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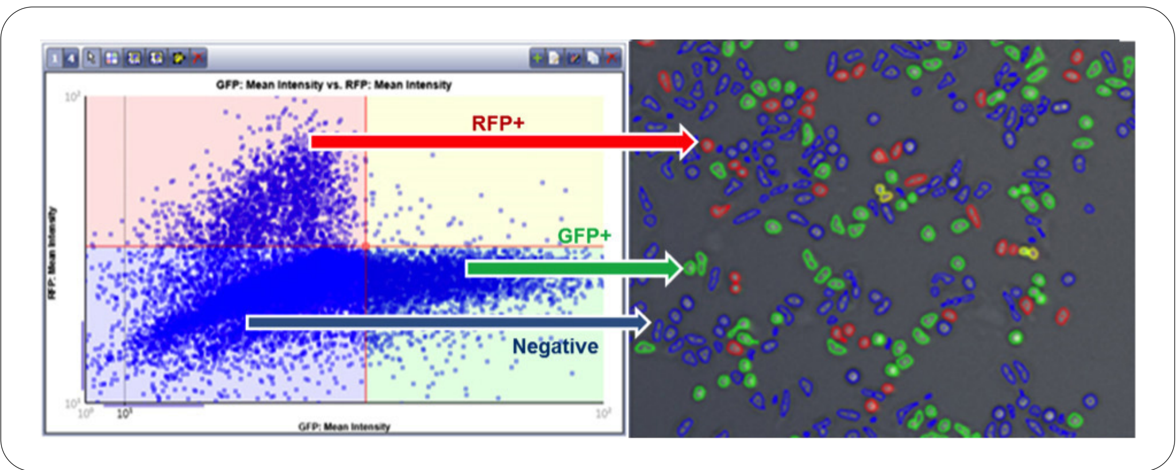
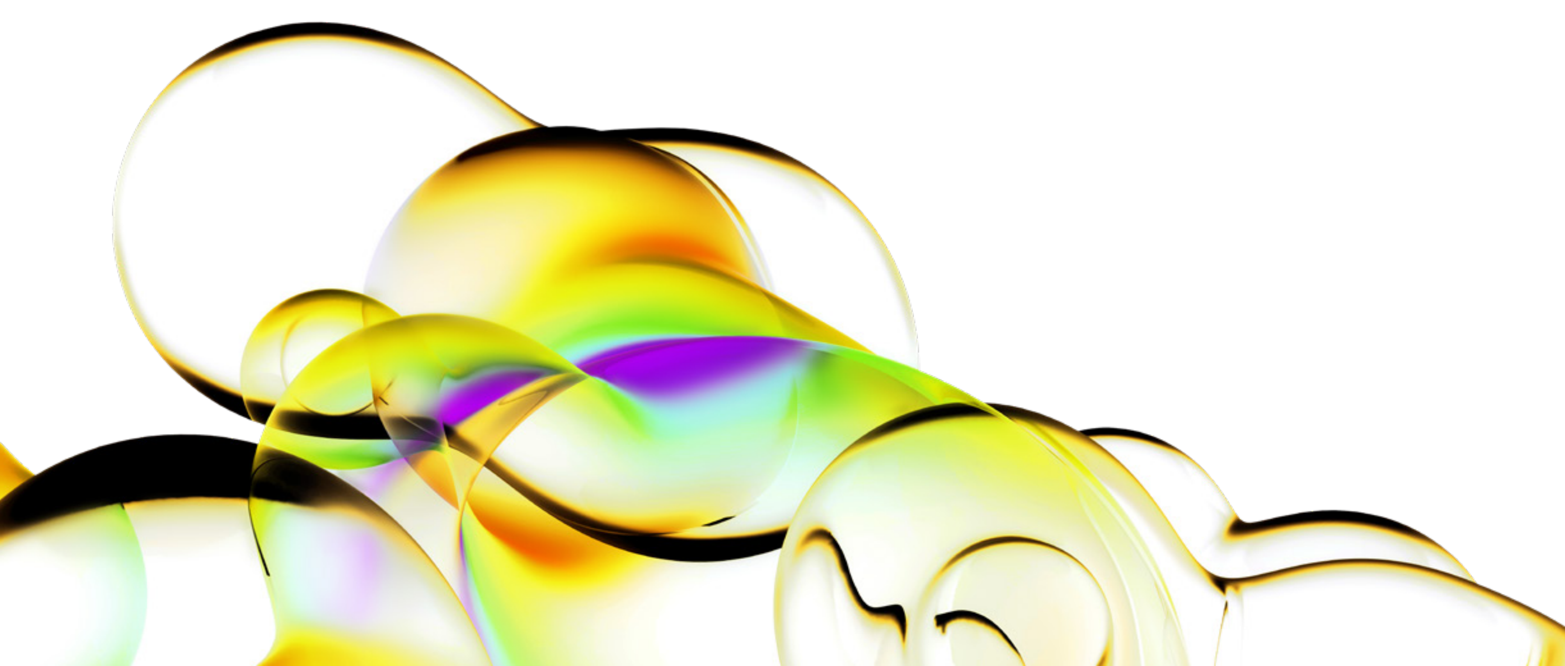
APPLICATION NOTE

Multiplex fluorescence assays for adherence cells without trypsinization.

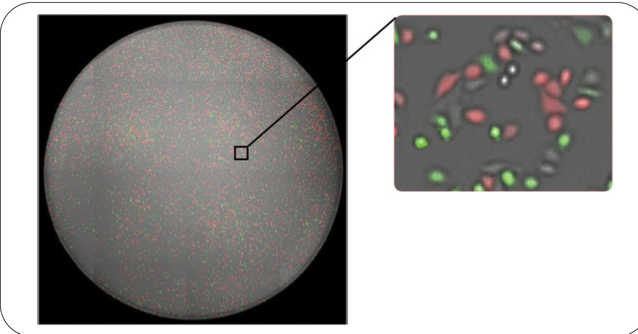
The combination of a brightfield and three fluorescent channels allows the Celigo® image cytometer to perform many multiplexed assays. A gating interface similar to flow cytometry provides great flexibility in data analysis. In addition, images of gated cells are displayed and updated with the gating operation.

