Single-cell dispensing and screening of cell lines for monoclonality verification using the impedance-based single-cell dispenser and high-throughput fluorescence-based imager

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Summary

There is an unmet need in the industry for a device that allows the fast and efficient isolation of single cells while preserving their integrity and providing insurance for their clonality. Performing gene editing, single-cell dispensing, and screening to develop a stable monoclonal cell line is a long and labor-intensive process. The optimization of these steps through different approaches, such as high-throughput screening and/or automation, can increase the efficiency and yield of the monoclonal cell-line development process. In this poster, we show the impedance-based single-cell dispenser and automated fluorescence-based high-throughput imaging screening to obtain monoclonal cell lines through robust single-cell selection.

Methods

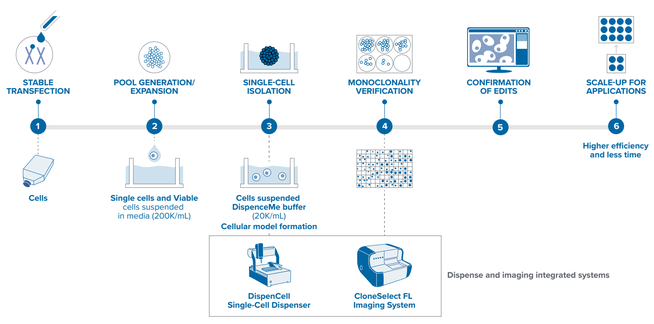
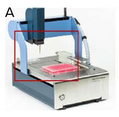


Figure 1. Cell line development workflow using DispenCell™ and CloneSelect® Imager FL (CSI-FL).

A B



C

Number of multiwell plates	Recommended loading time
1x 96 multiwell plates	30 sec
2x 96 multiwell plates	60 sec
1x 384 multiwell plates	120 sec

Figure 2. Image of a 96-well plate setup on dispenser holder being dispensed into A. and B. Plate 1 selected with a 96-well plate as the model. C. Recommended loading times for different plate types.

The workflow (Figure 1, steps 3 and 4) for single-cell dispensing, characterization of colonies, and monoclonality assessment includes an impedance-based single-cell dispenser (DispenCell™), and an automated cell imager (CloneSelect® Imager FL). Using this bundle, the cells were dispensed into 96- and 384-well plates imaged on Day 0 for double monoclonality assurance (impedance and microscopy), and later monitored regularly for growth using the fluorescence capability cellular imager. We used this workflow to isolate gene-edited CHO cell lines with GFP expression and HEK-293 cell lines(maintained as ATCC instructions) into both 96-well and 384-well plates. The DispenCell's analysis software identifies single cells and introduces optimization parameters such as time between two events and time per plate to maximize the efficiency of single-cell dispensing. The cells were isolated and imaged using the following steps.

Experimental setup

Prior to any cell sample preparation, prepared appropriate media and filter it through a 0.2µm filter to avoid particulates. Added 200µL of this media into each well of the 96-well plate and 100µL into the 384-well plate. Left the plates in the incubator at 37°C until use. Set up the instrument in the hood and link it to the computer as per the instructions provided in the manual.

Cell sample preparation

Cell culture flasks (75cm^2) with required cell lines were treated with Trypsin (2mL) for 5mins at 37°C in an incubator. The cells were then diluted with cell culture media and filtered through the 20µm (provided by seed biosciences) imaged on the countess cell counter and hemocytometer to get accurate counts of cell concentration per mL and viability. The viability was preferred to be above 90% and the cells needed to be in a single-cell suspension. The cell concentration is then adjusted to 200K cells/mL, concentration greater than 200K cells/mL would cause the time between events or cells during dispense to be lower than 3secs which could lead to higher than one cell in each well.

For non-GFP or RFP expressing cells: The cells were stained with Calcein AM with a final concentration of the dye at 250nM for 15mins. These cells were left at 37°C in an incubator and re-filtered before mixing with the dispensing buffer.

In this workflow, we used two cell lines: GFP-expressing CHO and non-GFP HEK-293 cells

Mix with DispenceMe buffer

DispenceMe buffer was provided by the kit and should be kept at 4°C before use. Gently pipetting up and down by using a wide bore tip to mix 15µL of the above-mentioned single cell suspension with 135µL of DispenceMe buffer without any delays (to prevent cell clumping). Avoid bubbles during the mixing.

