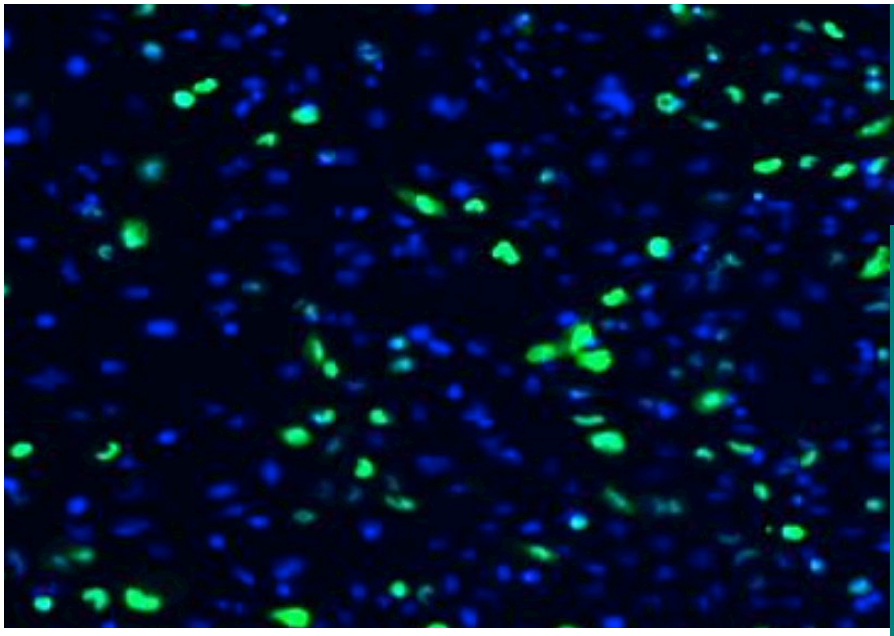

APPLICATION NOTE

Multimode Detection

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Comparing Transfection Reagent Efficiency by Imaging and Cell Counting on the EnSight Multimode Plate Reader

Introduction

Many cell-based assays and biotherapeutic production processes rely on the expression of exogenous molecules in a model cell line. This makes it highly desirable to have robust, reliable methods that are quick to perform and easy to reproduce for both the optimization of transfection rates and the monitoring of cytotoxic effects in and on the cell line(s) of interest.

There are a myriad of methods available for transfection (the process of introducing foreign DNA into a eukaryotic cell line) which can be classified into four major categories: chemical-, non-chemical-, particle- and viral- based methods. Here, we show chemical transfection of two commonly used cell lines, HeLa and CHO, comparing and contrasting the efficiency and cell health of two non-liposomal reagents from Mirus Bio LLC, *Trans*IT®-LT1 and *Trans*IT®-2020, to Lipofectamine® 2000.

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The EnSight™ multimode plate reader offers well imaging, label-free and labelled detection technologies, on a single system, and is perfectly suited to track and measure transfection efficiency across different cell types and with different protocol variations and optimizations. The combination of brightfield and digital phase contrast (DPC) imaging with up to four fluorescent colors in a high-density plate-based format allows the user to test many conditions quickly and with enough replicates to ensure the quality of the data generated. In this application note we demonstrate how the EnSight multimode plate reader with Kaleido™ data acquisition and analysis software can be used to simultaneously measure transfection rate and assess cell health in a rapid, easy to use, and reproducible manner.

Materials and Methods

An overview of the workflow for the transfection experiments is shown in Figure 1. Briefly, cells were plated and allowed to adhere overnight before transfection. Brightfield and DPC images were obtained prior to transfection and every 12 hours after transfection, to monitor cell growth and reagent toxicity. After 48 hours, the cells were stained and imaged in dual fluorescent mode, then analyzed for transfection efficiency.