- Reduce sample preparation and number of cells per test (96 or 384 wells)
- Cells stay in multi-well plates without trypsinization
- Images provide visualization of cell morphology, fluorescent distribution for assay development and quality control
- Gating is reflected on cell images





Fluorescence proteins



Whole well view GFP/RFP transfected Hela cells in a 96-well plate.

- Identify cells in brightfield image
- Measure fluorescent protein signals in green, and red channels
- Label-free, non-invasive – no need to trypinsize adherence cells
- Quantify fluorescent protein signals on a cell-by-cell basis repeatedly on the same plate providing temporal data.
- Add propidium iodide for viability of GFP transfect cells in the same well

Transfection and transduction optimization using Celigo adherent cell cytometer

The combination of a brightfield and three fluorescent channels allows the Celigo to perform many multiplexed assays. A gating interface similar to flow cytometry provides great flexibility in data analysis. In addition, images of gated cells are displayed and updated with the gating operation.

Example 1. GFP transfection optimization in 96 well plates without trypinization

HeLa cells were transfected with a range of concentrations of a plasmid encoding turbo-GFP1 and seeded out into a 96-well plate. To monitor cell death, propidium iodide was added to the wells in some experiments. The same plate was imaged daily over a 5-day period.

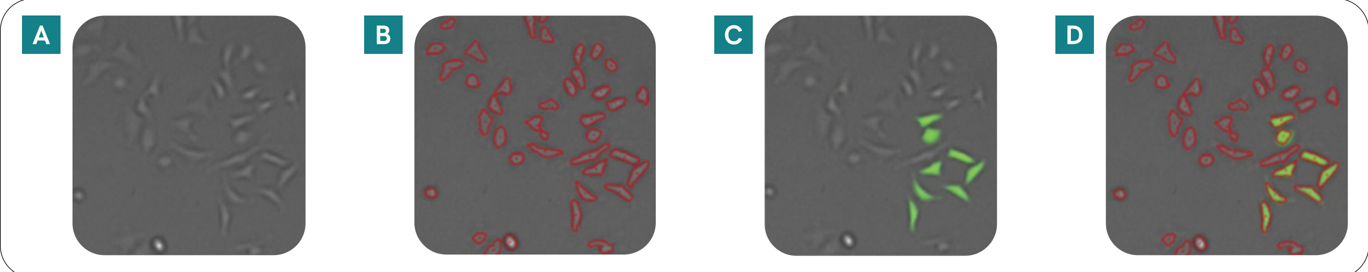


Figure 1: Cell Images of GFP transfected Hela in 96-well plate. (A) Brightfield cell image including transfected and non-transfected cells. (B) Celigo image process software identifies all the cells using brightfield image, indicated by the red outline. (C), (D) Within this red outline, green fluorescence intensity was measured to gate cell populations produce transfection efficiency.

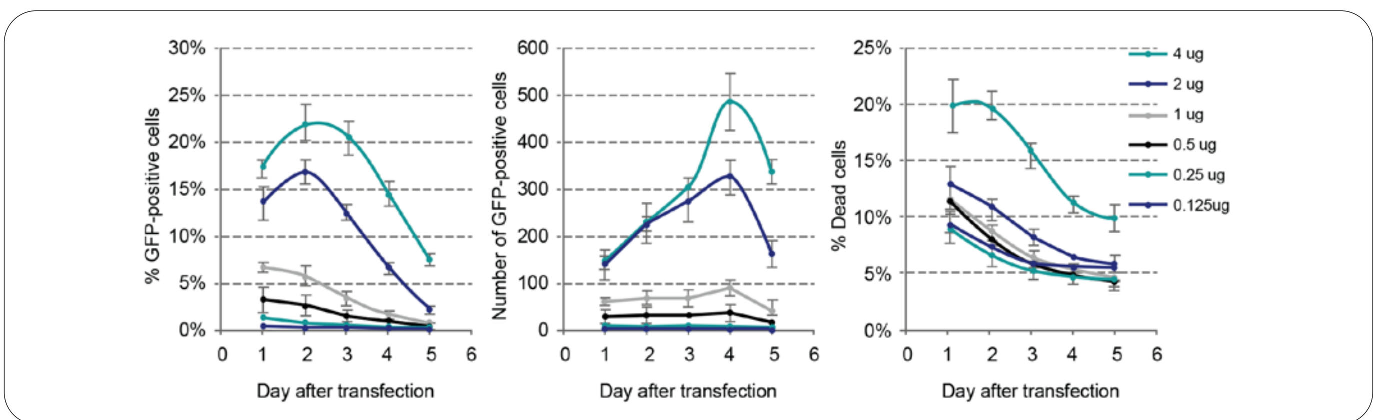


Figure 2: GFP-Positive Cell Quantification. Left: Percentage of GFP-positive cells vs. day after transfection. Middle: GFP-positive cell counts. Right: Percentage of dead cells (propidium iodide-positive). Error bars indicate standard deviation.

