APPLICATION NOTE

Assess transduction efficiency of CFP-tagged nuclear protein with automated cellular imaging

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Introduction

Since the discovery of green fluorescent protein in the 1960s, a wide variety of fluorescent proteins (FP) and variants have been developed to span the entire visible light spectrum. These genetically encoded fluorescent proteins sparked a revolution in the field of cell biology. Cyan fluorescent protein (CFP) has garnered significant interest because of its utility in multicolor applications such as FRET. This is because the excitation and emission spectra for CFP span wavelength ranges that are rarely used, making it ideal for multiplexing with a variety of fluorophores.

Advancements in fluorescent protein technology and automated microscopy have expanded researchers' ability to study gene function and dynamic processes in living cells. Fluorescent proteins are commonly used as reporter genes fused to a gene of interest. Once the FP-tagged gene product is successfully expressed by the cells, it can be imaged in order to study the function of and track the localization of proteins, organelles, and cellular compartments.[1]

Direct visualization of the FP fusion product can additionally aid in optimizing methods for expressing the novel genetic material in cells. Transfection and transduction are two common methods for introducing nucleic acids and proteins into cells. However, each method requires detailed planning, optimization, and time, and they are both limited by how efficiently the genetic material is introduced and then expressed by the cells. These factors necessitate the use of a robust and precise method for assessing transduction or transfection efficiency.

Benefits

- Perform multicolor applications with fluorophores in the CFP wavelength range
- Analyze cells captured in brightfield
- Quantitate transduction efficiency with preconfigured analysis modules
- Generate data while acquiring images with an on-the-fly analysis

In this application note, we highlight the utility of the ImageXpress® Pico Automated Cell Imaging System and CellReporterXpress® Image Acquisition and Analysis Software for the optimization of vector concentration via evaluation of transduction efficiency. We transiently transduced two cell lines with the CellLight™ Nucleus-CFP, BacMam transduction reagent, which utilizes a BacMam vector system for delivering genetic material. Here, we describe the acquisition of images using the brightfield and CFP channels on the ImageXpress Pico, and robust transduction efficiency analysis using CellReporterXpress software. We then highlight the utility of the CFP channel for multicolor imaging.

Materials

- ImageXpress Pico Automated Cell Imaging System (Molecular Devices)
- CellReporterXpress Image Acquisition and Analysis Software (Molecular Devices)
- HeLa cells (ATCC, P/N: CCL-2)
- U2OS cells (ATCC, P/N: HTB-96)
- 96-well microplates (Greiner, cat #: 655090)
- CellLight Nucleus-CFP BacMam 2.0 (ThermoFisher, cat #: C10616)
- MitoTracker Deep Red FM (ThermoFisher, cat #: M22426)
- AlexaFluor 546 Phalloidin (ThermoFisher, cat #: A22283)

Methods

Transduction of HeLa and U2OS cells with a BacMam transduction reagent

The workflow for conducting this transduction assay with the ImageXpress Pico system is outlined in Figure 1. HeLa and U2OS cells were plated at 8,000 cells per well in a 96-well, clear-bottom microplate. The cells were incubated overnight at 37°C, 5% CO_2. CellLight Nucleus-CFP, BacMam 2.0 (BacMam Nucleus-CFP) was then added to the wells in four different particle concentrations: 25, 50, 75, and 100 particles per cell (PPC). This reagent consists of a BacMam construct containing a SV40 nuclear localization sequence fused to CFP. Each PPC concentration treatment was run in triplicate, and the cells were incubated at 37°C, 5% CO_2 for 19 hours with the transduction reagent. Prior to imaging, the media plus BacMam Nucleus-CFP was removed from the wells and replaced with live cell imaging media.

Label-free and fluorescence imaging

10X images were captured using the ImageXpress Pico system with the brightfield and CFP channels. The exposure times for the channels were 3 ms for brightfield and 1000 ms for CFP. Nine sites were captured per well, which captured 47.52% of the well. The acquisition settings were optimized for brightfield analysis by means of slightly defocusing the brightfield image in order to enhance contrast (Figure 2 and 3).

Multicolor staining

Before cell fixation in 4% paraformaldehyde, the cells were stained with MitoTracker Deep Red for 30 min and then washed once with PBS. After fixation, the cells were washed, blocked, permeabilized, and then stained with AlexaFluor 546 Phalloidin. The cells were then washed twice with PBS prior to imaging with the ImageXpress Pico system with the 40X objective and the CFP, TRITC, and Cy5 channels.

