

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

Assay ID: Celigo_02_0014

Description: To demonstrate the capability of the Celigo to perform rapid, high throughput imaging and analysis of hematopoietic progenitor cell (HPC) proliferation using the 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.

Stains: Bright Field, DyLight488 (Green) and DAPI (Blue)

Imaging channels: Bright Field, Green, and Blue

Image analysis algorithm: Celigo software Target 1 + 2 + Mask

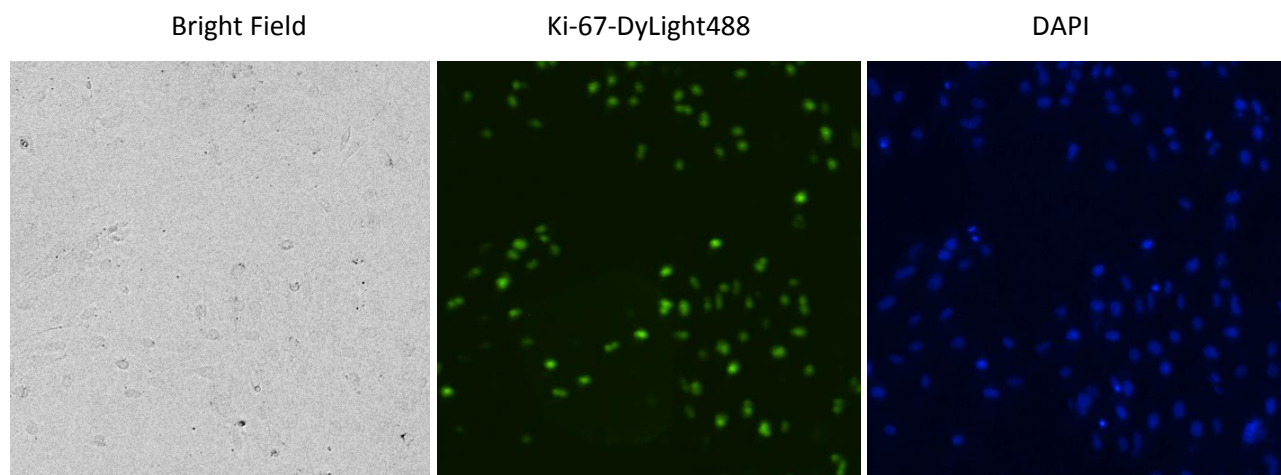
Methods:

1. Collect different samples of iPSCs isolated from donors
2. Plate HPCs into 6-well plates and incubate for 2 days
3. At end of incubation, fix cells with 4% formaldehyde, permeabilize with 0.2% Triton X-100
4. Stain cells with primary human anti-Ki-67 overnight, then stain with secondary DyLight488 antibody for 1 hour
5. Finally, counterstain the cells with DAPI
6. Image and analyze the stained cells on Celigo for end point reading