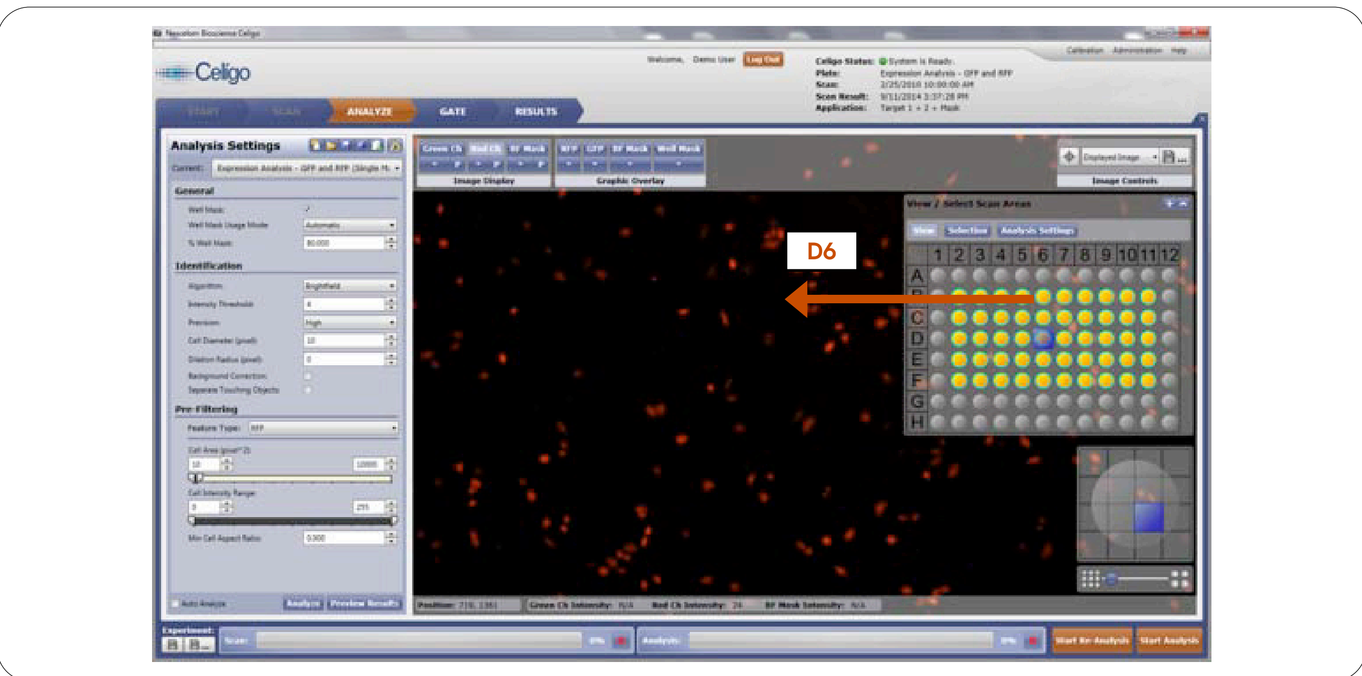
Example 2: Measure % RFP positive cells in samples containing GFP, RFP and non-expressing cells

96-well containing samples with varied % of RFP, GFP and non-expressing Hela cells were counted using Celigo. brightfield, green fluorescence and red fluorescence channels were used.

Step 1: Acquire & view images on Celigo image cytometer

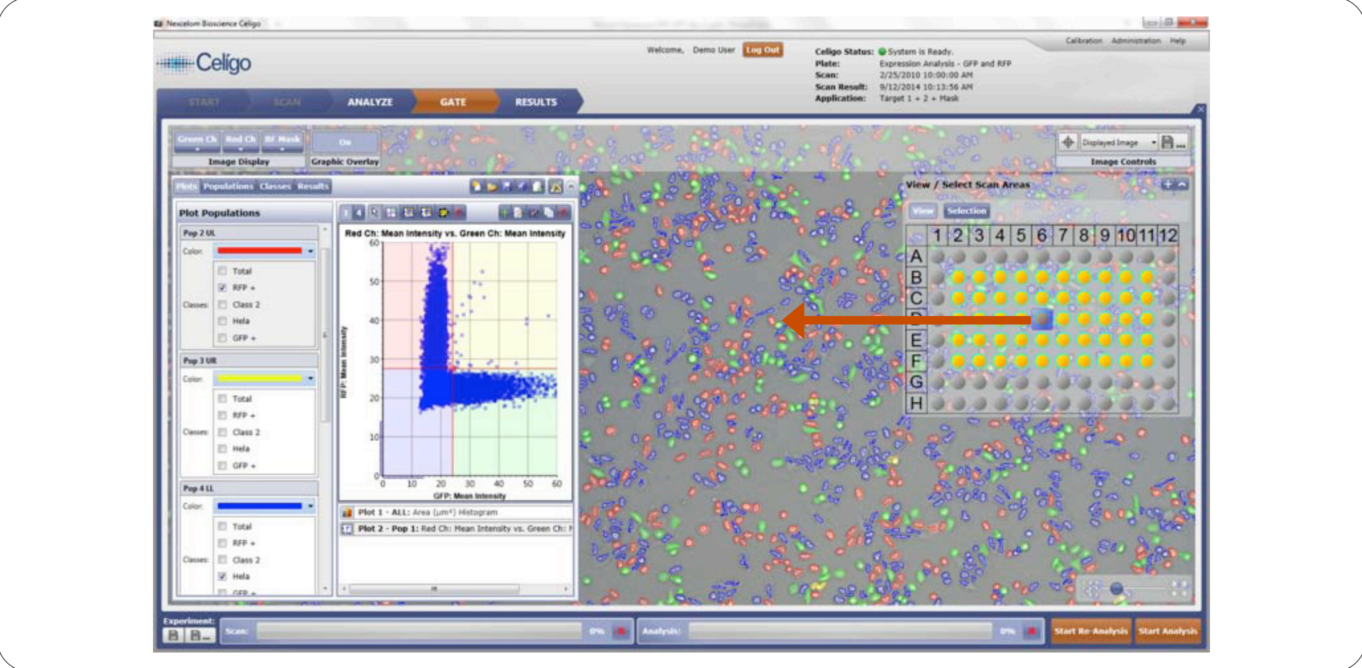
Brightfield image of well D6, zoomed view.

RFP fluorescence image of well D6, zoomed view.



Step 2: Gate and circle counted cells automatically

Overlay of brightfield, GFP+ and RFP+ cells identified by Celigo image processing software. GFP vs RFP intensity scatter plot is also shown. When the gate on the scatter plot is modified by dragging, the counted cell image is updated automatically.



View whole well cell images

Double click any well on the well map to display whole well images. Zoom in to see details as listed in Table 1.

