

## Assay Name: Single Cell Detection of FACS-sorted cells

**Assay ID:** Celigo\_07\_0001

**Description:** Single cell detection following serial dilution or single cell fluorescent-activated cell sorting

**Stains:** CellTracker™ Green (for metabolically active cells)

**Imaging channels:** Bright field and Green

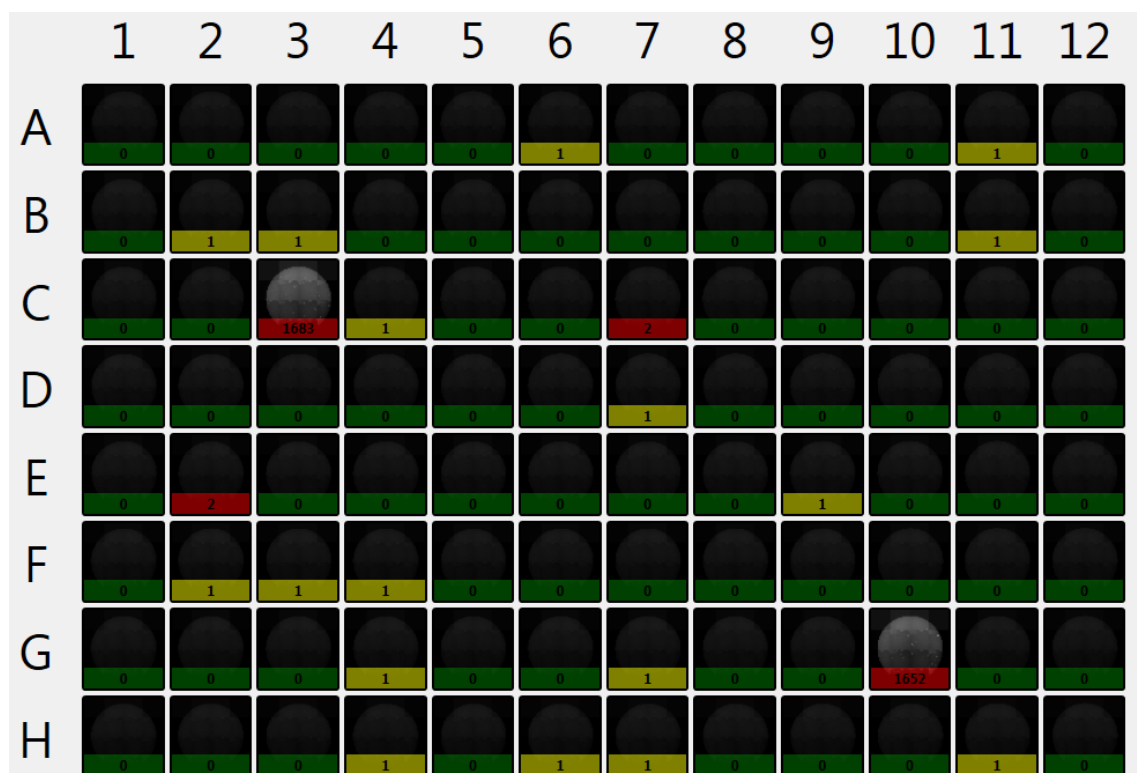
**Image analysis algorithm:** Target 1 + 2

### Methods:

1. Culture and collect CHO-S cells and stain with CellTracker™ Green (CTG)
2. Seed single cells in the wells of 96-well microplate via serial dilution or fluorescent-activated cell sorting
3. Assign at least one reference well containing 1,000 – 2,000 cells for focus registration
4. Allow cells to settle for 10 mins or pulse spin using a centrifuge 30 seconds 1,000 rpm
5. Use Celigo to capture images and analyze the total number of live cells per well

