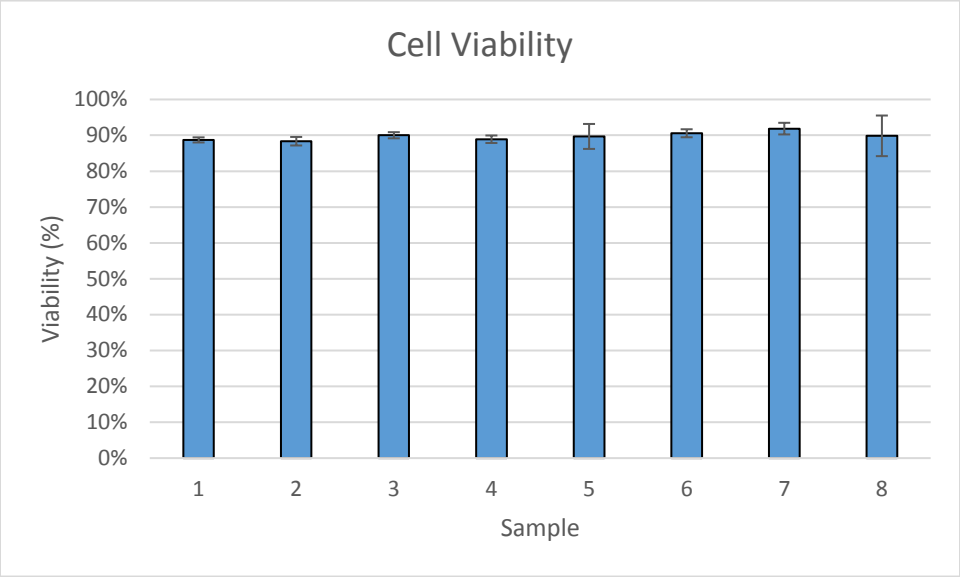


Zoom Counted Image




Captured Image
Counted Image
Bright-Field
Hoechst
PI

- The cell viability was measured directly on the microcarriers



Conclusion

- Celigo was able to directly measure total cell count and viral infected cell counts of cells on the microcarriers in a 96-well plate format
- The Celigo was also used to quantify virally infected cells per bead in a high throughput manner
  - The Celigo analyzed 20 samples in less than 10 minutes total for scanning and analysis
- Celigo was also able to capture blue and red fluorescent images to perform total and dead cell counting using Hoechst and PI
  - In addition, the number of microcarriers was also counted in the bright field images
  - Overall, the Celigo was able to capture and analyze 64 samples in 15 min
- Finally, high quality images can be saved and reviewed for record keeping