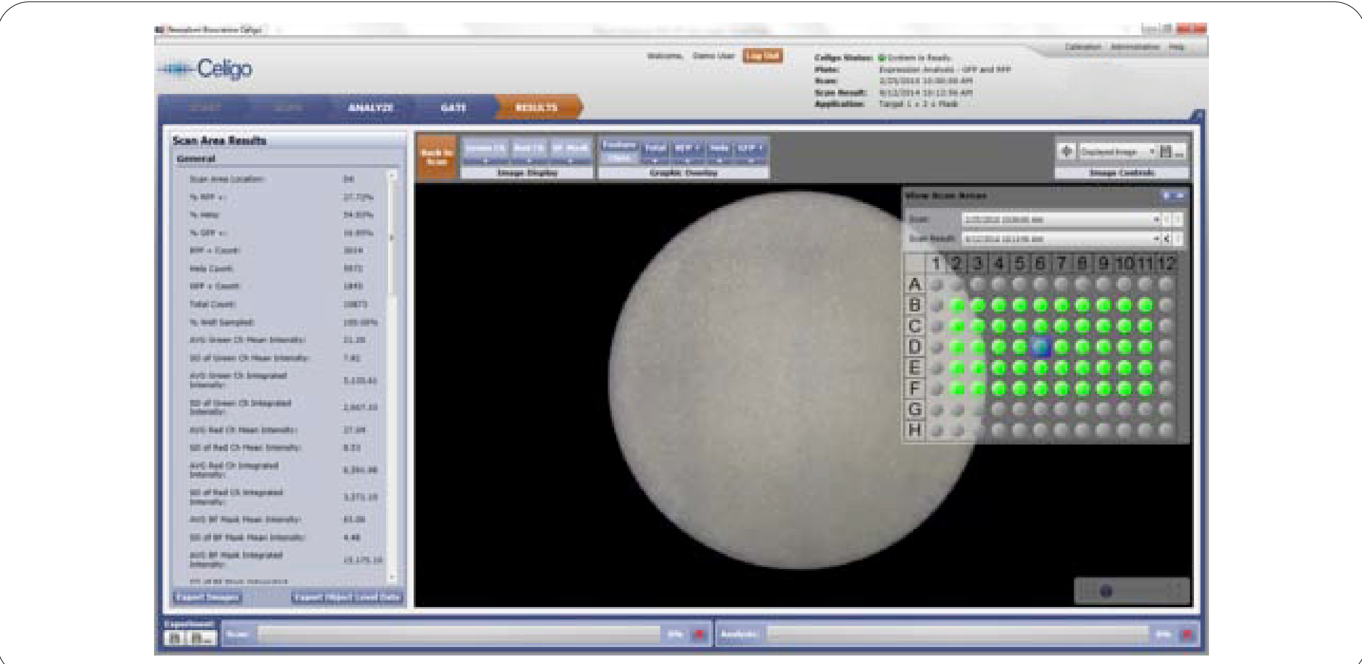
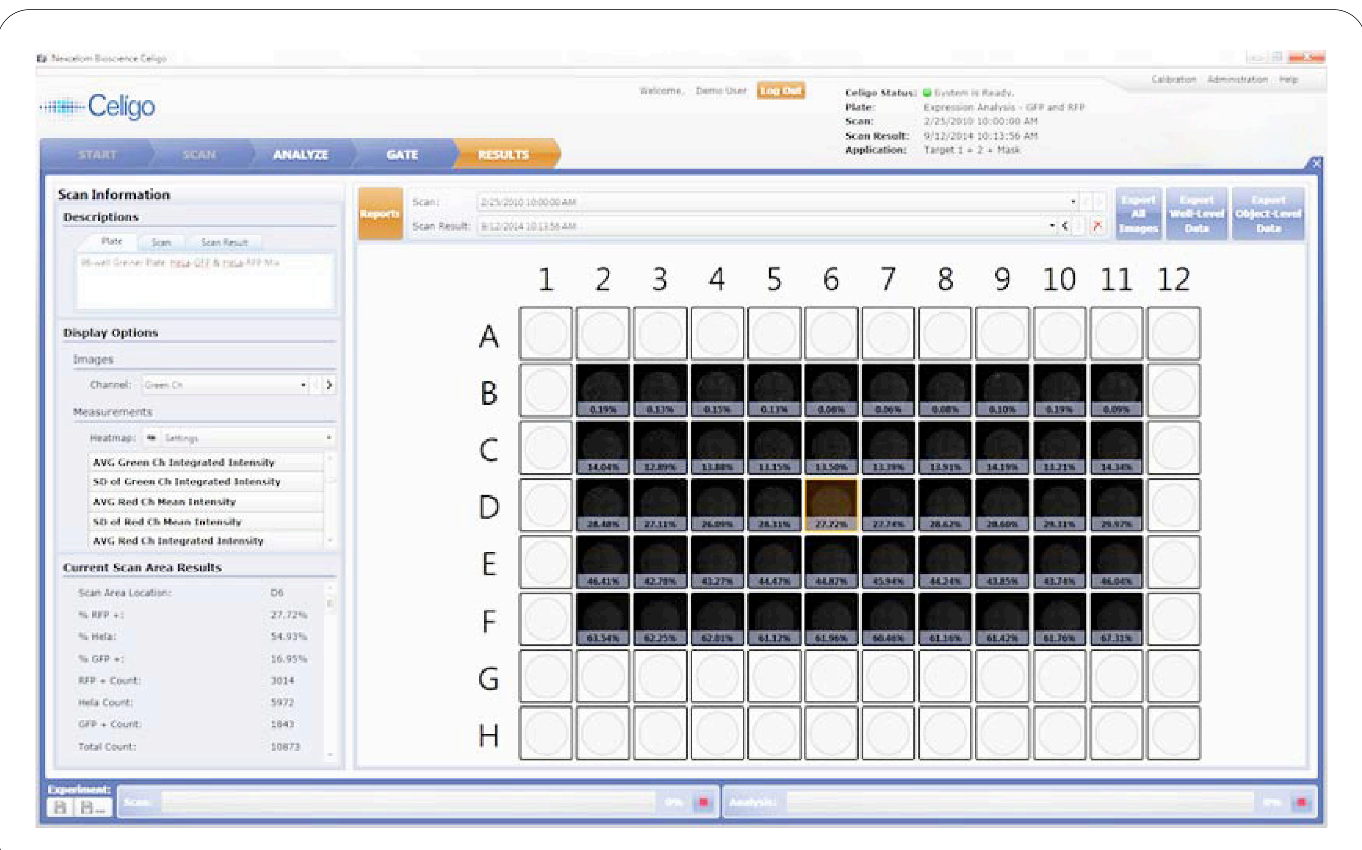


Table 1: Cell image examples from wells with varied RFP+ cell populations.