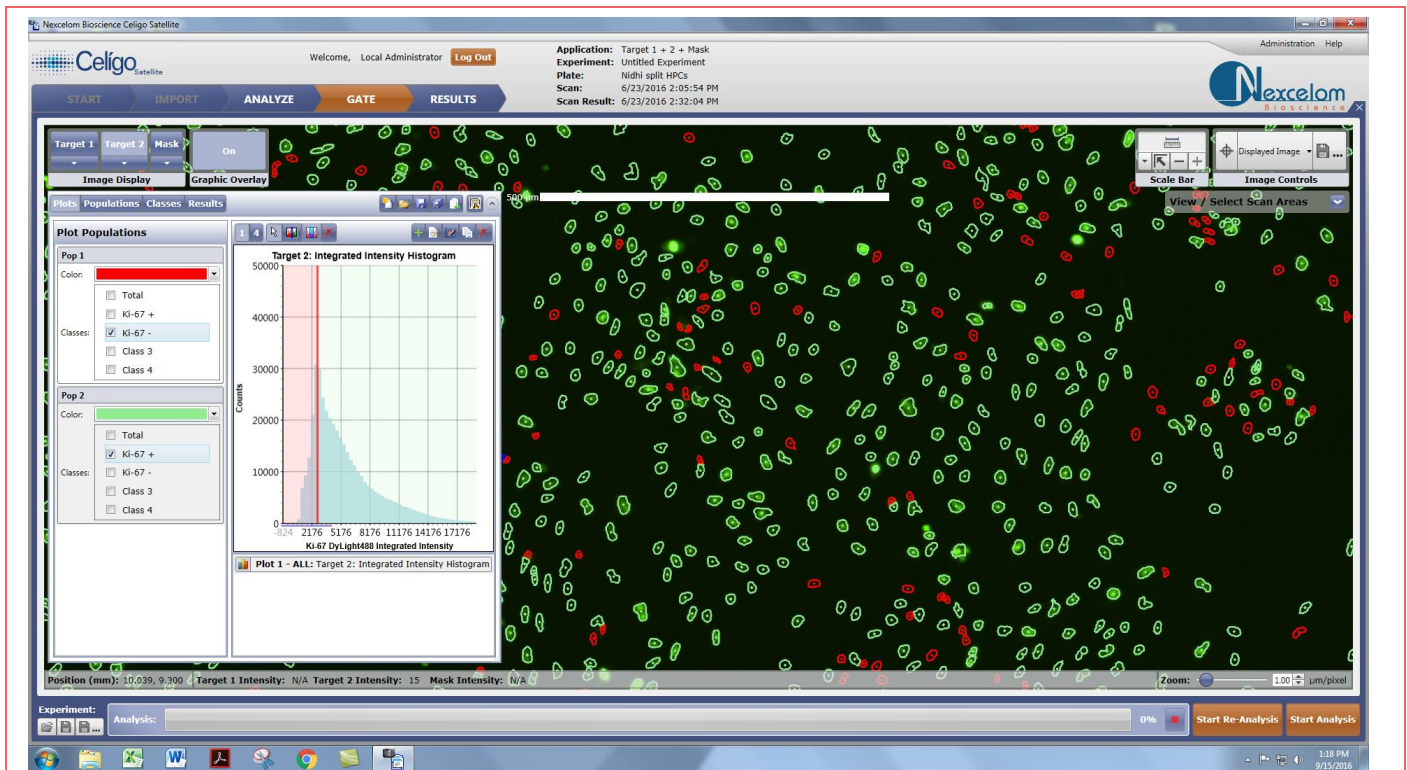
Results:

Celigo-captured bright field, DyLight488 and DAPI fluorescent images

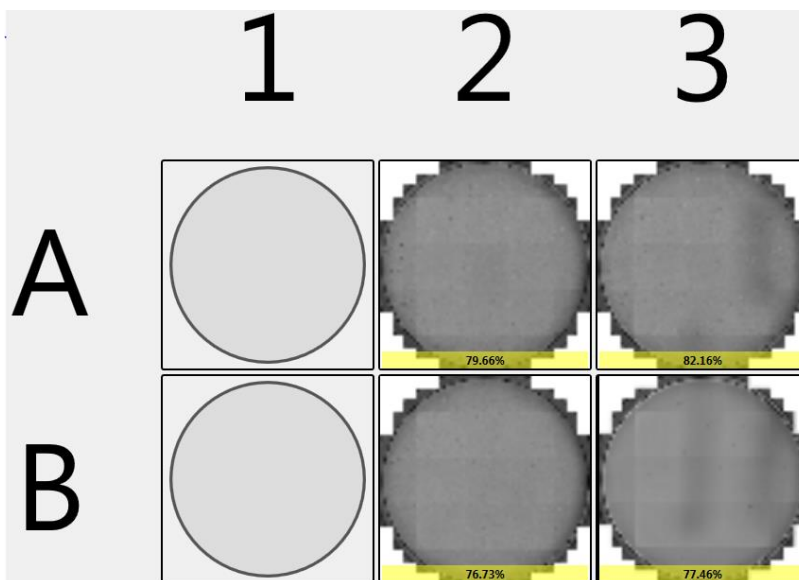
- Examples of bright field, Ki-67-DyLight488 and DAPI stained fluorescent images



- Celigo used the counted DAPI-positive cells as the total, then used 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 image and identify Ki-67-positive cell population percentages with the Celigo gating function
- The percent of Ki-67-positive cell population is automatically generated by Celigo software