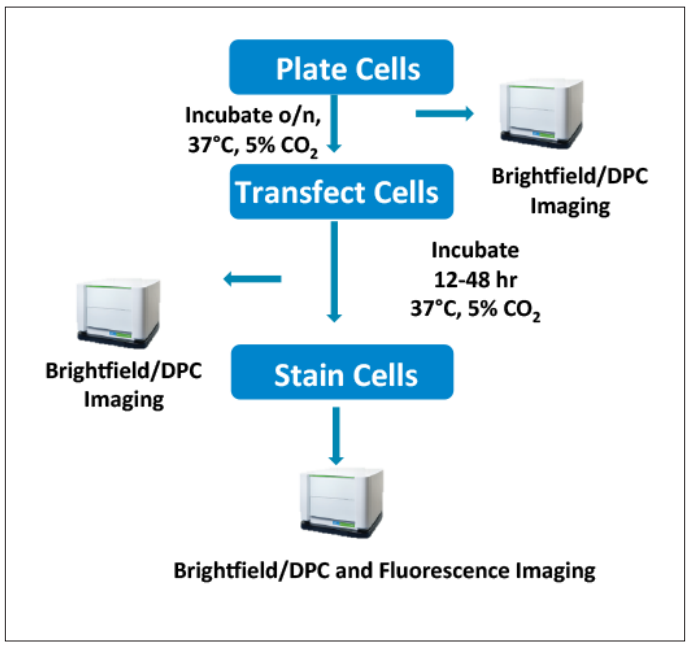

Figure 2. Overview of Transfection Experiment Workflow.

Cell Seeding

Both cell lines used, HeLa and CHO-K1, were purchased from ATCC, catalog numbers CCL-2 and CCL-61, respectively. Cells were maintained according to standard cell culture practices listed by ATCC. 50 µL of HeLa cells were seeded at a cell density of 2500 cells/well in a PerkinElmer 96-well black ViewPlate™ (catalog number 6005182). 50 µL of CHO cells were seeded at a cell density of 2000 cells/well in a PerkinElmer 96-well black CellCarrier™ plate (catalog number 6005558). Both cell types were seeded 18-24 hours prior to transfection. Optimal cell densities and seeding times were determined in separate experiments (data not shown).

Transfection

The chemical transfection reagents used were: Lipofectamine® 2000 from Thermo Life Sciences (catalog number 11668-027), *Trans*IT®-LT1 and *Trans*IT®-2020 from Mirus Bio, LLC. The pEYFP-nuc plasmid DNA was used to monitor transfection efficiency and as a bacterial cloning vector for mock DNA transfection. For Lipofectamine® 2000 transfections, DNA and reagent were mixed in a 5:3 (w:v) ratio in Opti-MEM® (Thermo Life Sciences, catalog number 31985062), incubated for 5 minutes, then added to cells. For *Trans*IT® transfections, DNA and reagent were mixed in a 1:3 (w:v) ratio in Opti-MEM®, incubated for 15 minutes, then added to cells. For both transfection reactions, the final DNA concentration was 0.1 µg per well (100 µL total well volume). Cells were incubated at 37 °C at 5% CO_2 for the duration of the study. Optimal DNA:reagent ratios were determined in separate experiments (data not shown).

Cell Staining

Approximately 40-48 hours after transfection, the cells were stained with Hoechst 33342 (Life Technologies, catalog number H3570). The Hoechst stain was prepared at 3X in the same medium used for cell seeding, with a 50 µL addition to 100 µL of cells in a 96-well plate. Final dye concentration was 5 µg/mL. Cells were incubated for at least 60 minutes at 37°C at 5% CO_2 prior to imaging.

Image Acquisition and Evaluation

Images were acquired using the EnSight multimode plate reader, equipped with well-imaging module, and Kaleido data acquisition and analysis software. Brightfield and DPC imaging were performed before transfection and monitored approximately every 12 hours after transfection. Fluorescent imaging was performed at an optimal time point, as determined in preliminary experiments, 48 hours post-transfection. Transfection analysis was performed using YFP (excitation 525 nm) as the signal and Hoechst (UV excitation 385 nm) for object and nucleus detection, respectively, in combination with the Custom Transfection Analysis method provided by Kaleido software. Confluency and foreground roughness mean were determined using the brightfield images and a pre-defined Brightfield Confluency method. Number of cells per well and median object area were determined in the DPC images by a pre-defined Count Cells v1.3 method provided by the Kaleido software. For all data analysis, the error bars depicted represent that standard error measurement for four replicates.