Cell images		
GFP+, RFP+, GFP-RFP-	RFP+	Counted RFP+

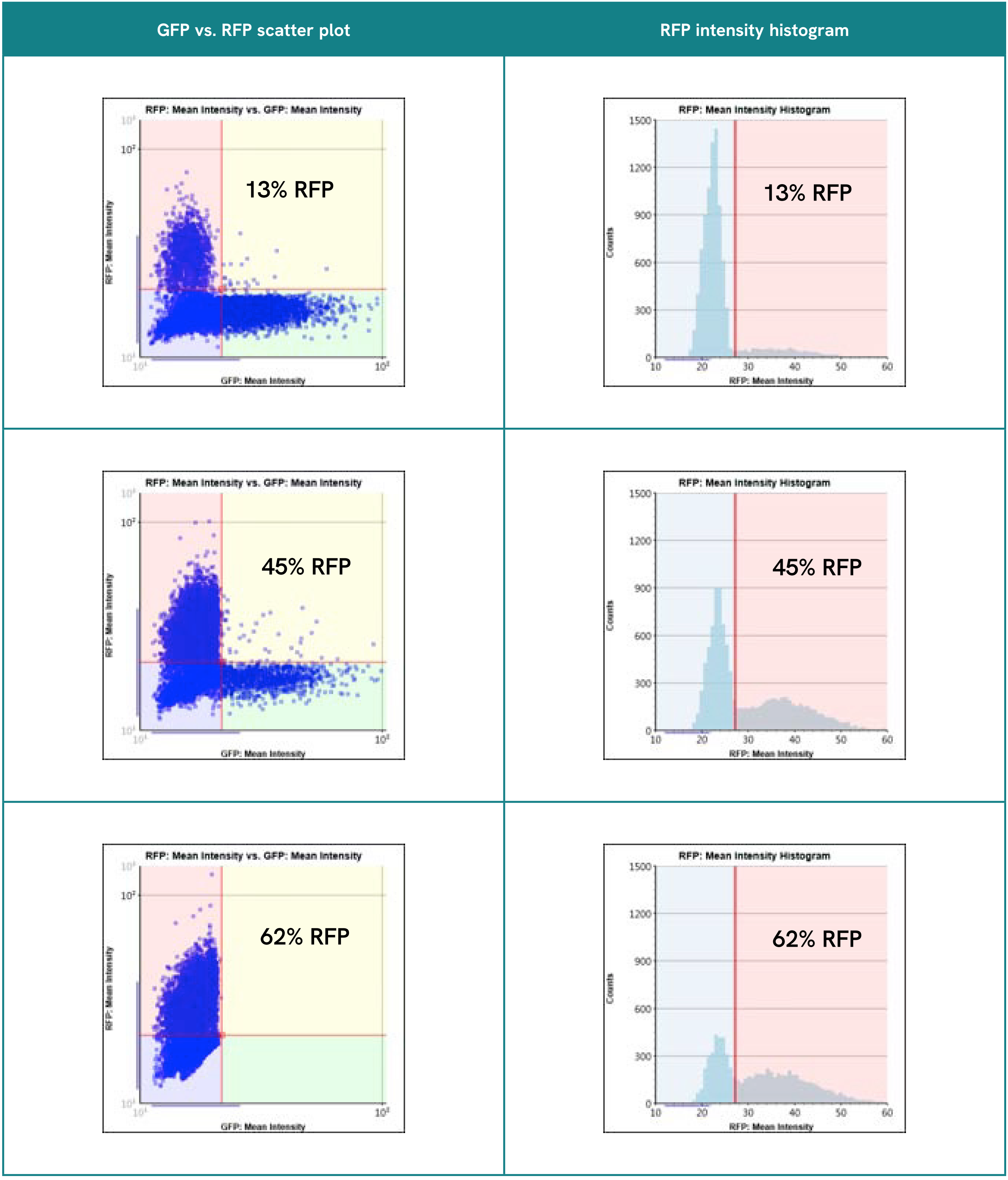
Step 3: Review Plate Level Results for Each of the Wells in the 96-Well Plate



Reported data	%	Count	Mean intensity AVE & SD	Integrated intensty AVE & SD
Total		•		
RFP+	•	•	•	•
GFP+	•	•	•	•
Non-FL HeLa	•	•	•	•
Green Channel			•	•
Red Channel			•	•
Brightfield Mask Channel			•	•

More plots are available from Celigo software, as listed in Table 2.

Table 2: Example plots



Step 4: Export Results for Each of the Wells in the 96-Well Plate and Produce Data Plot

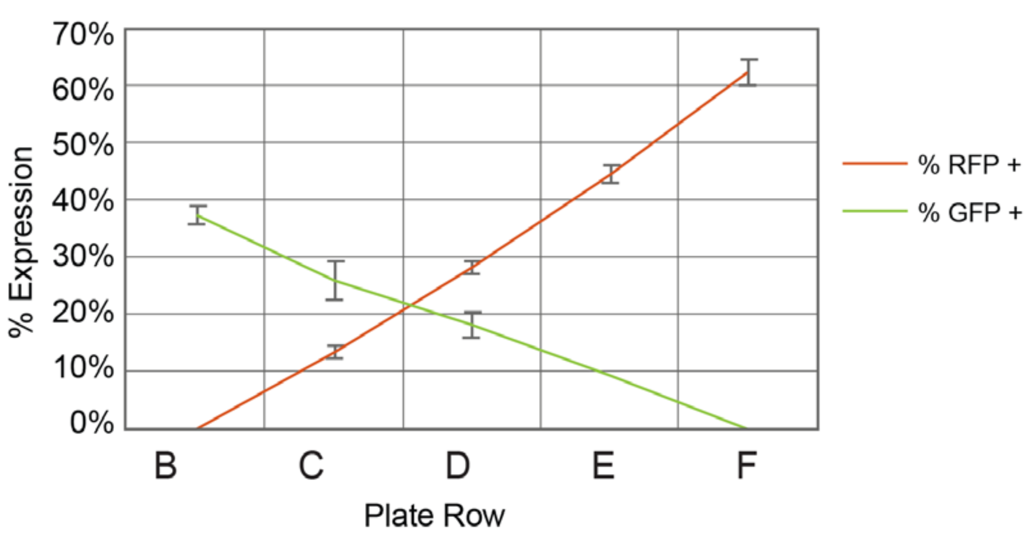
Open Well Level Data in Excel / Calculate AVE & SD / Create Plot with Ave & Std

