TECHNICAL NOTE

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Acoustophoresis-based Cell Focusing Enables Enhanced Single-cell Dispensing

Clara Siber, Julia Scherzinger, Elly Sinkala, PhD, Lena Lautscham, PhD
CYTENA GmbH, Freiburg, Germany

Introduction

Single-cell isolation is a crucial step in cell line development (CLD) and in single-cell omics. The UP.SIGHT™ enables controlled and gentle single-cell isolation while providing multiple levels of assurance that a single cell was dispensed, including confirmation images from the nozzle during isolation and from the target well once dispensed. The UP.SIGHT also meets important user needs like high clonal recovery rates and sorting of cells by size, roundness and fluorescence signal. Cell Focusing, a new feature available with the UP.SIGHT, enables even more control over the dispensing process. The cells are aligned as they approach the nozzle where they are identified and classified before being dispensed within picoliter droplets (**Figure 1**).

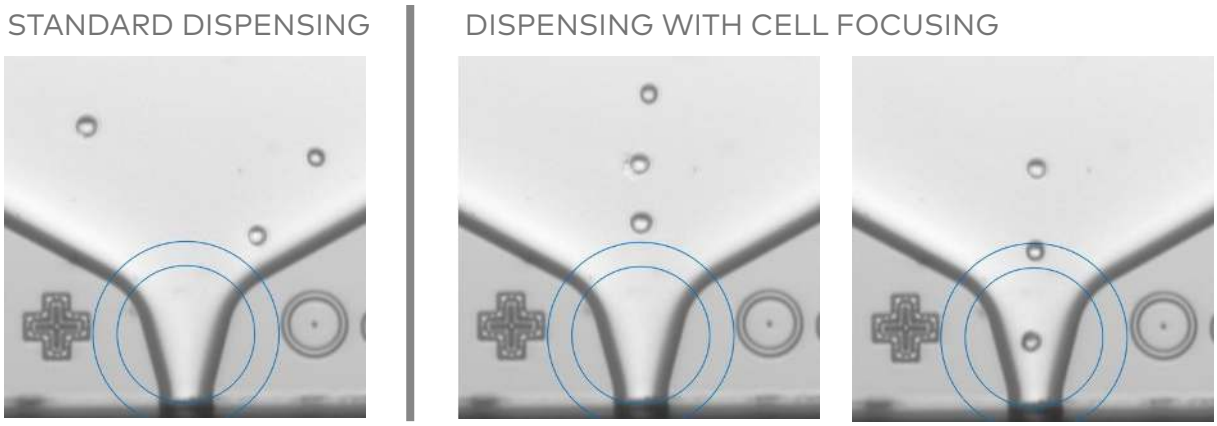

Figure 1. *Cell cam images of the nozzle of the cartridge. In the standard workflow, cells approach the nozzle from different directions (left) while they are all aligned in the middle when acoustophoresis-based Cell Focusing is applied (right).*

Cell Focusing is achieved by acoustophoresis, the manipulation of a particle's position in standing acoustic waves. Particles with a positive acoustic contrast to the surrounding fluid are driven toward the pressure node (Bruus, 2012), or in the case of the UP.SIGHT, the nodal line of the so-called λ/2-resonance of the fluid cavity of the dispense chip. In the presented system, this λ/2-resonance is formed with acoustic waves of a frequency in the range of 535–570 kHz, which are coupled into the chip. Acoustophoresis is compatible with a wide range of particle and fluid combinations and does not impact the viability of the processed cells. In the UP.SIGHT workflow, the precision of measured cell properties and the robustness of cell sorting are increased when using Cell Focusing. Additionally, this advanced feature enables dispensing under more difficult conditions, such as low cell concentrations, while decreasing dispensing time.

Increased measurement precision of cell properties

Determination of the cell size and roundness is vital information for the identification of a cell in the UP.SIGHT. Moreover, the measurement of fluorescence signal allows for sorting of labeled cells with respect to certain properties. To enable a robust sorting of the cells, a high precision of these values is desirable and can be increased by using the Cell Focusing feature, which ensures that cells reach the nozzle in a more controlled way and reduces the probability that they are located close to the edge where the measurement can be influenced by shadows (**Figure 2**).

To test the increased precision of using the Cell Focusing feature with the UP.SIGHT, bead dispensing was performed with polystyrene beads (FSDG009, Bangs Laboratories Inc.), that exhibit a constant size (15.25 µm) and roundness and fluorescence. Measurements were taken using the UP.SIGHT with and without Cell Focusing and for three different bead concentrations.