Results

Transfection Analysis Reveals Different Efficiency with Cell Line and Reagents Tested

Representative images of EYFP-nuc transfected HeLa and CHO cells using *Trans*IT®-LT1 (LT1), *Trans*IT®-2020 (2020), and Lipofectamine® 2000 (LF) are shown in Figure 2. We observed the highest transfection efficiencies in HeLa cells with LF; whereas CHO cells had the best transfection efficiency with LT1. This result was confirmed by the image analysis as indicated in Figure 3A. The calculated transfection rate was significantly higher when transfecting the HeLa cells with the LF reagent, while in the CHO cells the transfection rate was highest using the LT1 reagent. Similarly, the median signal intensity of transfected cells, shown in Figure 3B, further confirms

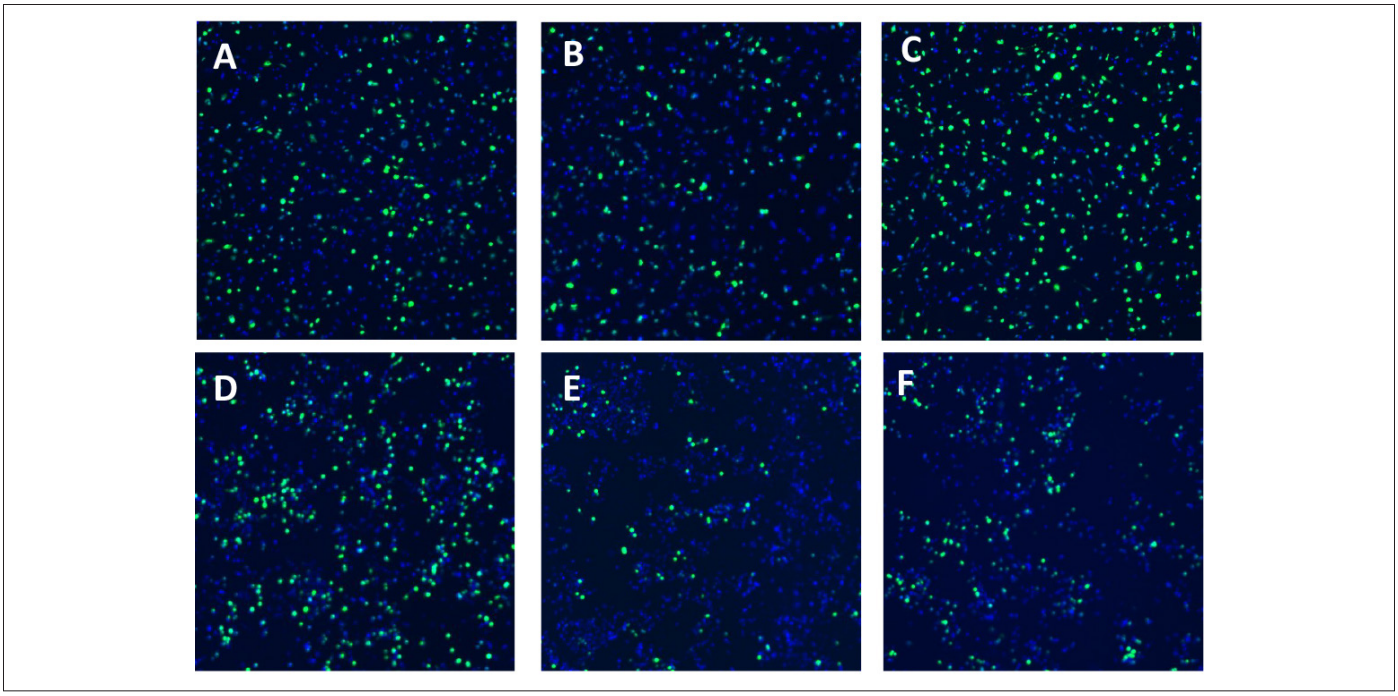

Figure 2. Representative fluorescent images of transfected HeLa and CHO cells. HeLa (A-C) and CHO (D-F) cells were transfected with YFP using either LT1 (A,D), 2020 (B,E), or LF (C,F) reagent. YFP shown in green, Hoechst stain shown in blue.

that the LF transfection was most effective in the HeLa cells, while the LT1 transfection was most effective in the CHO cells. Combined, these data generated by the transfection analysis method provide both quantitation and confirmation of the visual appearance of relative transfection rates observed in Figure 2.