Measurement Plate Maps

% RFP +	1	2	3	4	5	6	7	8	9	10	11	12	% RFP +	
A													AVE	SD
B		0.2%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.2%	0.1%		0%	0%
C		14.0%	12.9%	13.9%	13.2%	13.5%	13.4%	13.9%	14.2%	13.2%	14.3%		14%	0%
D		28.5%	27.1%	26.1%	28.3%	27.7%	27.7%	28.6%	28.6%	29.3%	30.0%		28%	1%
E		46.4%	42.8%	43.3%	44.5%	44.9%	45.9%	44.2%	43.8%	43.7%	46.0%		45%	1%
F		63.5%	62.3%	62.0%	61.1%	62.0%	60.5%	61.2%	61.4%	61.8%	67.3%		62%	2%
G														
H														

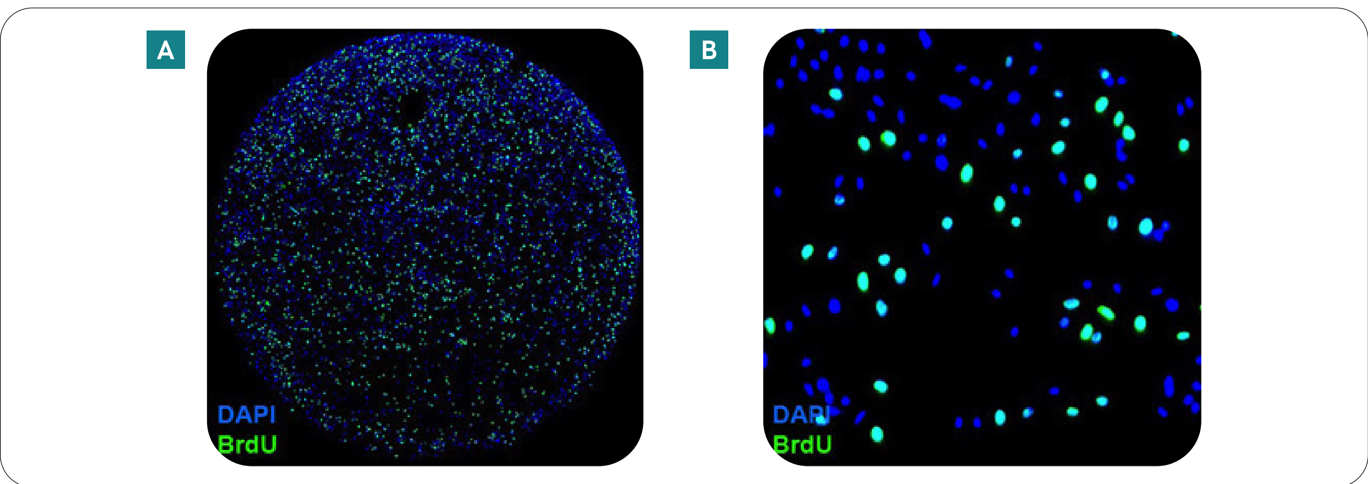
% Hela	1	2	3	4	5	6	7	8	9	10	11	12	% Hela	
A													AVE	SD
B		62.1%	62.7%	63.0%	61.9%	64.4%	60.8%	61.4%	63.0%	61.1%	60.2%		62%	1%
C		61.0%	69.5%	58.6%	60.6%	58.8%	58.0%	58.5%	58.5%	58.4%	55.0%		60%	4%
D		53.8%	53.9%	55.1%	52.6%	54.9%	59.4%	51.6%	51.2%	51.6%	48.2%		53%	3%
E		44.1%	47.3%	46.2%	45.3%	45.2%	43.9%	45.2%	46.4%	46.9%	43.4%		45%	1%
F		36.3%	37.6%	37.8%	38.7%	37.8%	39.2%	38.3%	38.3%	37.7%	32.5%		37%	2%
G														
H														

% GFP +	1	2	3	4	5	6	7	8	9	10	11	12	% GFP +	
A													AVE	SD
B		37.4%	37.1%	36.7%	37.8%	35.3%	38.9%	38.3%	36.6%	38.5%	39.5%		38%	1%
C		24.7%	17.3%	26.9%	25.9%	27.1%	28.1%	27.2%	26.9%	28.0%	29.8%		26%	3%
D		17.2%	18.3%	18.4%	18.6%	17.0%	12.5%	19.1%	19.6%	18.5%	21.0%		18%	2%
E		9.0%	9.4%	10.0%	9.7%	9.4%	9.6%	9.9%	9.3%	8.9%	9.5%		9%	0%
F		0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.2%	0.0%		0%	0%



