Assay Name: HPC proliferation measurement using Ki-67 cellular marker

Assay ID: Celigo_02_0014

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Experiment: HPC proliferation measurement using Ki-67 cellular marker

Purpose	To demonstrate the capability of the Celigo to perform rapid, high throughput imaging and analysis of hematopoietic progenitor cell (HPC) proliferation using Ki-67 cellular marker. The Ki-67 protein is a biomarker for cell proliferation which are present in all the active phases of cell cycle such as G1, S, G2, and mitosis, but not in G0 phase.
Current Method(s)	Flow cytometry, but is tedious to setup multiple samples, not idea for high-throughput assays
Target Cell Type	HPCs derived from iPSCs (one healthy and one diseased patient)
Experiment Plan	Cells were cultured in 6-well plates, samples from diseased and healthy donors, fixed, permeabilized, and stained for Ki-67 expression, and counterstained with DAPI.
Hypothesis	Celigo will be able to perform rapid, whole-well imaging and analysis of Ki-67 expression levels to compare between groups.

Celigo Setup

Plate Type	6-well Corning
Scan Channels	Bright field, Green (DyLight488), Blue (DAPI)
Resolution	1 um/pixel
Scan Area	Whole well
Analysis Method	Target 1 + 2 + Mask
Scan Frequency	Once (endpoint)
Scan Time	10 minutes

Assay Protocol and Plate Setup

Goal

To demonstrate the capability of the Celigo to perform rapid, high throughput imaging and analysis of cell proliferation using Ki-67 cellular marker.

Protocol

Cell and stain preparation

1. Collected different samples of iPSCs that were isolated from either healthy and diseased donors
2. HPCs were plated into 6-well plates and incubated for 2 days (See plate map below)

	1	2	3
A		Healthy Donor Samples	
B		Diseased Donor Samples	

3. At end of incubation, cells were fixed with 4% formaldehyde, permeabilized with 0.2% Triton X-100
4. The cells were then stained with primary rabbit anti-human Ki-67 overnight
5. After overnight staining, the cells were washed and stained with secondary DyLight488 goat anti-rabbit IgG antibody for 1 hour
6. Finally, they were washed and counterstained with DAPI for 30 min in the dark
7. The stained cells were imaged and analyzed on Celigo for end point reading

Data Collection

1. Immediately after, the plate was scanned in Celigo using Target 1 (BF) + 2 (Green) + Mask (Blue) for an end point scan
2. Fluorescent gating was set up based on the DAPI mask to determine mean fluorescent intensity and % of Ki-67 positive cells

Data Analysis

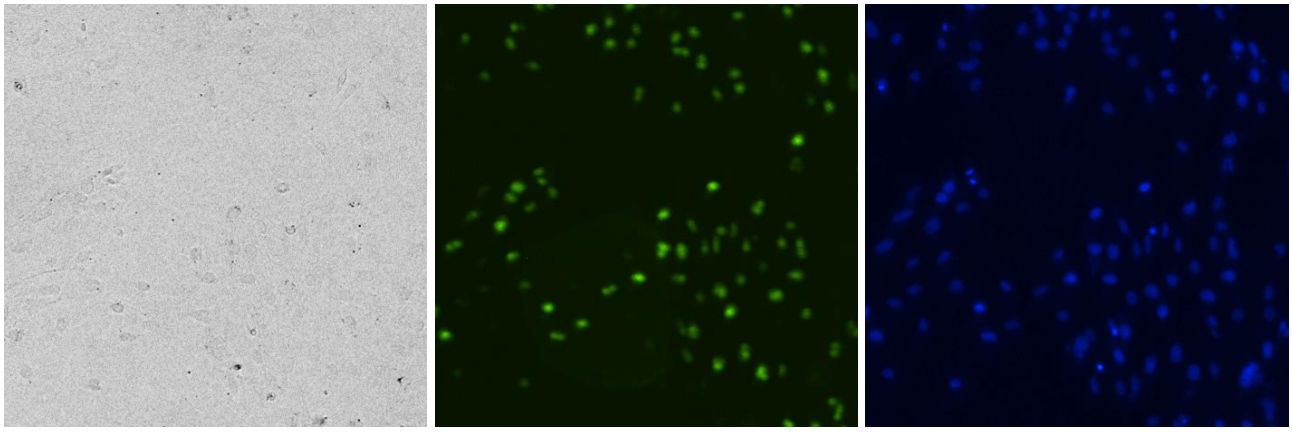
- The images for each HPC sample were analyzed by using the DAPI-positive cells as the mask
- Next, the DyLight488 fluorescent intensities within the identified DAPI-positive cells were plotted under the Gate tab to determine the DyLight488-positive cell population percentages

