

Introduction/Background

Cell transfection and transduction refer to an array of techniques used to introduce foreign genetic material, or cloning vectors, into cell genomes. The application of these methods has revolutionized biological research and industry including through the enabling of:

- The selective modification (addition/deletion/modification) of genes at a cellular level to determine the corresponding role in cell function and disease.
- The generation of genetically modified “transgenic” organisms that allow the study of gene function at the systemic level, the ability to use organism in the production of drugs, and the strengthening of crops for food production.
- The use transfected cell lines as hosts for the mass-scale production of protein therapeutics and purified proteins for use in research.
- Research, and developing clinical applications, in the prevention of disease progression through “gene therapy” treatment.



Figure 1 – Orflo's Moxi GO II – Next Generation Flow Cytometer. Image shows user loading a sample into the two-test disposable flow cell. The Moxi GO II is a configurable flow cytometer with a 488nm laser and up to two fluorescence recording channels (2 PMT's with emission filters at 525/45nm filter and 561nm/LP, respectively).

Numerous transfection/transduction approaches have been developed for introducing the cloning vectors into the cells, ranging from viral (i.e. viral transduction) to chemical (e.g. lipofection), and physical (e.g. electroporation).¹ These techniques are coupled with the use of DNA restriction endonucleases or, alternatively, the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas-9 subsystem (an RNA restriction enzyme approach) to ensure the proper insertion of the sequence into the host genome. The CRISPR approach, in particular, has received tremendous momentum over the past couple of years due to its low cost, versatility and simplicity.²

Ultimately, with all transfection techniques, there is a limit to the efficiency of the overall transfection approach. This constraint demands that the researcher verify the efficiency of their transfection efforts. One common approach is to include the DNA encoding fluorescent proteins, such as green fluorescent protein (GFP), into the cloning vector. The co-transfected GFP protein is then produced along with the target protein, acting as a “reporter” in providing a linked fluorophore, with known excitation and emission spectra, with fluorescence that can be monitored to determine the degree of transfected protein expression. This imparts the ability to not only determine the efficiency of the transfection approach but also, particularly with transient transfection, the peak gene expression time-point and corresponding decline in production. As, for transient transfection, gene expression peaks can be variable (typically 36 to 96 hours³ but can peak as fast as 6 hours⁴ in some hosts) researchers ideally need to use continual discrete measurements to monitor progression of the gene expression. That approach can be very prohibitive (both cost and time) in labs that aren't equipped with continually-operated flow cytometry instrumentation.

Orflo's newest, "Next Generation Flow Cytometer", the Moxi GO II (Figure 1), is ideally and uniquely suited to fulfilling researchers needs for apoptosis monitoring. The system combines the Coulter Principle, the recognized gold standard for precise cell sizing and counts, with simultaneous fluorescent measurements using a 488nm laser, coupled with two PMT detection channels, one filtered to detect at 525/45nm and the other at with 561nm/LP. This fluorescence configuration is ideal for many of the most common fluorophores including phycoerythrin (PE, immunolabeling, Annexin V), propidium iodide (PI for viability), Calcein-AM (Cell Health), FITC (immunolabeling, Annexin V), and GFP (transfection efficiency). The Moxi GO II utilizes a disposable flow-cell architecture, does not require warm-up, runs test in under 10 seconds, and does not require cleaning/shutdown procedures. Its low cost and very small footprint make it easy for researchers' to acquire one for their own lab, placing it on the bench-top or even the cell culture hood. This enables researchers to easily acquire temporal flow data over short or long periods. The result is an affordable flow cytometer that delivers "Assays on Demand," including transfection and transduction efficiency verification and monitoring.

GFP Transfection Data

The figures below present some example GFP transfection data from de-identified Orflo customers/partners, highlighting the utility of the Moxi GO II in this application. Specifically, Figure 2 provides examples of transfection efficiency monitoring for four separate cell lines: HEK-293 (Fig. 2, row 1), HCT-15 (Fig. 2, row 2), ID8 (Fig. 2, row 3), and LS 174T (Fig. 2, row 4). For each cell line, direct user-generated (cropped in Photoshop) screenshots are displayed showing the GFP vs. Coulter Sizing scatter/dot plot (Fig. 2, column 1), the GFP fluorescence intensity histogram (Fig. 2, column 2), an on-unit comparison of the scatter plots of the transfected vs. the non-transfected cells (Fig. 2, column 3), and an on-unit comparison of the GFP fluorescence intensity of the transfected vs. the non-transfected cells (Fig. 2, column 4). For each cell type, the Moxi GO II is able to rapidly (~10 second test time) provide precision information on percentages of transfected cells (GFP+ vs GFP-), Coulter-Principle-based cell sizing (for both transfected and non-transfected populations), and exact (Coulter Principle) cell concentrations. Beyond that, the system provides qualitative information in the histogram distribution shape such as the bi-modal GFP expression visible in the scatter plot and fluorescence histograms of the ID8 transfected cell population. Additionally, as a critical part of monitoring transfection efficiency, researchers need to compare transfected populations to non-transfected, two post-transfection time-point/states of transfected cells, or two cell populations that were transfected with differing transfection approaches. This sort of data comparison is readily enabled in seconds by the Moxi GO II through the Saved Test "Compare/Overlay" feature (examples in Fig. 2, columns 3 & 4).