DispenCell dispense

Steps on the screen were followed for loading, plate settings, and device setup prior to dispensing (Figure 2C). A threshold of 200 and above was selected based on the particle size histogram generated during the sample loading.

Tcc recommended range ~ 2–5secs

Process (plate dispense) time ∝ Tcc ∝ Process Efficiency

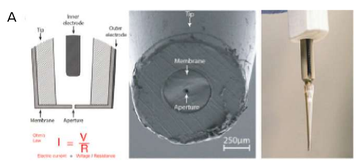
CSI-FL imaging

The dispensed plates were carefully moved to the incubator and imaged an hour later using the multi-florescence channel on the Fusion software of CSI-FL on Day 0 for cell counts and then on Day 5 for colony counts/ confluence in case of CHO-GFPs and Day 14 in case of HEK-293s. The cells expressing high amount of florescent protein or stained on Day 0 of imaging were readily detected by the CSI-FL Day 0 monoclonality report feature. We needed a manual count or verification for the cells with low expression / not readily detected in software counts.

Results

We demonstrated the feasibility of workflow using DispenCell and CSI-FL as a bundle. CHO-GFP and HEK-293 cell lines were dispensed in 96-well plates and 384-well plates. We obtained the overall single-cell deposition data from DispenCell and confirmed by image-based data for the same on Day 0 in addition, we monitored colony outgrowth using CSI-FL.

A



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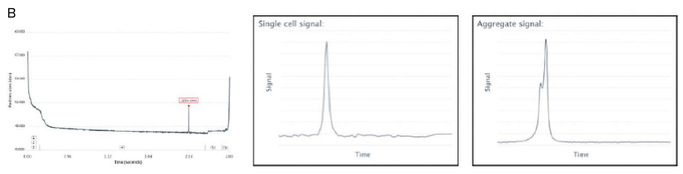


Figure 3. A. DispenCell tip with a 30µm and gold electrode with an impedance measurement feature. B. Typical peak generated when a cell passes through the tip (left) and comparison between a symmetric single cellular peak and an aggregate peak.

Results

CHO cell dispensing and imaging

The overall single-cell efficiency data for adherent CHOs into 96-well plates and 384-well plates were at least 60% and went as high as 90% with sample and TCC optimization. The impedance accuracy, also a comparison between peak information and the cell images was always higher than 70%.

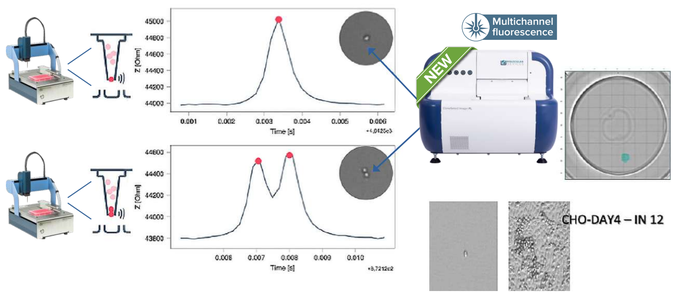


Figure 4. A pictorial representation of the peak information produced by the dispenser of two wells beside their images from CSI-FL. One with a single peak and cell (top) and one with a double peak and two cells (bottom). The single cell developed into a colony and its monoclonality report was generated using the monoclonality report feature on CSI-FL on Day 4.

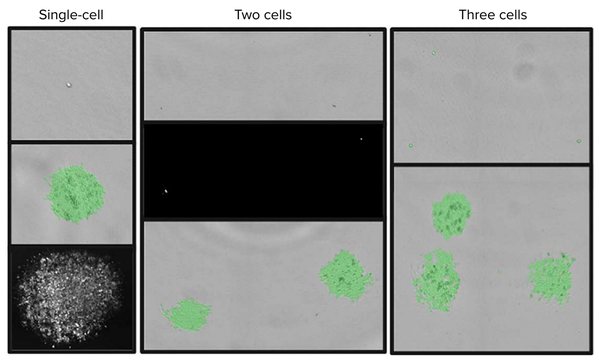
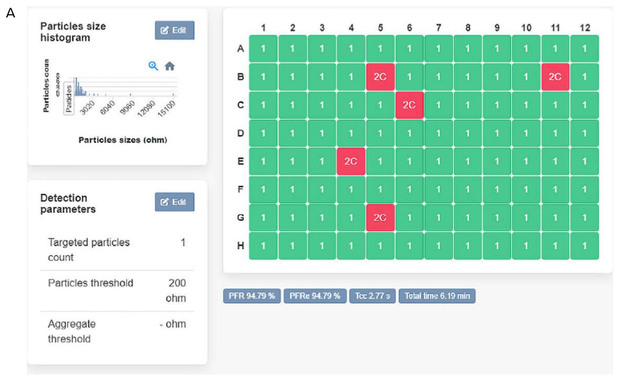


