#2953 – Acoustic Droplet Ejection and Laser Scanning Platforms Enable High-Throughput Cell Dispensing and Quantitation

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ABSTRACT

Objective: Assess performance of acoustic droplet ejection and whole-well fluorescence imaging for quantitative high-throughput cellular dispensing and measurement.

Methods: Source plates contained CMFDA (Invitrogen)-labeled CHO cells resuspended in PBS from 1- to 2x10⁶ cells/mL. Cells were dispensed over a range of 0-400 droplets of 2.5 nL each (i.e., final dispensed volumes of 0-1000 nL) using acoustic droplet ejection (ADE; Echo® 555 liquid handler, Labcyte Inc.). The cell concentration curve was dispensed in both forward and reverse direction across the receiving plate to determine whether cells settled in the source wells over the dispensing time. The receiving 384-well plates contained 40 µL/well growth medium + 10% FCS and after dispensing, the plates were incubated at 37°C/5% CO_2. Whole-well images of the 384-well plate were acquired using a 488 nm laser scanning platform (IsoCyte™, Molecular Devices, Inc.). Total cell counts per well was determined using the IsoCyte software. A solution of saponin (Sigma Aldrich) and propidium iodide (Invitrogen) was added at 40 µL/well and the plates were scanned again to determine nuclear counts.

Results: These tests demonstrated that the cell counts per well increased linearly with increasing dispense volume with both CMFDA and PI stains. A starting concentration of 1x10⁶ cells/mL yielded about one cell per 2.5 nL droplet across all dispense volumes, with the expected doubling at 2x10⁶ cells/mL. Among 24 replicates for each dispense volume above 5 nL, the average CV for the cell counts was 19%.

Conclusions: The Echo ADE liquid handling system can be used to dispense live cells into high-density microplates in a quantitative manner. Total cell counts in each well can be rapidly determined using fluorescent dyes and the IsoCyte laser scanning platform. Some applications for high-throughput low-cell-number dispensing and fluorescent quantitation include cell health measurements, cell cloning, and RNAi studies.

DISPENSING TECHNOLOGY

Cell suspensions were dispensed by acoustic droplet ejection (ADE) with the Echo 555 liquid handler (Labcyte Inc.) The Echo platform uses focused ultrasonic energy to eject 2.5 nL droplets of liquid from 384- and 1536-well microplates into 96-, 384-, 1536- and 3456-well plates (Figure 1). Larger volumes are transferred as multiple droplets at frequencies up to 500 droplets per second. The acoustic mechanism eliminates the need for pipette tips, pin tools or nozzles. There is no contact between the ejection mechanism and the ejected sample, therefore there is no need for washing and no chance of cross-contamination. ADE saves time and money, while also improving both precision and accuracy over other low-volume dispensing technologies. ADE is especially well suited to biological applications in which precision measurements are critical and cross-contamination of samples can interfere with accurate interpretation of the results.

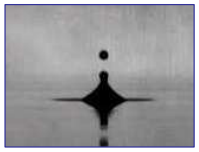

Figure 1. Stroboscopic image of 2.5 nL droplet ejection by ADE.

DETECTION TECHNOLOGY

Whole-well images were acquired with the IsoCyte laser scanning platform (Molecular Devices, Inc.) This scanning platform enables integrated image acquisition, analysis and data output from up to four channels. The results shown here used two channels to quantify fluorescent dyes discriminating live and dead cells. Whole plates can be scanned in less than five minutes, with image analysis occurring concurrently with scanning. Results can be viewed immediately after the scan. The results are saved as a list file (.csv) enumerating cell-by-cell data for each well, and a summary text file showing the number and percent of live and dead cells in a plate layout format.

QUANTITATION AND REPRODUCIBILITY

M1R-CHO cells were washed and resuspended in 1X PBS to a cell density of 2x10⁶ cells/mL. Using the Echo platform, 5-400 nL aliquots of cell suspension were dispensed into a 384-well plate containing 40 µL/well of growth medium. To quantify the number of cells per well we then added 40 µL/well of 0.02% saponin / 2 µg/mL propidium iodide and incubated the cells for 30 min. The plates were scanned on the IsoCyte scanner, with 488 nm excitation and the fluorescence emission filtered through a 600 nm long-pass filter. Results indicate that an average of two cells are dispensed in each 2.5 nL droplet (Figure 2.) Reproducibility tests yielded an average CV = 17%.