Cell proliferation assay using BrdU incorporation

Image data



Gated data

Total: Mean Intensity vs. DNA Synthesis(+): Mean Intensity

Total: Mean Intensity

DNA Synthesis(+): Mean Intensity

Total: Mean Intensity vs. DNA Synthesis(+): Mean Intensity

Total: Mean Intensity

DNA Synthesis(+): Mean Intensity

BrdU with DAPI Total Stain

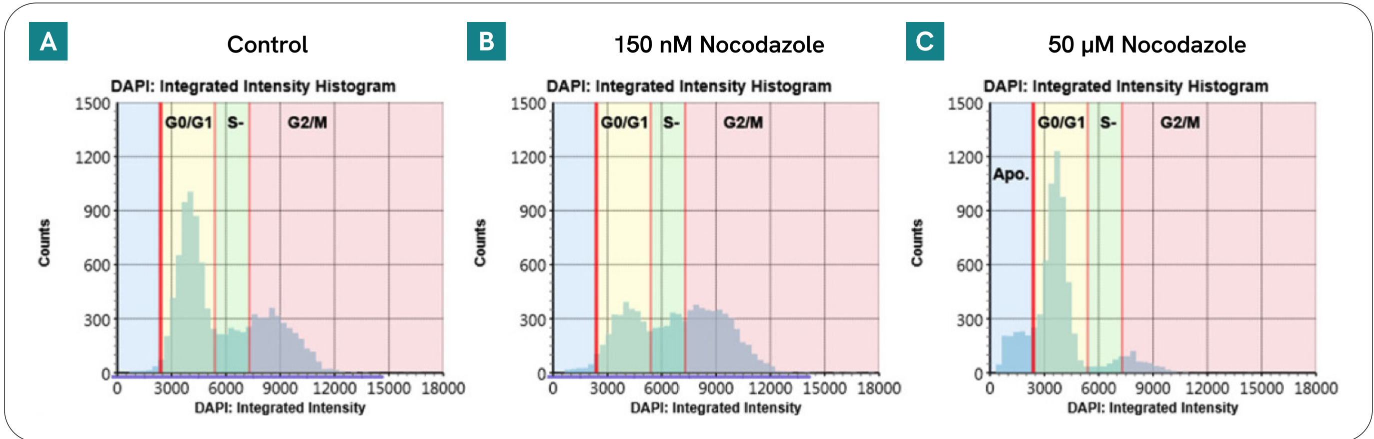
% BrdU Positive

log M [Aphidicolin]

Cell cycle analysis of adherence cells in 96-well plates using Celigo

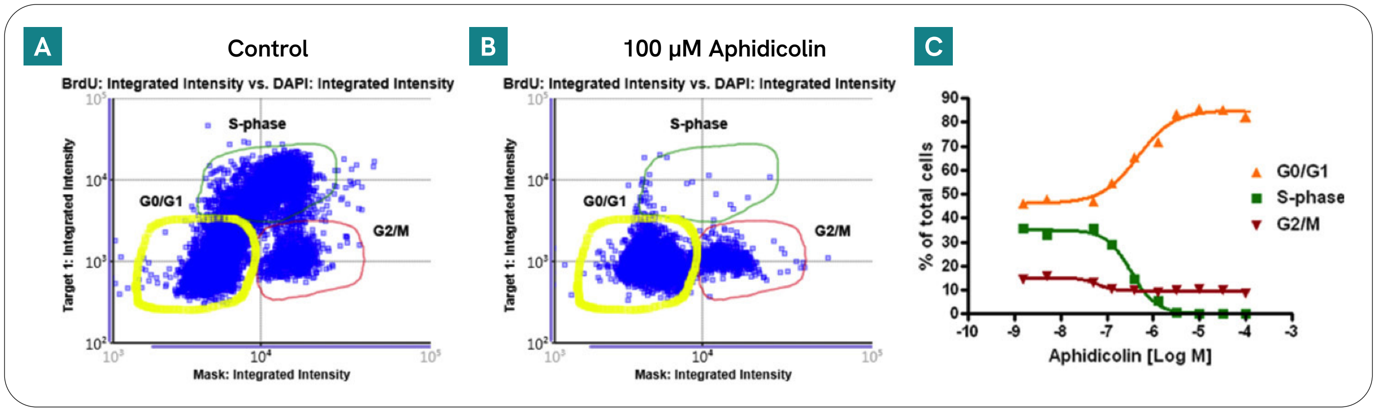
Cells were stained with DAPI, a marker for DNA synthesis. The multi-fluorescent channel analysis and gating interface allows identification of cells in the G0/G1, S-Phases and G2/M phases of the cell cycle.

Cell cycle - Method 1: Measure DNA content using DAPI



Cell cycle - Method 2: Using DAPI and BrdU

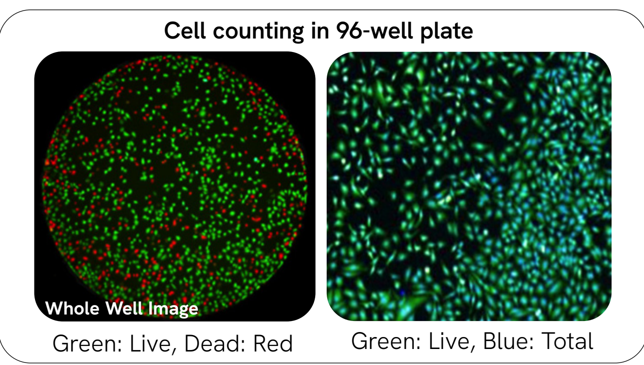
- Cell cycle analysis is examined by measuring the amount of DNA per cell
- Incorporation of DAPI and BrDU
- Double peak characteristic peak of cell cycle consisting of G0/G1, S and G2/M phases

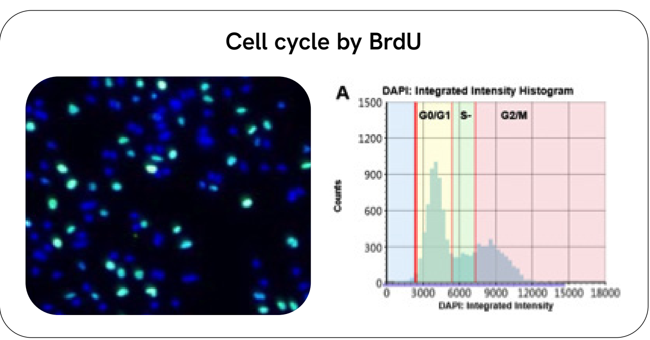


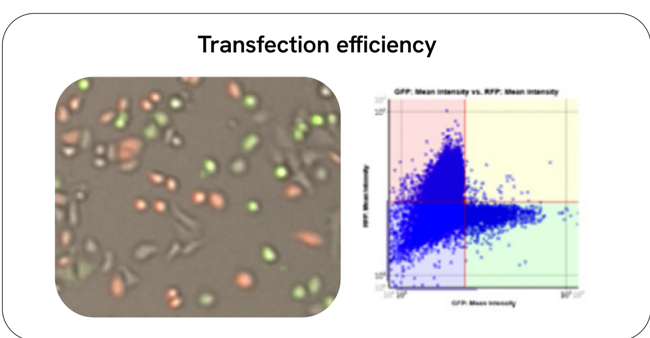
Imaging data can be used to generate dose response curves to characterize drug activities on the cell cycle (right panel).