Figure 3 additionally shows an example of the system applied to the comparison of the transfection efficiency of CHO cells using either polyethylenimine (PEI) or electroporation transfection approaches. In this data, the improved efficiency of electroporation (77.6% transfection efficiency, Fig. 3c) vs. PEI (7.4%, Fig. 3b) is readily observed. Such data is useful in not only determining the end-result transfection efficiency but also in iterative analysis during transfection protocol optimization. The various transfection parameters underlying each technique can be formed into a "design of experiment" (DOE) permutation format and rapidly assessed by the Moxi GO II at the researcher's hood or bench-top. This approach can also be applied to the monitoring of peak transfection expression following transient transfection.

As can be observed from both Figures 2 and 3, data is easily output for publication or future analysis in exact system screenshot (bitmap, BMP) format. Beyond that, data is also provided in the industry-accepted flow cytometry standard (FCS 3.1) for further offline analysis (e.g. FlowJo).

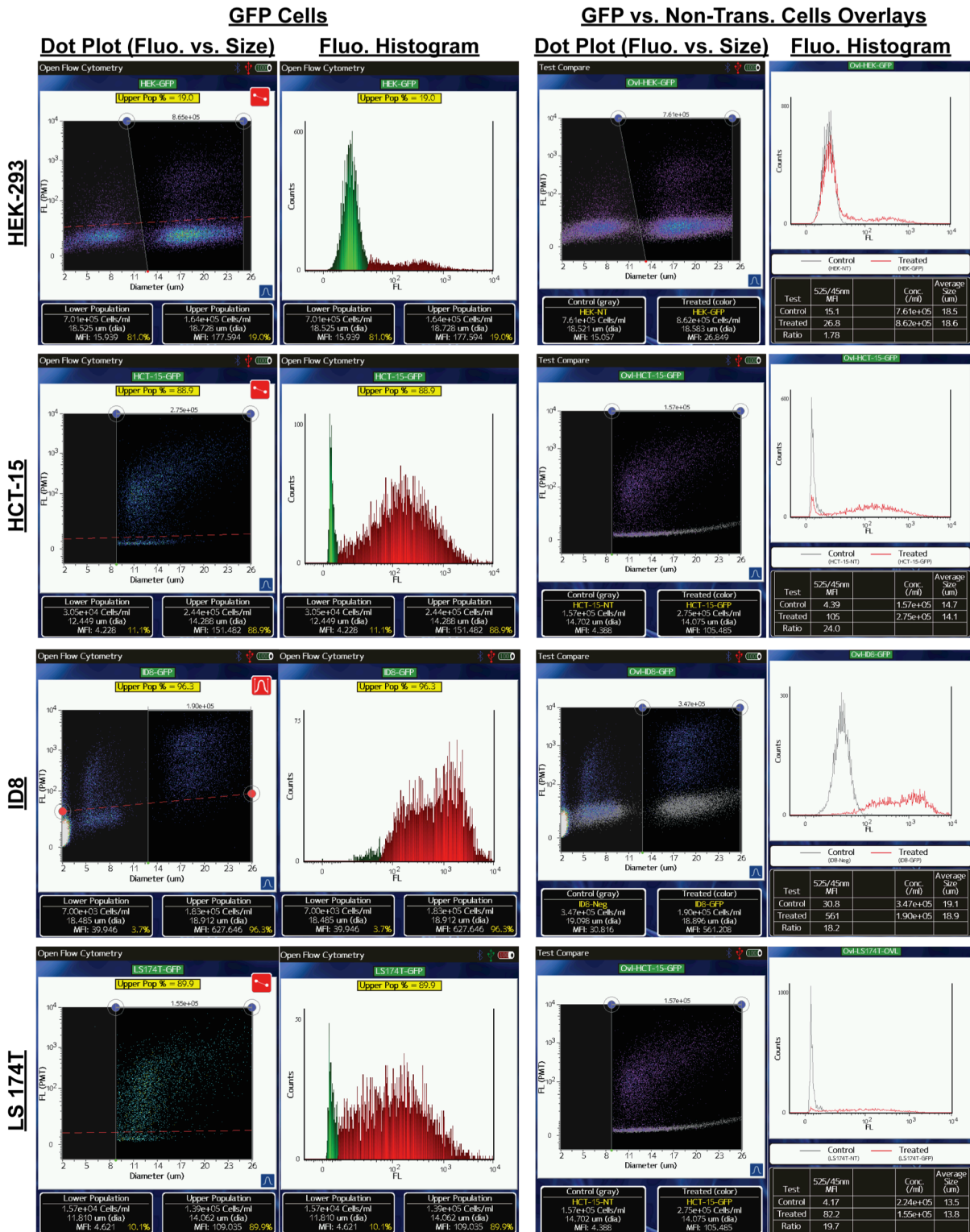


Figure 2 - Examples of transfection efficiency monitoring for four separate cell lines: HEK-293 (row 1), HCT-15 (row 2), ID8 (row 3), and LS 174T (row 4). For each cell line, direct user-generated (cropped in Photoshop) screenshots are displayed showing the GFP vs. Coulter Sizing scatter/dot plot (column 1), the GFP fluorescence intensity histogram (column 2), an on-unit comparison of the scatter plots of the transfected vs. the non-transfected cells (column 3), and an on-unit comparison of the GFP fluorescence of the transfected vs. the non-transfected cells (column 4).