Results

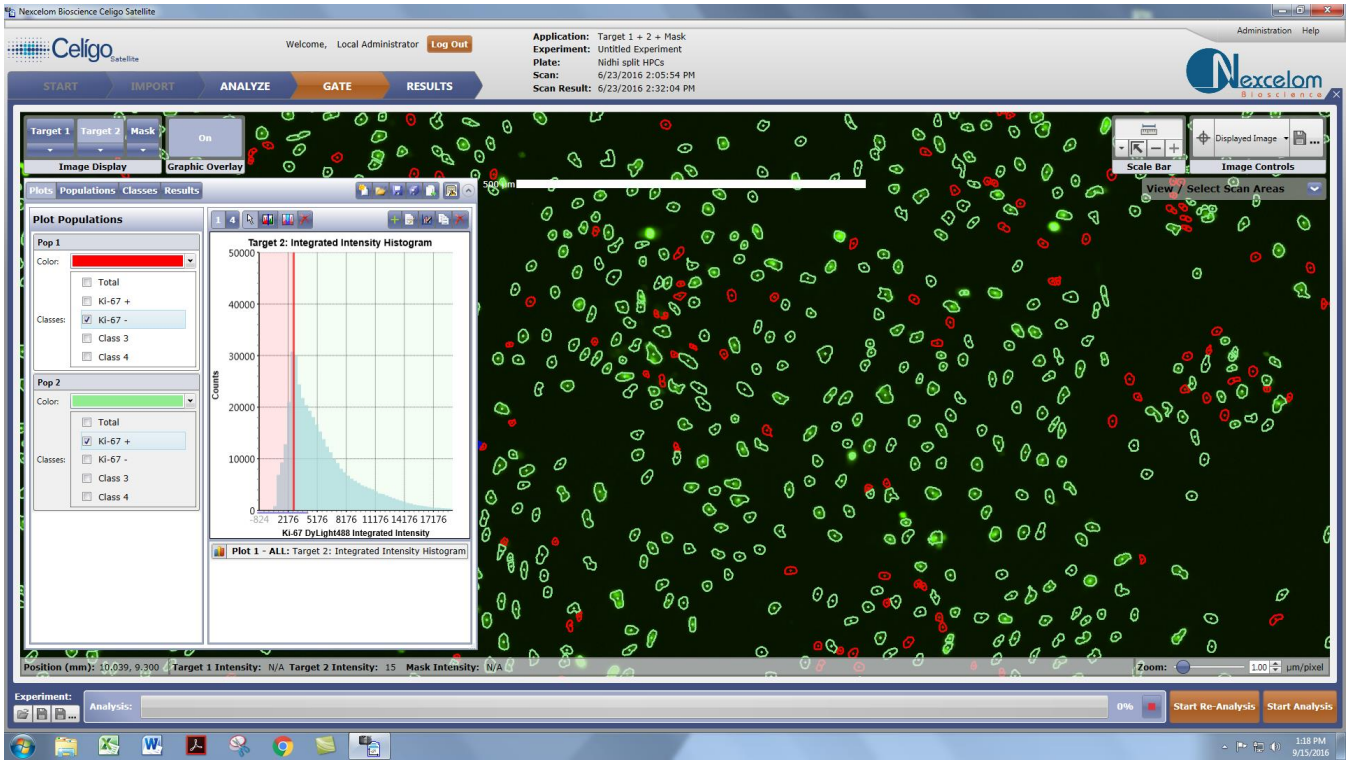
1. Celigo-captured bright field, DyLight288 and DAPI fluorescent images