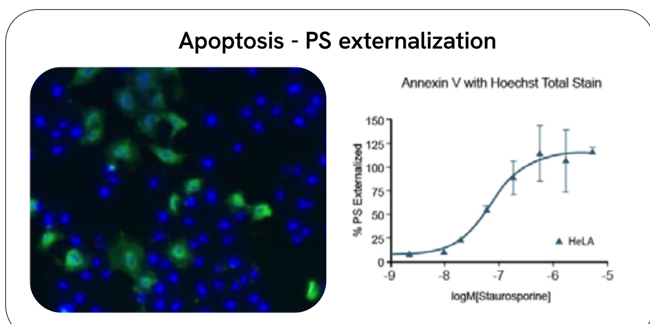
Cell viability determined by staining cells with live and dead cell-specific dyes

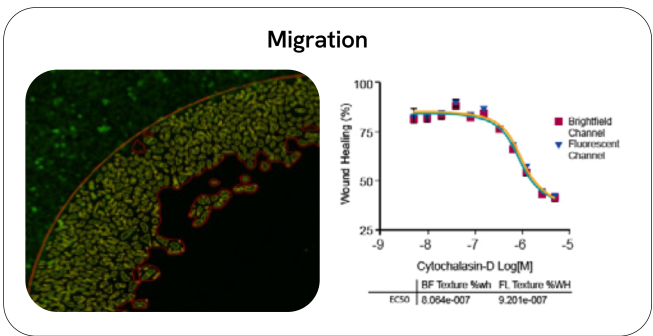
Briefly, cells are simultaneously stained with a mixture of calcein AM, propidium iodide, and Hoechst 33342 for respective staining of live, dead and all cells. Images are acquired and analyzed using the Celigo software. Markers are identified in each fluorescent channel and for each well of a microtiter plate, live and dead cell counts as well as the percentage of live and dead cells are automatically reported.

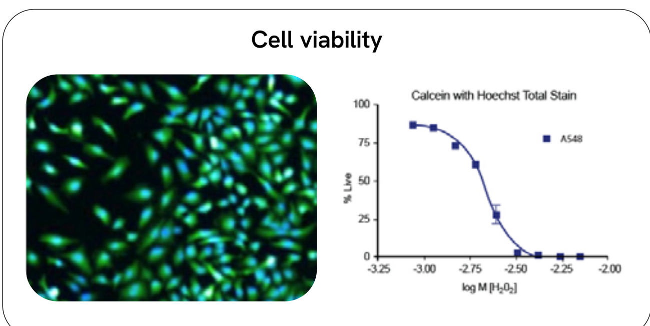


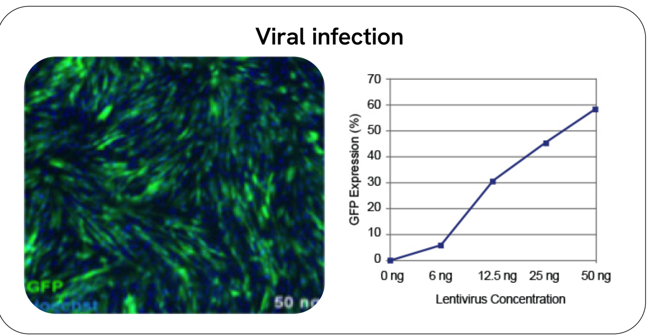


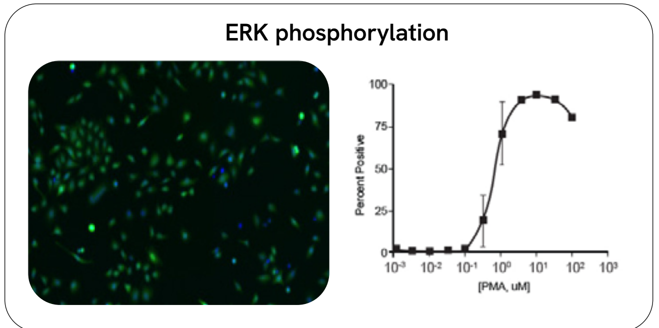


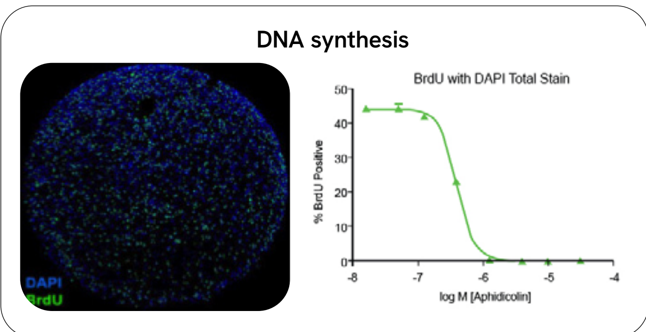












- Receptor internalization
- Phagocyosis
- Receptor detection: CD71, CD54 ICMA-1

- ERK Phophorylation
- Nuclear antigen detection

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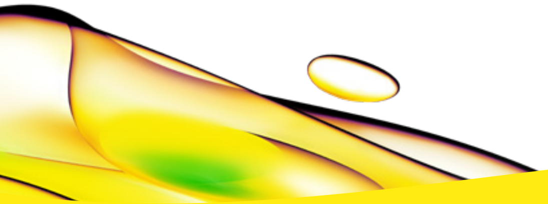

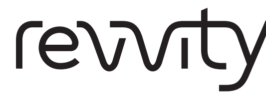

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