### Results:

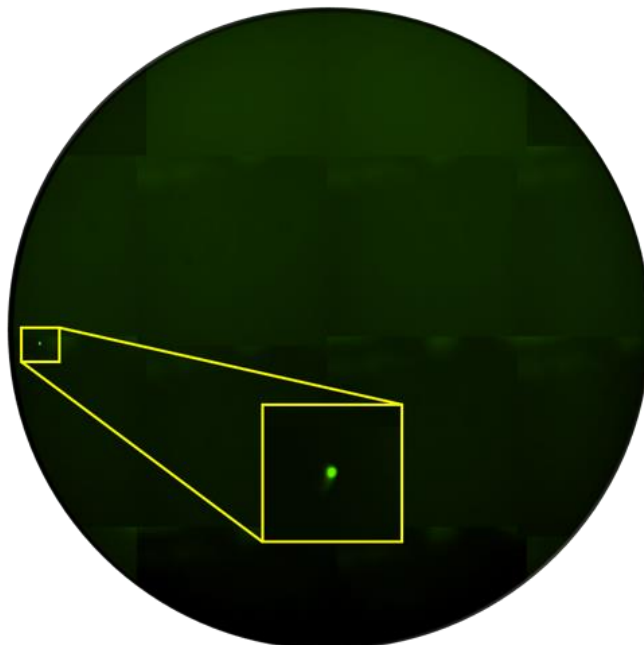
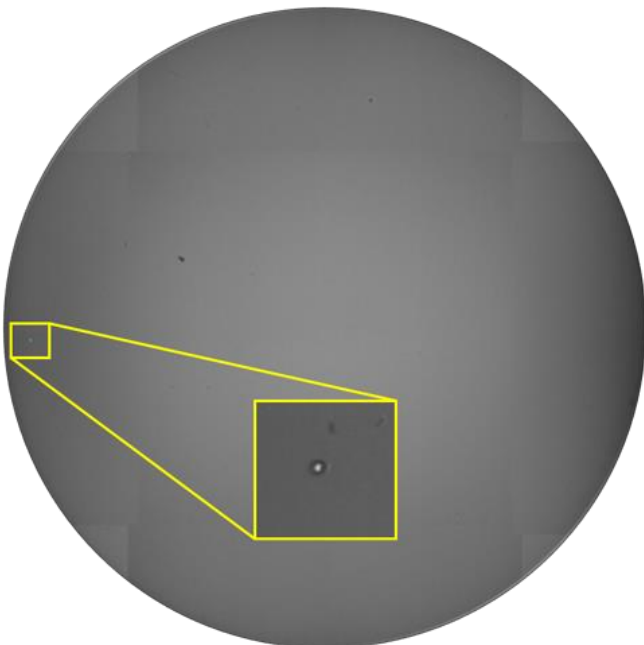
96-well microplate heat map showing the number of CTG-labelled cells per well



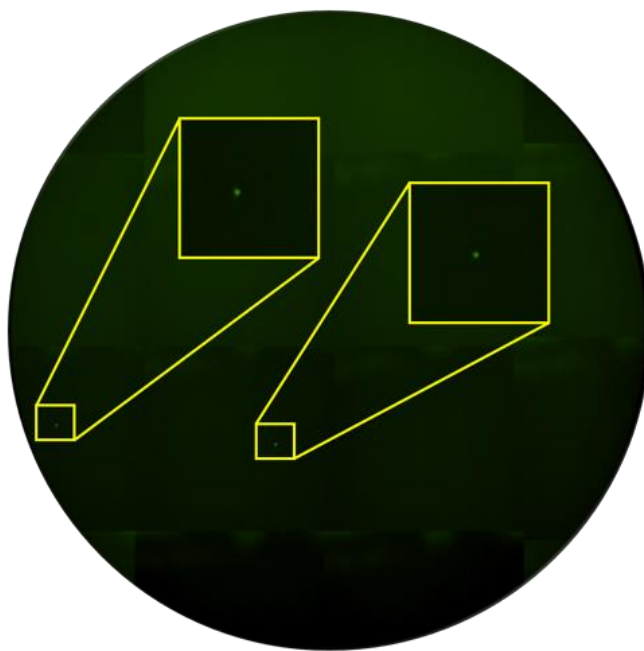
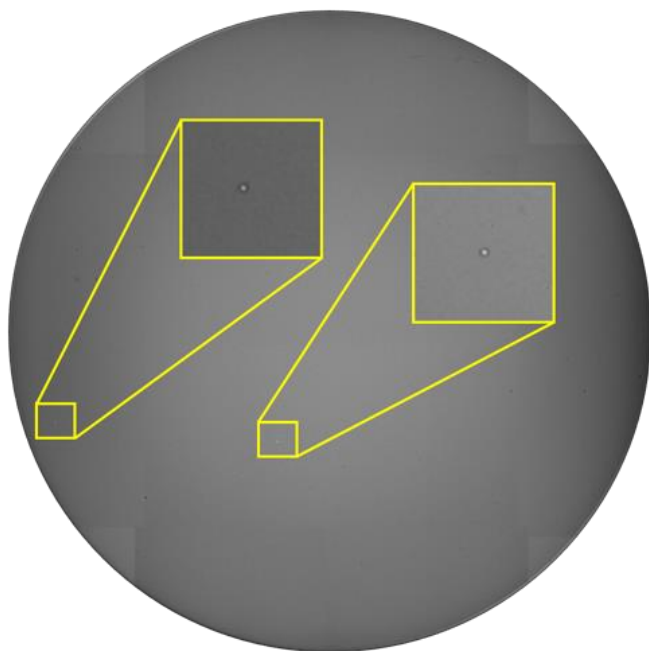
- The heat map feature allows for quick visualization of which wells contain single cells, as depicted in yellow. Red wells have more than 1 cell and Green wells contain no cells.
- Wells C3 and G10 were selected as reference wells for focus registration

Whole-well and zoomed images in bright field and fluorescence

Confirmation of a single cell in well D7



Confirmation of two cells in well C7



- Celigo's whole-well imaging allows for simple validation of successful single cell plating or the presence of more than one cell