Automated image analysis of transduction efficiency

The Transmitted Light Cell Scoring, General analysis module in CellReporterXpress was run on-the-fly during the acquisition to analyze both HeLa and U2OS cells for transduction efficiency. With minimal user intervention, the analysis module was configured to quantify the cells using the brightfield channel and then score the cells positive or negative for CFP expression. Slightly defocusing the brightfield image provided optimal contrast for analysis, as seen in Figure 3, where the analysis was able to accurately count the low contrast, thin U2OS cells. Multiparametric readouts were generated from the analysis and provided a comprehensive view of the transduction experimental results (Figure 4A). These readouts included summary measurements like positive vs. negative cell counts and percentages, and all cell and positive cell area and intensities, in addition to individual cellular measurements.

Plate cells and incubate at 37°C, 5% CO_2 overnight

Seed the cells with the CellLight transduction reagent and incubate for ≥16 hours

Image on the ImageXpress Pico

Run an on-the-fly analysis in CellReporterXpress to generate and review data while acquiring images

Figure 1. *CellLight, BacMam transduction efficiency assay workflow with the ImageXpress Pico system and CellReporterXpress software. U2OS and HeLa cells were incubated with the BacMam reagent at different particle per cell (PPC) concentrations for 19 hours. Images were captured with the 10X objective, and a transmitted light cell scoring analysis module was run on-the-fly.*

Results

Comparison of transduction efficiency results in HeLa and U2OS cells

Analysis results revealed that increased BacMam Nucleus-CFP PPC concentration correlated to increased transduction efficiency, with the U2OS cells exhibiting a greater susceptibility to transduction by this BacMam system (Figure 4). The most significant increase in transduction efficiency was noticed between the 25 and 50 PPC treatments for both cell types, yet this trend was more significant for the HeLa cells. Although 50 and 75 PPC treatments didn't yield statistically different results for HeLa cells, 100 PPC increased the HeLa cell transduction efficiency by approximately 10%. Regarding the U2OS cells, this increase in transduction efficiency was not significantly different between the 50, 75, and 100 PPC treatments (Figure 4B). Each PPC concentration yielded 90% or greater transduction efficiency in the U2OS cells.

Similarly, overall expression of the CFP-SV40 nuclear localization sequence increased with increasing PPC concentration. This was assessed by measuring the CFP intensities for the positively transduced cells (Figure 4C). Individual cell data was also probed to identify characteristics of both HeLa and U2OS cells with the highest levels of CFP intensity, which were found to be cells with a small cell area and rounded appearance (Figure 5). This rounded cell morphology is characteristic of cells undergoing division, yet staining with viability, proliferation, or cell cycle markers would be necessary to confirm this.

In addition, visualization of the CFP-SV40 fusion by automated cellular microscopy revealed the localization of this inserted chimeric protein. With increasing CFP expression, we noticed increased cytosolic localization of the SV40 protein, as seen in Figure 5C. This indicated that

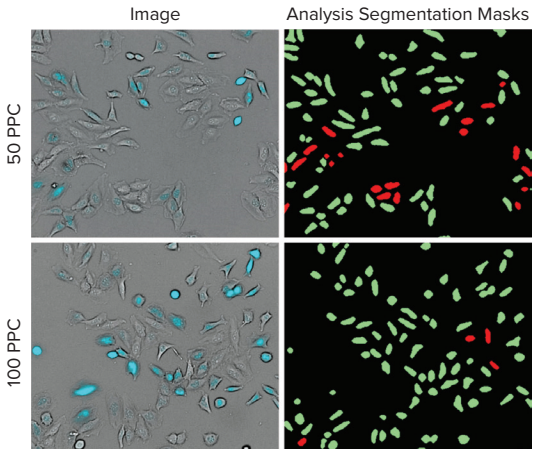

Figure 2. *Representative 10X images of HeLa cells 19 hours after the addition of CellLight Nucleus-CFP, BacMam. (Left) Images are from wells treated with 50 PPC or 100 PPC. Transduced HeLa cells (brightfield and cyan) can be clearly visualized in comparison to the non-transduced cells (brightfield only). (Right) The Transmitted Light Cell Scoring, General Module in CellReporterXpress software, was utilized to score the cells positive (green) or negative for transduction based on CFP expression.*

