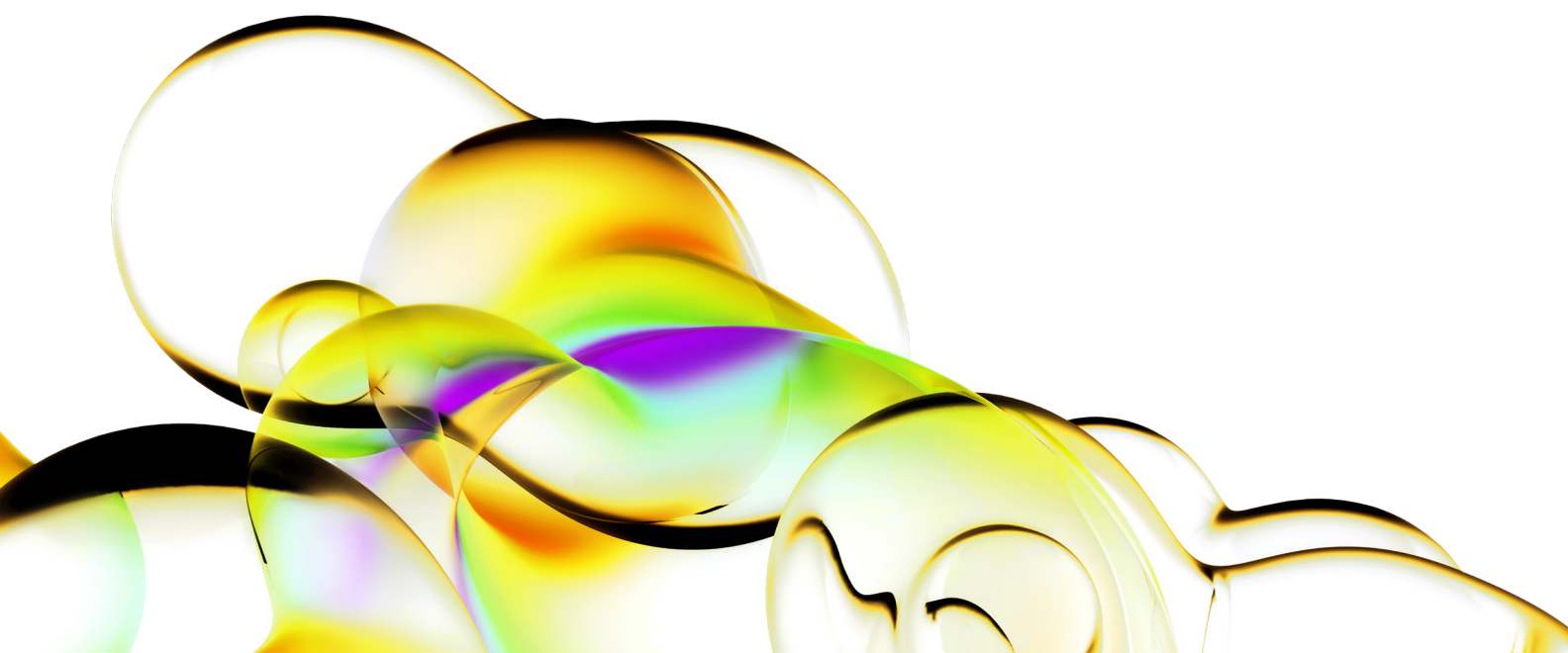


Transfection optimization using expression analysis.

Introduction

Optimization of cell transfection typically includes determining the optimum mass of plasmid to be transfected and the time point after transfection that provides the maximum expression. Too much plasmid can be toxic and lead to compromised cell growth; too little plasmid will reduce the overall transfection efficiency. As cells proliferate, the number of transfected cells will increase at the expense of diluting the plasmid in each transfected cell. Thus there will be an optimum time point for maximum number of expressing cells.

The Celigo® image cytometer is a benchtop in situ cellular analysis system that rapidly provides high integrity whole well images for routine brightfield and fluorescent cellular analysis. The Celigo image cytometer provides brightfield and three channels of fluorescence capabilities for visualizing and quantifying cellular responses in 1,536- to 6-well plates and flasks. The system provides full resolution at 1 μm /pixel (Figure 1). Developed for brightfield and fluorescence imaging, it incorporates proprietary optics and software to image and identify cells in brightfield with consistent contrast across the whole well all the way up to the well edge. This enables the ability to use the brightfield image for defining the position and area of the cells without the need to add additional fluorescent probes.