- Examples of bright field, Ki-67-DyLight488 and DAPI-stained fluorescent images

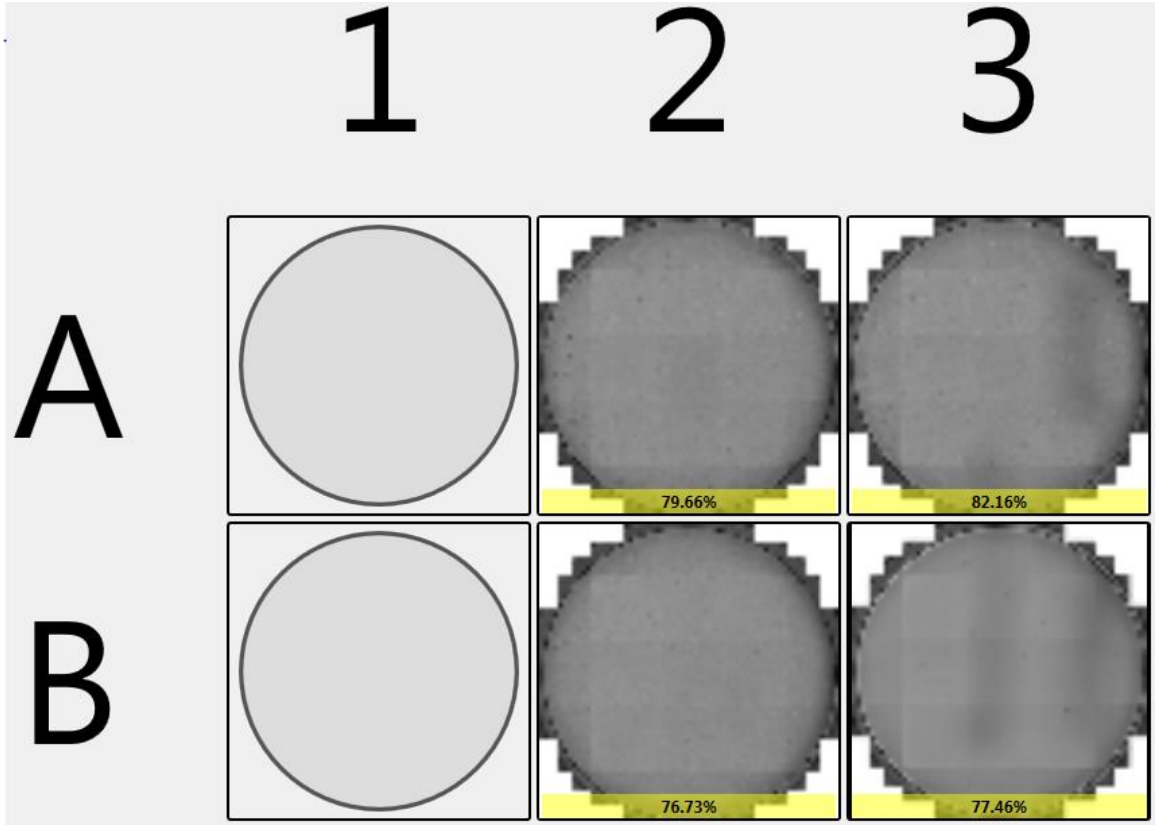
Bright Field | Ki-67-DyLight488 | DAPI



- Celigo was able to count DAPI-positive cells, to determine the total population, use the gating function to identify Ki-67-positive cells and calculate the percent of Ki-67-positive cells for the whole plate

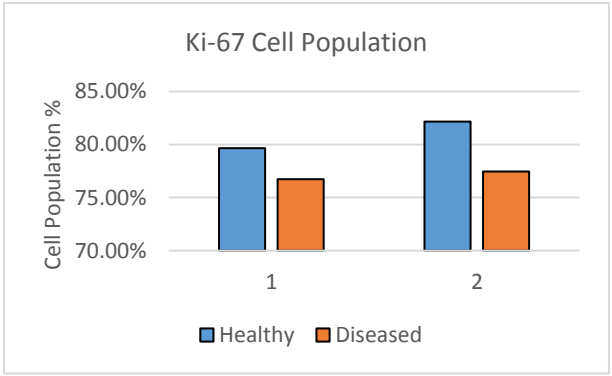


- Celigo was able to calculate the percent of Ki-67-positive cells for the individual wells of the entire plate



- Celigo was able to generate reports in table format

% Ki-67 +	1	2	3
A		79.66%	82.16%
B		76.73%	77.46%
% Ki-67 -	1	2	3
A		20.29%	17.77%
B		23.22%	22.47%



Conclusion

- Celigo was able to image and identify Ki-67-positive cell population percentages with the Celigo gating function
- The percent of Ki-67-positive cell population was automatically generated by Celigo software
- In this experiment, the cell proliferation for diseased patient samples was not significantly lower than the healthy patients shown in the Ki-67 cell population percentages above
- Celigo image cytometer allowed rapid bright field and fluorescent imaging of HPCs labeled with DyLight488 and DAPI