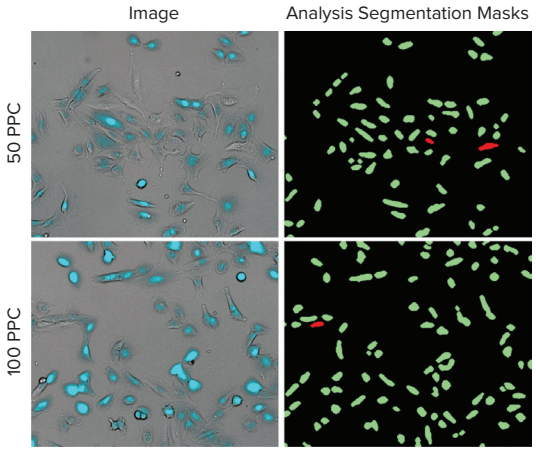

Figure 3. *Representative 10X images of U2OS cells 19 hours after the addition of CellLight Nucleus-CFP, BacMam. (Left) Images are from wells treated with 50 PPC or 100 PPC. Transduced U2OS cells (brightfield and cyan) can be clearly visualized. (Right) The Transmitted Light Cell Scoring, General Module in CellReporterXpress software, was utilized to score the cells positive (green) or negative (red) for transduction.*

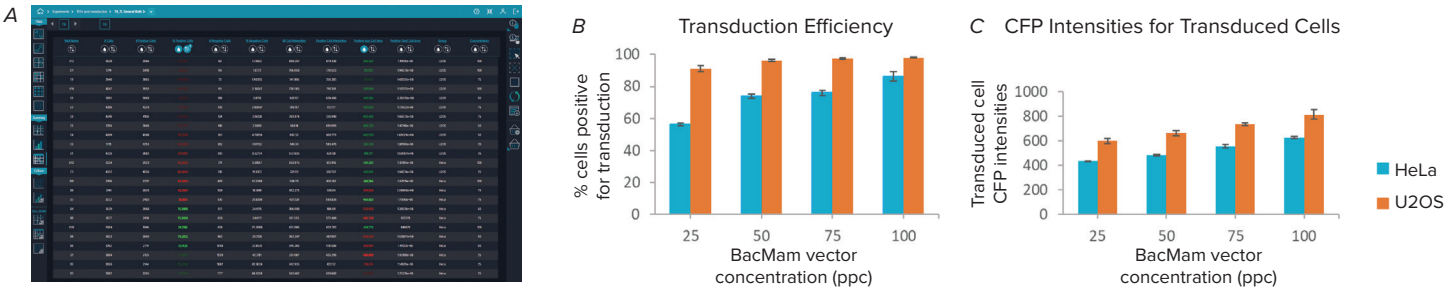

Figure 4. *Comparison of transduction efficiency results for HeLa and U2OS cells treated with varying concentrations of the CellLight Nucleus-CFP, BacMam. (A) Multi-parametric readouts were generated from the transduction efficiency analysis and measurements were displayed on an Interactive Data Table, which can be easily exported into a CSV file with one click. A heatmap was applied to the % positive cells (positive for transduction) and positive cell average area measurements, and the data table was sorted using the % positive cells measurement – the wells with the highest % positive cells appearing at the top of the table. This data demonstrated that U2OS were more easily transduced as compared to HeLa cells, as determined by the (B) percent of cells positive for transduction and (C) the positive cell CFP intensities.*