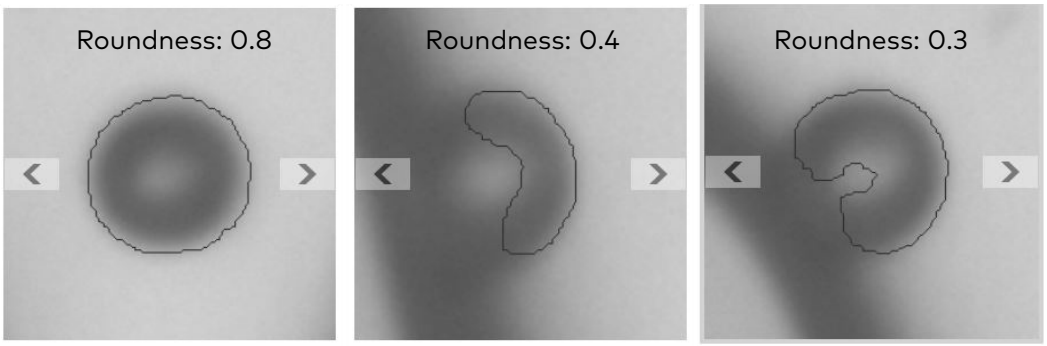

Figure 2. *Example images of bead detection in the nozzle region of the dispense chip and determined roundness of the detected bead. In the image on the left, the bead was recognized nicely, while the images in the middle and on the right show examples where the bead is located close to the edge of the nozzle and therefore is not detected properly.*

Looking at the distribution of the measured values, a single narrow peak was expected for all three parameters since all beads have the same properties. Dispensing with Cell Focusing turned on resulted in more precise measurements than standard dispensing, which can be seen by the single and more distinct peak in the probability density for all three parameters (**Figure 3**). Especially, the roundness measurement exhibited a second peak at around ~0.3 for standard dispensing because of beads positioned close to the edge illustrated in **Figure 2** which disappeared when Cell Focusing was activated. In **Figure 4**, the data points are shown in scatter plots that can help the user identify different cell populations and set the parameter limits for cell sorting accordingly. In the scatter plots obtained when Cell Focusing was activated, the cluster formation is more prominent and facilitates choosing parameter limits for more robust and efficient sorting.

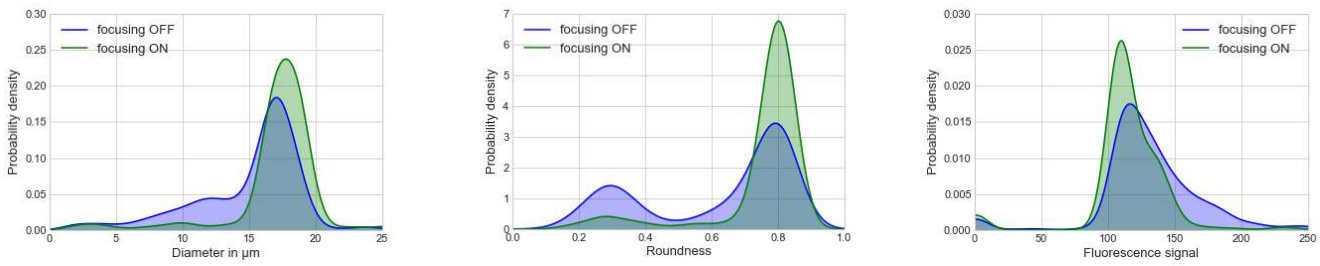

Figure 3. *Comparison of distribution of measured values for size, roundness and fluorescence signal of polystyrene beads for standard dispensing with the UP.SIGHT and dispensing with Cell Focusing. The shown probability density is calculated from >500 data points.*

Faster single-cell isolation at low concentrations

When processing samples that have a limited number of cells, such as clinical samples, the dispensing time without Cell Focusing can increase since the frequency of a single cell in the nozzle is reduced. However, Cell Focusing increases the probability of the cell being clearly detected in the nozzle area, effectively increasing the local concentration of the sample.