DPC Cell Counting and Brightfield Confluency Analysis Indicate Reagent Toxicity

Transfection-induced cytotoxicity was determined by measuring total cell counts and cell confluency of each well. Indeed, in Figure 4, in both the HeLa and CHO cells, treatment with LF caused lower total cell counts and lower confluency levels indicating a larger extent of cell death. This effect was more pronounced in the CHO cells, which seemed to exhibit lower cell numbers in the presence of the YFP containing plasmid with the LT1 and 2020 reagents tested. Surprisingly, when using LF, CHO cells showed reduced cell number and confluency in both the mock and YFP transfected as further illustrated in the brightfield images shown in Figure 5.

Reduced Rate of Cell Growth in LF Transfected CHO Cells

The Ensight Multimode Plate Reader is able to monitor cell number and cell confluency using brightfield imaging without having to stain or perturb the cells. Figure 6 depicts both cell number and percent cell confluency over the 40 hour time period after transfection. While all wells containing each of the three reagents displayed similar starting number of cells (3000/well), it is clear that treatment with LF reduces the overall growth rate of the cell line, with essentially no increase in cell number or cell confluency in the 24-40 hour time range (Figure 6C), while both the LT1 and 2020 reagent-treated cells continued to grow over the entire time period (Figure 6A and 6B). This indicates that while both *Trans*IT® YFP-transfected CHO cells had lower overall cell numbers compared to the mock transfection, after an initial period of growth inhibition they recovered and were able to continue to replicate at a similar rate to the control conditions. It's possible that the expression of YFP in a shorter doubling time line such as CHO cells may have deleterious effects, due to the fact that the cells are expending resources necessary for mitosis on over-producing the exogenous protein.

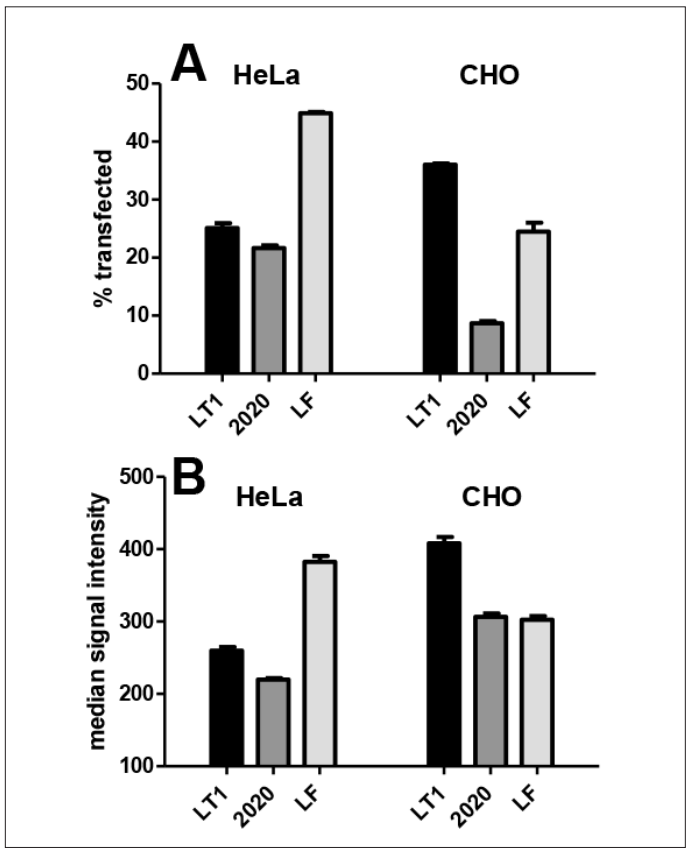

Figure 3. Transfection rates and signal intensity of YFP transfected cells. (A) Calculated percent of YFP transfected HeLa and CHO cells for each transfection reagent tested (B) Median signal intensity of YFP transfected HeLa and CHO cells for each reagent tested.