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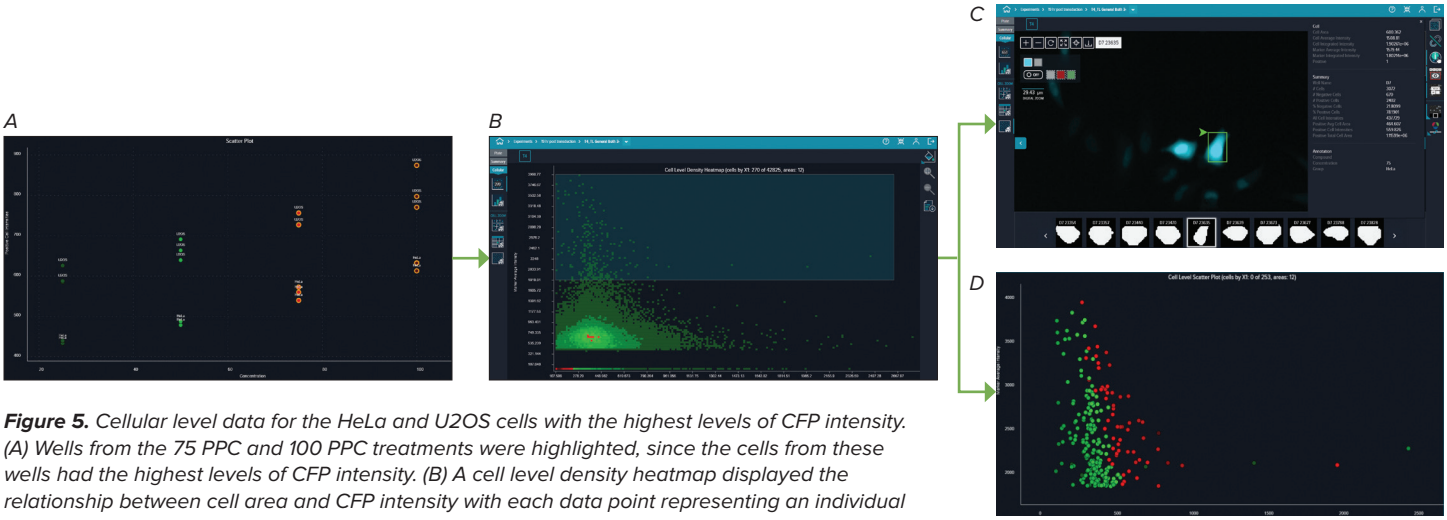

Figure 5. *Cellular level data for the HeLa and U2OS cells with the highest levels of CFP intensity. (A) Wells from the 75 PPC and 100 PPC treatments were highlighted, since the cells from these wells had the highest levels of CFP intensity. (B) A cell level density heatmap displayed the relationship between cell area and CFP intensity with each data point representing an individual cell. The cells with the highest levels of CFP intensity were selected in order to view these cells in the (C) Cell Image Gallery and in a (D) Cell Level Scatter Plot. The heatmap target in the Cell Level Scatter Plot is the CFP integrated intensities.*

there is a balance between optimal PPC concentration for highest transduction efficiency or for optimal expression and localization of the introduced genetic material. These high transduction efficiencies and the persistence of the CFP signal post-fixation further demonstrated the utility of this BacMam reagent for both live cell imaging and endpoint, fixed cell staining applications. Furthermore, the ability to image CFP labeled nuclei with the ImageXpress Pico freed the other more commonly used channels for imaging additional cellular structures stained with other fluorophores (Figure 6).

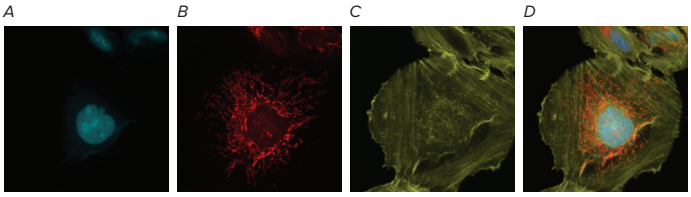

Figure 6. *Multicolor staining of U2OS cells highlighted the utility of the CFP channel when multiplexing with additional fluorophores. 40X images of U2OS cells transduced with (A) 75 PPC of the BacMam Nucleus-CFP transduction reagent (nuclei, cyan), and then stained post-transduction with (B) MitoTracker Deep Red (mitochondria, red) and (C) AlexaFluor 546 Phalloidin (actin, yellow). (D) The merged three channel (CFP, TRITC, Cy5) overlay is presented here.*

Conclusion

The BacMam reagent effectively transduced the HeLa and U2OS cells and offers a suitable alternative to more commonly used nuclear stains for live cell imaging applications. We demonstrated the utility of the ImageXpress Pico system and CellReporterXpress software in the evaluation and quantitation of nuclear transduction. With robust focusing methods and preconfigured analysis modules and data reports, the ImageXpress Pico system served as an integral tool in the assessment and optimization of transduction/transfection assay protocols. Furthermore, the ability to image CFP offers a suitable alternative to more regularly used fluorescent proteins like GFP, opening up the more commonly used fluorescent channels for the staining and visualization of additional cellular markers or structures.

Resource

1. Haney SA, Bowman D, Chakravarty A. An introduction to high content screening. Wiley. 2015.

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