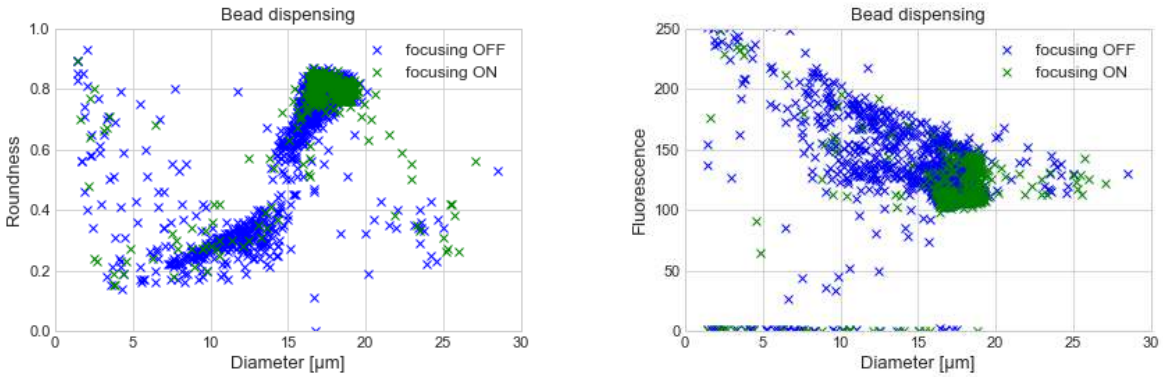

Figure 4. *Measured values for size, roundness and fluorescence signal of polystyrene beads. The distribution of the measurements is shown for standard dispensing with the UP.SIGHT (blue) and dispensing with Cell Focusing (green).*

In order to compare the time required for single-cell isolation, dispensing was performed with polystyrene beads (FSDG009, Bangs Laboratories Inc.), which served as a cell replacement model. Single-bead isolation was performed with the UP.SIGHT with and without Cell Focusing. The time needed to process a 384-well plate was measured for both cases and for three different bead concentrations.

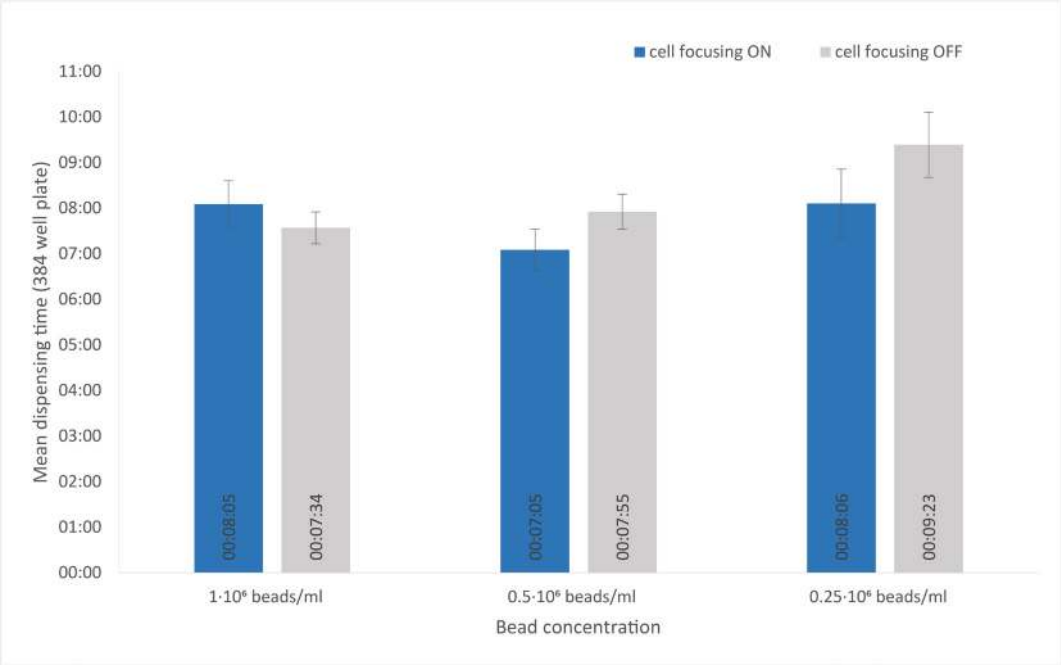

Figure 5. *Mean dispensing times for a 384-well plate with the standard workflow (gray) and a workflow with Cell Focusing (blue). The comparison is shown for samples with different bead concentrations: 1x10⁶ beads/mL, 0.5x10⁶ beads/mL and 0.25x10⁶ beads/mL. Beads with a diameter of 15.25 µm were used and served as a cell replacement model. Error bars indicate the standard deviation resulting when averaging between three different print runs. The resulting deviations are in line with the normal time/duration fluctuations expected between print runs per plate from three different printing runs in each case and fit to normal fluctuations in time needed per plate.*