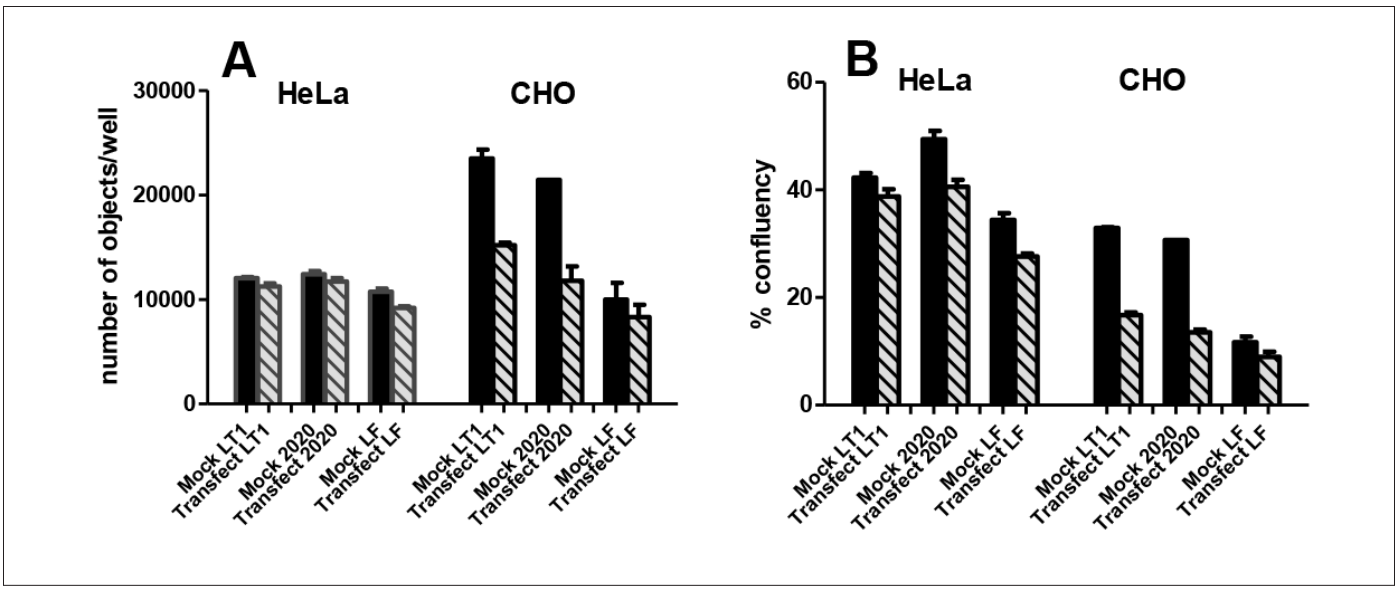

Figure 4. Number of cells and cell confluency of transfected HeLa and CHO cells. (A) Counted cells per well for both mock (black bars) and YFP transfected (striped grey bars) HeLa and CHO cells (B) Percent confluency for both mock (solid black bars) and YFP transfected (striped grey bars) HeLa and CHO cells. Data shown was measured 40h post-transfection.