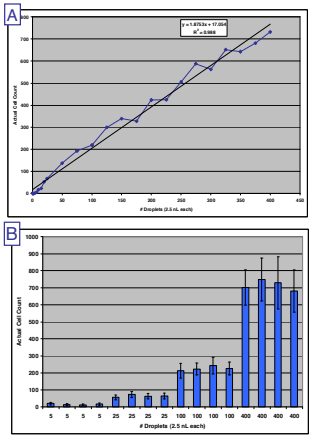

Figure 2. Quantitative fluorescence imaging reveals that approximately two cells are dispensed in each 2.5 nL droplet. A) 24-point droplet dispensing curve showing cell counts/well vs. droplets dispensed. B) Cell counts from individual source well into four replicate wells for each of four dispense volumes (5, 25, 100, 400 droplets.) Error bars represent ± 1 SD.

LIVE-DEAD ASSAY

M1R-CHO cells were washed and resuspended at 2x10⁶ cell/mL in PBS. Using both Echo and manual cell dispensing, cells were plated at 400 cells/well in 40 µL of growth medium in 384-well, black walled, clear-bottomed polystyrene plates (Greiner.) Cells were incubated for 2 h at 37°C in 5% CO_2, and then exposed for 22 h to various concentrations (0-30 µM) of staurosporine (Calbiochem.) After treatment, the cells were stained by adding 40 µL/well of 0.5 µM calcein-AM / 0.5 µM ethidium homodimer (EthD-1) solution (ToxCount Cell Viability Assay Kit, Active Motif). Live and dead cells were quantified by imaging on the IsoCyte scanner using 488 nm excitation, a 510-540 nm band-pass filter (Channel 1) for calcein-AM and a 600 nm long-pass filter (Channel 3) for EthD-1 (Figure 3.) The zero time point samples were prepared prior to adding staurosporine by staining cells with 40 µL/well of 0.02% saponin / 2 µg/mL propidium iodide. The effect on cell viability with increasing staurosporine concentration is shown in Figure 4.

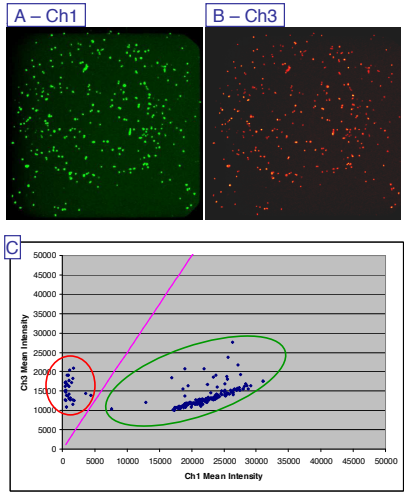

Figure 3. IsoCyte whole-well images of Echo dispensed cells stained with calcein-AM and EthD-1. A) Untreated cells in Channel 1 (510-540 nm band-pass) and B) Channel 3 (600 nm long-pass.) C) Quantitation of the live (green circle) and dead (red circle) cells based on Ch1/Ch3 ratio.

COMPARISON TO MANUAL DISPENSING

To compare the efficiency of acoustic droplet ejection to manual dispensing for live cells, we dispensed 250 nL (100 droplets) of M1R-CHO cells in 2x10⁶ cells/mL using the Echo system and 40 µL of M1R-CHO cells in 1x10⁴ cells/mL using a manual pipettor, with eight replicates of each staurosporine condition. Cell counts were comparable for both dispensing methods, while variability was less among the Echo dispensed wells (CV = 3.9%) than the manually dispensed wells (CV = 4.7%.) (Figure 4.) A similar staurosporine dose-response was observed with both dispense methods, indicating no adverse effect on cell viability from acoustic droplet ejection.

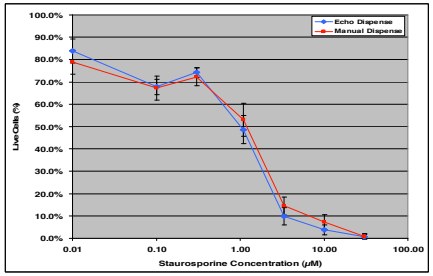

Figure 4. Percent live cells after 22 h incubation with increasing concentrations of staurosporine, quantified on the IsoCyte scanner. Results from Echo dispensing (average CV= 3.9%) are comparable to manual dispensing (average CV = 4.7%)

CONCLUSIONS

- The Echo 555 liquid handler is suitable for transferring live cells in 2.5 nL droplets for cell-based assays.
- IsoCyte laser scanning platform detects quantitative differences in cell counts over whole wells from different dispense volumes.
- A live-dead assay reveals quantitative results from staurosporine-induced cell death, demonstrating a practical cell-based application of these instrument systems.

REFERENCES

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2. *Array Production by Acoustic Ejection of Single and Multilayer Fluids and Cell Suspensions.* Mutz et al. Proceedings SmallTalk 2002.
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