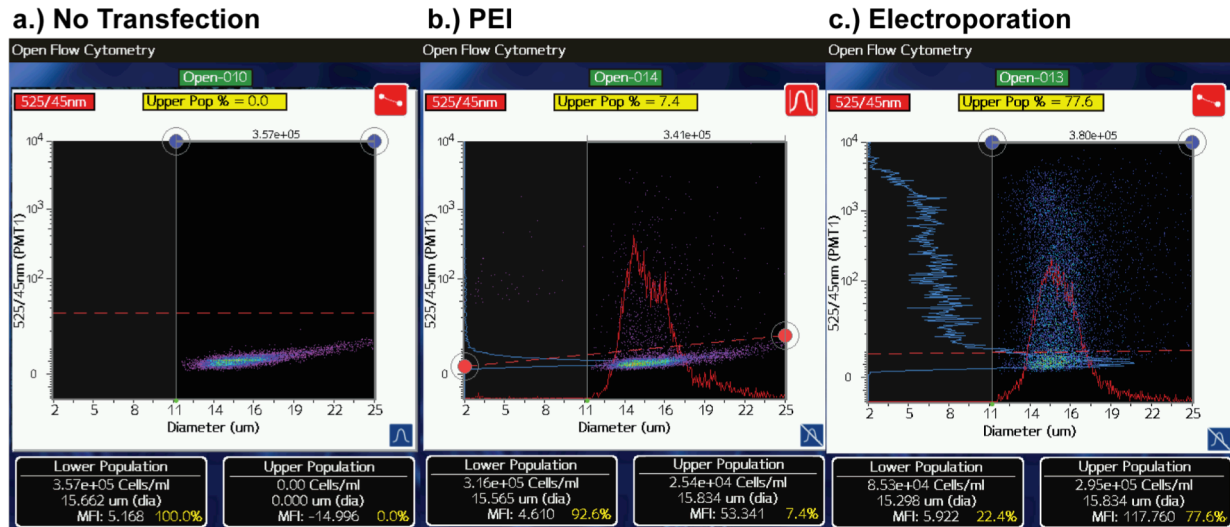


Figure 3 – Screenshots from the Moxi GO II showing scatter plot (GFP fluorescence vs. size) examples for a.) Non-transfected CHO cells. b.) PEI-transfected CHO cells c.) Electroporation-transfected CHO Cells.

Summary

In this application note, we describe the application of the Moxi GO II towards the application of transfection and transduction monitoring. De-identified customer/partner data is presented to demonstrate the utility of the system in both the quality of the data produced as well as the monitored parameters. One of the most powerful features of the Moxi GO II instrument is the ease-of-use and versatility in collection of data. With an ability to run tests without the need for system warm-up, maintenance, or shutdown procedures, the Moxi GO II is ideally suited for transfection efficiency “spot checks”, transfection protocol optimization, and transfection time course monitoring. In addition, the Moxi GO II’s small footprint enables researchers to place the Moxi GO II in the culture hood or lab bench-top, allowing for more immediate and frequent flow analysis of their systems over (potentially) long periods of time. Finally the Moxi GO II touchscreen GUI is designed to make even complex flow analysis accessible to researchers, regardless of their flow expertise. These features, combined with the system’s low price point, should establish the Moxi GO II as a staple in any lab performing transfection or transduction efficiency monitoring as well as any lab that has need for cell-based flow cytometry analysis.

References

1. Kim, T. K. & Eberwine, J. H. Mammalian cell transfection: the present and the future. *Anal. Bioanal. Chem.* **397**, 3173–3178 (2010).
2. Travis, J. Making the cut: CRISPR genome-editing technology shows its power. *Science*. **350**, 1457 (2015).
3. Assur, Z., Hendrickson, W. A. & Mancia, F. Tools for Co-expressing Multiple Proteins in Mammalian Cells. *Methods Mol. Biol.* **801**, 173–187 (2012).
4. Yee, J. & Nash, T. E. Transient transfection and expression of firefly luciferase in *Giardia lamblia*. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 5615–5619 (1995).

Transfection Efficiency Monitoring - Orflo Product List

- Orflo Moxi GO II Next Generation Flow Cytometer ([Orflo Cat #MXG102](#))
- Compatible Orflo Cassette (any of the following would work):
 - Type CST (Orflo Cat# MXC040)
 - MF-S (Orflo Cat# MXC020)
 - MF-S+ (MXC030)
- [Cat #MXA080 – Orflo Flow Reagent](#)