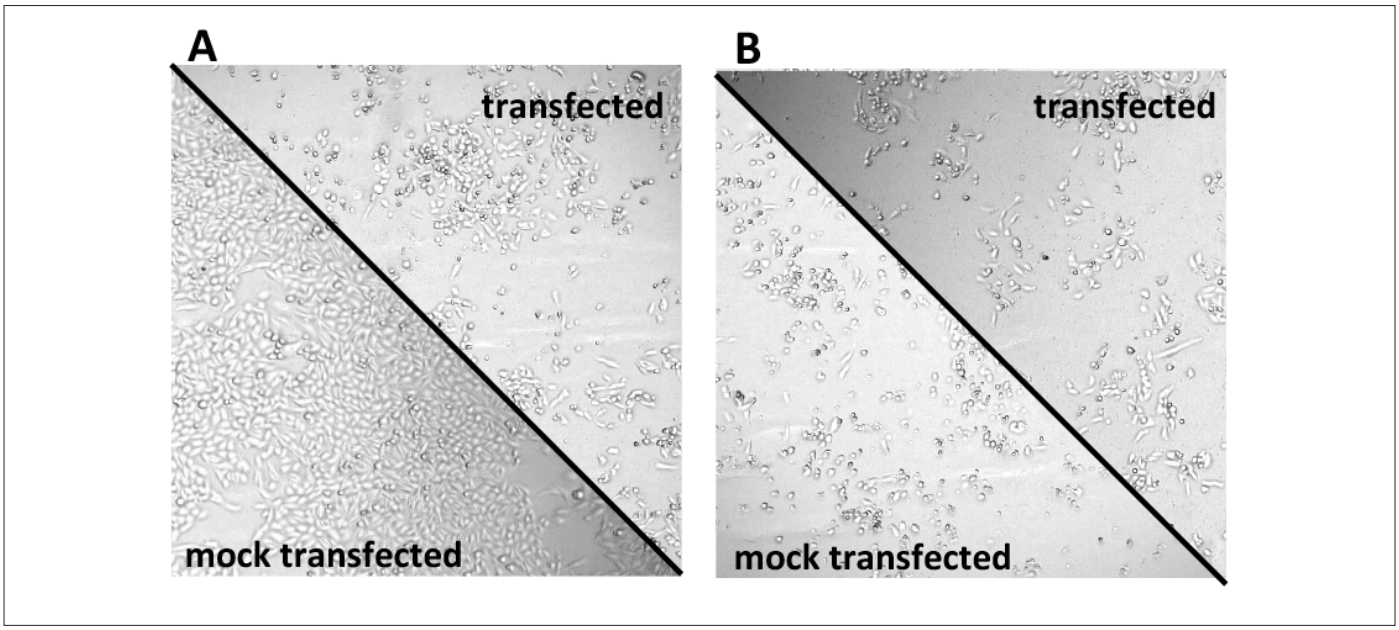

Figure 5. Representative brightfield images of mock and YFP-transfected CHO cells. Cells mock or YFP-transfected using LT1 reagent (A) or LF reagent (B).

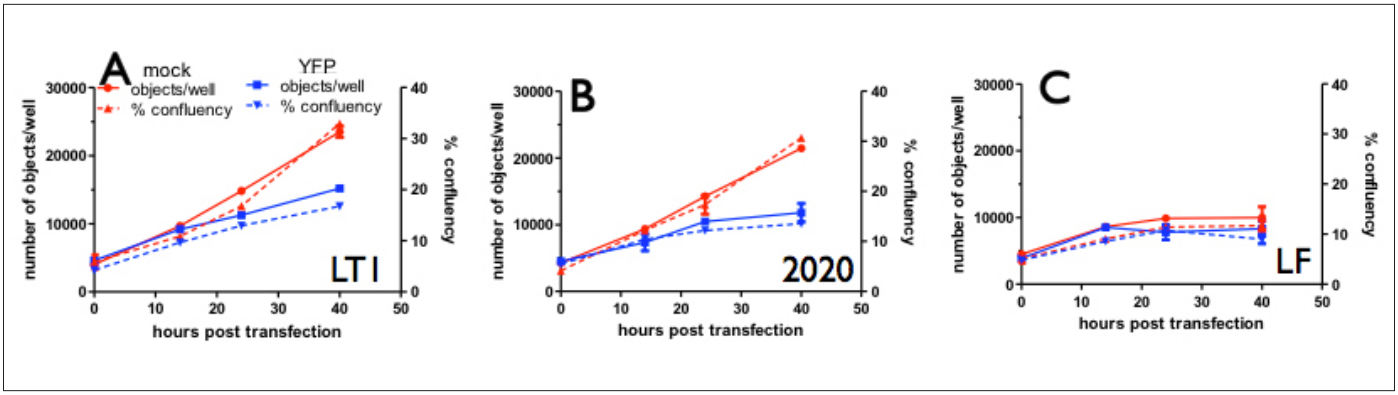

Figure 6. Cell number and cell confluency of mock and YFP-transfected CHO cells tracked over time. Number of objects per well for mock transfected (red circle, solid line) or YFP-transfected (blue square, solid line) CHO cells treated with either LT1 (A), 2020 (B), or LF (C). Percent confluency for mock transfected (red triangle, dotted line) or YFP-transfected (blue inverted triangle, dotted line) CHO cells treated with LT1 (A), 2020 (B), or LF (C).