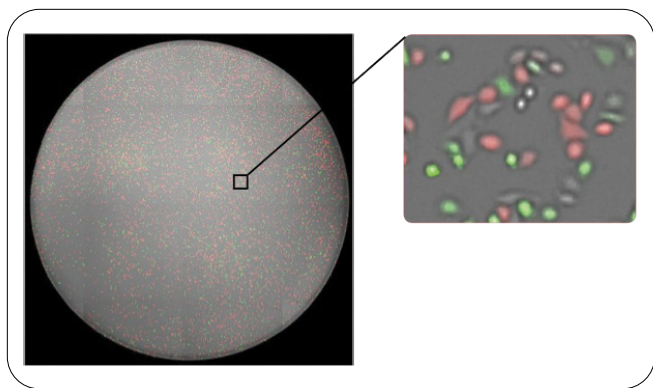


Figure 1: Whole-Well Brightfield and Multi-Color Fluorescence Imaging on the Celigo Image Cytometer. Left: Whole-well view (96-well plate). Right: Brightfield and two-channel fluorescent imaging of cells expressing GFP or RFP.

The Expression Analysis application identifies cells in brightfield and creates an outline, or mask, of each individual cell (Figure 2). The fluorescence signal in one to three fluorescent channels can be then measured within the brightfield mask. This provides a label-free, non-invasive method to quantify fluorescent signals on a cell-by-cell basis and can be performed repeatedly on the same plate providing temporal data.

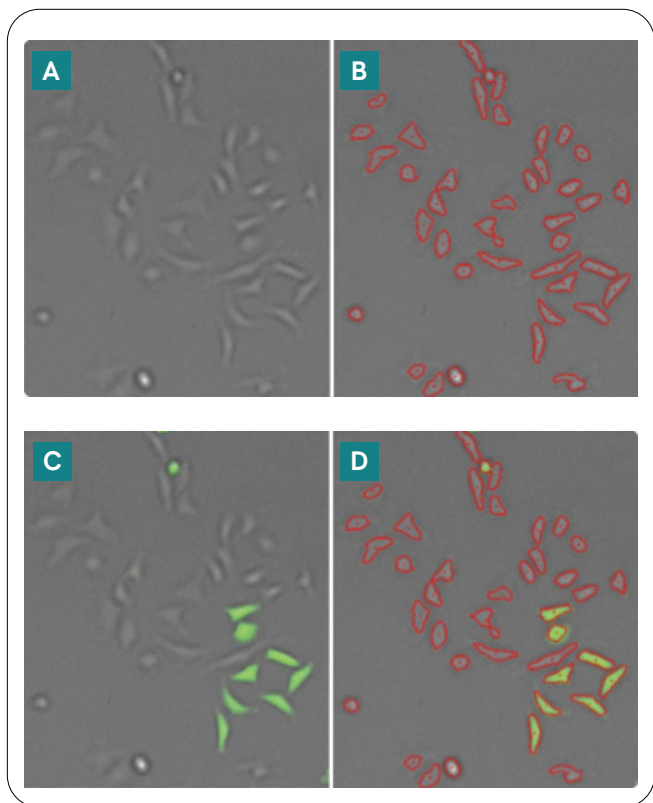


Figure 2: Brightfield Segmentation on the Celigo Image Cytometer. Cells are imaged (A) and identified (red mask in B) in brightfield. Fluorescence signal (GFP in C) is measured within the red mask defined by the brightfield image (D).

Experiment

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. Imaging the plate in three channels took approximately 20 min, after which the plate was returned to the incubator.

Results

The Expression Analysis application reports a range of cell based parameters (intensity and morphology) as well as absolute cell numbers and percentages for the whole cell population, providing a direct readout of the required information. In studies targeting multiple transfections, the application reports the number of double and triple transfectants within the population. A complete assessment can be performed using a single microplate. An important consideration for studies spanning several days is the need to seed cells sparsely. This often leads to uneven distribution of cells (Figure 3) in the well which, in turn, necessitates full-well imaging to ensure accurate quantitation of all cells within the well.

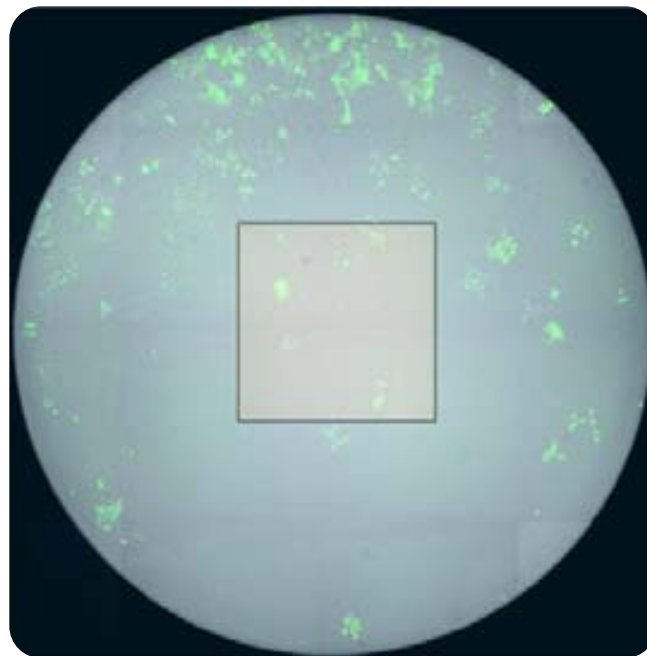


Figure 3: Full-Well Imaging (light blue-shaded area) is necessary to accurately quantify cells at low density that often disperse unevenly in the well. The clear center tile represents a single field-of-view sample. The image is a composite of brightfield and green fluorescence (GFP).