The results in **Figure 5** show that the Cell Focusing feature offered faster single-cell isolation for lower sample concentrations. In this study, the shortest time of 7:05 minutes for single-bead dispensing of a 384-well plate could be achieved when Cell Focusing feature was activated at a concentration of 0.5x10⁶ beads/mL. At this bead concentration, single-bead isolation was 10.5% faster when applying Cell Focusing, and at a concentration of ~0.25x10⁶ beads/mL was 13.6% faster. For a higher bead concentration of ~1x10⁶ beads/mL, isolation was 6.8% slower. One explanation is that an increase in the effective concentration in the nozzle is achieved by bringing the beads from the slower outer streamlines near the wall toward the middle. Furthermore, with the Cell Focusing feature on, bead detection is more robust because the cells or beads are guided toward the middle of the nozzle. The probability that the measured cells size and roundness are affected by the shadows and thereby lie outside the specified range is decreased and thus even less single-cell events in the nozzle are missed.

Cell viability and outgrowth

In many applications of the UP.SIGHT, subsequent colony growth from isolated single cells is needed. Therefore, the assurance that acoustophoresis-based Cell Focusing does not have a negative impact on the viability of cells processed is of the highest interest for many users. In literature, evidence can be found that acoustic pressure waves do not affect cell viability (Wiklund, 2012). To ensure that this is also valid for the system presented here, the colony formation of single cells isolated with the application of acoustophoresis-based Cell Focusing was benchmarked to cells processed by the standard workflow without Cell Focusing on the same device. For both cases, a 384-well plate preloaded with Dulbecco's Modified Eagle Medium (DMEM) and high glucose (Sigma-Aldrich) that was supplemented with 10% fetal bovine serum (Gibco) and 1% of 10 mM Non-essential Amino Acid (NEAA) (Gibco) and 10,000 U/mL Penicillin-Streptomycin (Gibco) was dispensed, and the same sample of CHO-K1 cells (Innoprot) in phosphate-buffered saline (PBS) was used. Monitoring of the plates for colony growth was performed at day 7 after single-cell dispensing.

Workflow	Standard Workflow	Cell Focusing Workflow
Wells with colony formation in 384-well plate	78%	83%

Figure 6. *Comparison of probability of colony formation from a single cell (CHO) between the standard workflow and a workflow applying Cell Focusing.*

For the workflow with Cell Focusing, colony outgrowth was obtained for 83% of the 384 wells while the standard workflow resulted in an outgrowth of 78% (**Figure 6**). Both numbers are comparable, and the difference lies within the normal range of variation for this type of experiment, typically a standard deviation of ~3.6%. It can be concluded that the application of acoustophoresis-based Cell Focusing does not impact the viability of cells.

Conclusions

- As a feature of the UP.SIGHT, acoustophoresis-based Cell Focusing allows for better control of the single-cell dispensing process by ensuring that the cells approach the nozzle from one specific direction.
- The measurement of cell properties like size, roundness and fluorescence signal is more precise when acoustophoresis-based Cell Focusing is applied, enabling a more robust classification and sorting of cells.
- Cell Focusing can reduce the time required for dispensing single cells at low concentrations in the sample. To achieve short dispensing times, a concentration of 0.5x106 beads/mL in combination with activated Cell Focusing is recommended.
- It has also been confirmed that acoustophoresis-based Cell Focusing does not negatively impact the viability of cells processed with the UP.SIGHT.

References

1. Bruus H. Acoustofluidics 7: The acoustic radiation force on small particles. Lab on a Chip. 2012; 12(6): 1014. DOI:10.1039/C2LC21068A.
2. Wiklund M. Acoustofluidics 12: Biocompatibility and cell viability in microfluidic acoustic resonators. Lab on a Chip. 2012; 12(11): 2018–2028. DOI:10.1039/C2LC40201G.

CONTACT US

CYTENA
Neuer Messplatz 3,
D-79108 Freiburg,
Germany

Phone: +49 761 70 88 90-0
E-mail: info@cytena.com
www.cytena.com

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