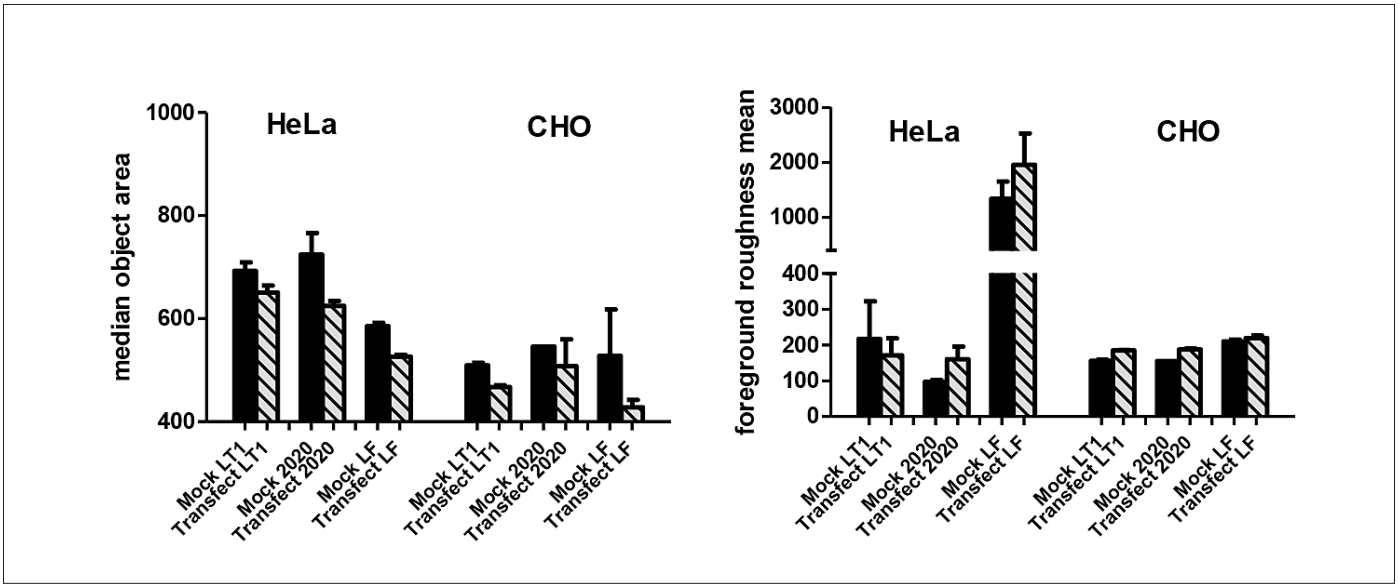

Figure 7. Object area and foreground roughness as measures of cell health. (A)Median object area values for each of the three transfection reagents tested in both HeLa and CHO cells (B) Foreground roughness values for each of the three transfection reagents tested in both HeLa and CHO cells.

Additional Analysis Output Parameters Confirm Cell Health Changes Post-Transfection

In addition to monitoring cell number and confluency, additional parameters can be used to assess overall cell health on the EnSight system. Both "median object area" and "foreground roughness mean" refer to the cell size and shape, respectively. As the cells round up, their object area decreases while as the cells become more round and uniform, the roughness parameter increases. Using these parameters in our assessment of the cells tested, it is apparent that the LF-treated HeLa cells show both a smaller object size and an increase in roundness and uniformity compared to the LT1 and 2020 transfected cells (Figure 7). This indicates that the LF reagent reduces the overall cell health of the HeLa cells. Interestingly, the LF transfected CHO cells showed a minor decrease in object size, but no change in foreground roughness compared to the TransIT® transfected cells. This demonstrates that there is little difference in cell health between all three reagents in the CHO cell line.

Conclusions

In this application note, we have demonstrated the benefit and utility of using the EnSight multimode plate reader with well-imaging capability, to monitor transfection efficiency in two different cell lines, HeLa and CHO, based on the fluorescence analysis method in Kaleido software. Using these methods, measured transfection efficiency was highest in HeLa cells using Lipofectamine 2000, while in CHO cells the highest transfection efficiency was obtained with *Trans*IT®-LT1. Simultaneous imaging in brightfield allows for the additional tracking of cell health and reagent toxicity, either at fixed end points or over longer time periods. By tracking these parameters in brightfield mode, we were able to determine that treatment with Lipofectamine 2000 caused a decline in cell health in HeLa cells and a decline in the rate of cell growth in CHO cells. Strikingly, use of the *Trans*IT®-LT1 and *Trans*IT®-2020 reagents had little effect on HeLa cell health, while all three of the reagents tested caused a decline in cell health in the CHO cell line.

The EnSight is an ideal system for the assessment of transfection methods for both transient assay systems and stable cell line generation, and may also be used for the purpose of high throughput screening using other measurement technologies such as Alpha, LANCE® or luminescence.

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