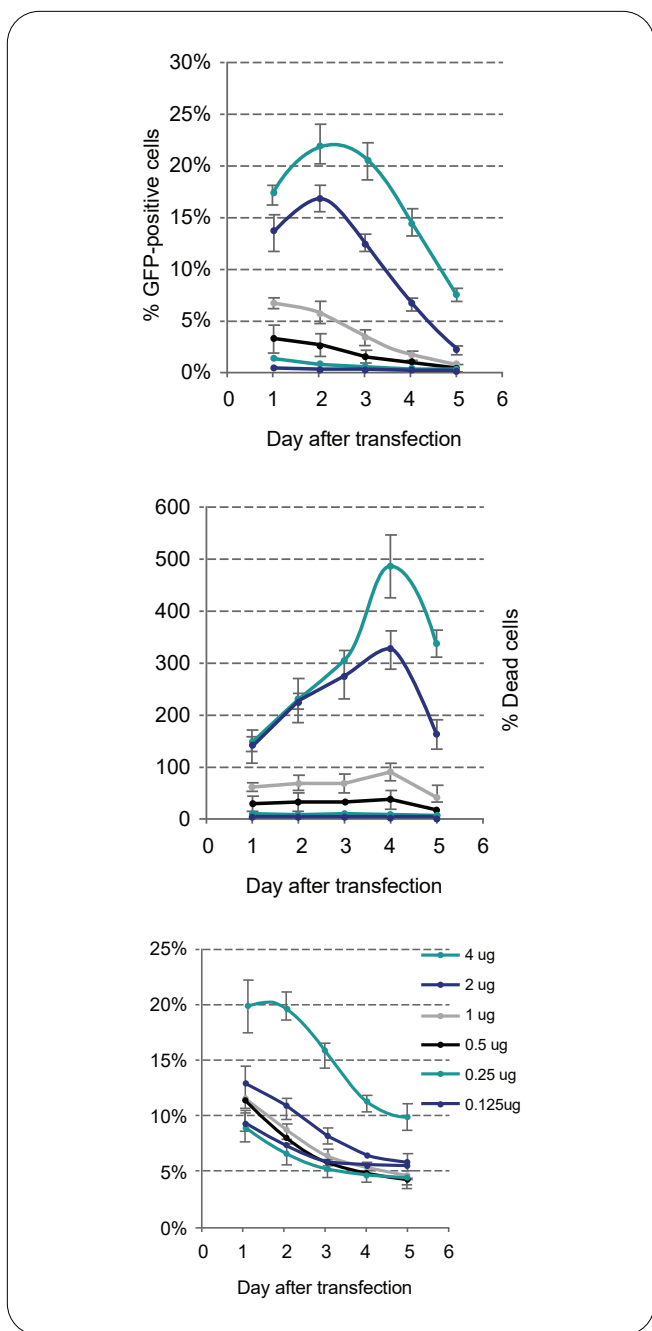


Figure 4: Streamlined cell culture maintenance using Celigo. Example of workflow and output for monitoring adherent cell cultures in T-25 flasks. Cells can be imaged and counted in culture vessels without staining or harvesting. Celigo updates and returns growth curves automatically.

As expected, increasing the mass of plasmid increased the percentage of GFP-positive cells (Figure 4). Maximum percentage of GFP-positive cells was obtained on day 2 after transfection. Looking at GFP-positive cell counts, however, the maximum number of GFP-positive cells was obtained on day 4 after transfection. This is due to proliferation of transfected cells. Eventually, the plasmid is diluted out and expression is lost. This was seen as a decrease of GFP-positive counts on day 5. Cell death was assessed in the same wells by quantifying propidium iodide uptake. The data show that 4 μg of plasmid caused significantly higher cell death compared to other concentrations. Compromised cell health may interfere with a downstream functional assay, e.g. cell proliferation or migration, and may be an important aspect of the optimization process.

Conclusions

Brightfield imaging and cell identification enables cell-based fluorescence quantification in a variety of multiwell formats and flasks without the requirement for counterstains. Cells can be left undisturbed in the plate or flask, imaged within 20-30 minutes, depending on the format, and then returned to the incubator for continued culturing. This facilitates time-course studies, such as monitoring fluorescence after a transient transfection, dramatically reducing the effort and time to perform the experiments.

References

1. Fluorescent proteins used in this demonstration are licensed from EVROGEN, Moscow, Russia. For further information on the proteins, please visit www.evrogen.com.

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