Figure 5. An example of how the fluorescence feature on the CSI helps differentiate and assure (Day 0 assurance) the presence of a cell versus debris on Day 0. The middle image with what looks like three cells in brightfield was only two cells in the fluorescence channel. These were later tracked until colony formation.

A



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	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	1	0	1	1	2	1	1	1	3	3
B	2	test	2	3	1	1	1	1	2	1	2	1
C	2	2	1	1	2	2	2	1	3	1	3	3
D	1	1	2	1	1	1	1	1	1	1	2	1
E	1	1	2	2	2	1	2	1	2	1	1	2
F	1	0	2	1	1	1	1	1	2	1	2	2
G	2	1	1	1	1	2	1	1	2	0	1	1
H	1	1	1	2	2	1	1	2	1	1	1	1

C

Instrument number	Single Cell efficiency (%)	Impedance accuracy (%)
12	69	74
37	70	75
39	66	74
Combined	68	74

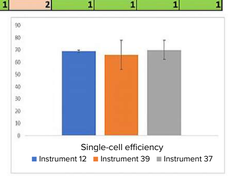


Figure 6. A. Impedance peak values generated by the DispenCell software for a 96-well plate after dispensing CHO-GFP cells and analysis B. CSI-FL Day 0 manual counts from images for the same plate using GFP detection for Day 0 count assurance. C. Averages of single-cell efficiency and Impedance accuracies of 96-well plates of individual instruments and an average of the instruments and representation of Single-cell efficiency (%) of all three instruments based on Day 0 images.

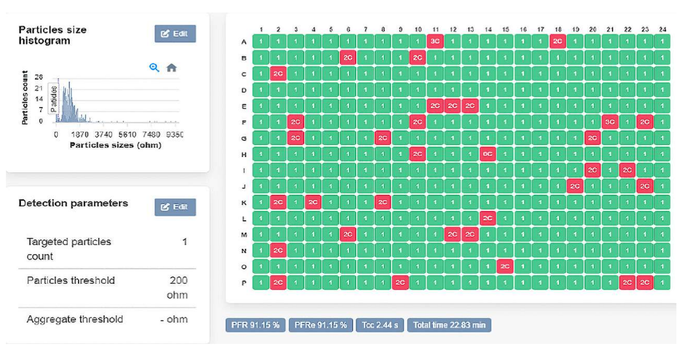


Figure 7. Impedance peak values generated by the DispenCell software for a 384-well plate after dispensing analysis

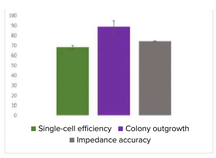
Colony outgrowth and screening

The colony outgrowth is a very important criterion in terms of cell line development. It is desired to be high as the fate of successfully edited and isolated single cells gets decided once it grows into a colony. Many a time these cells undergo stress based on the method of isolation and do not grow into a colony. We assessed the number of cells that grew into colonies on Day 5 after printing in the case of CHOs. The overall percentage of the total number of single cells effectively dispensed that grew into healthy colonies was 89% (Figures 8 and 9). Combined single cell deposition efficiency and colony outgrowth, the colonies obtained from single cell was found to be still >60% which was still significantly higher than the Limited dilutions in general.

A

Instrument number	Colony Outgrowth (%)
12	92
37	94
39	81
Combined	89

B



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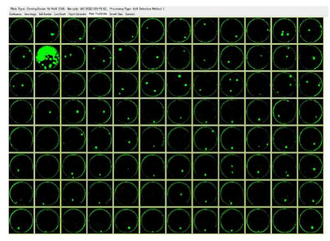


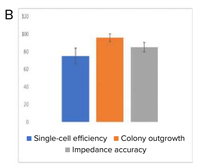
Figure 8. A. Average colony outgrowths of 96-well plates of individual instruments and an average of the instruments. B. Graphical representation of averages of all three instruments data of 96-well plates. C. CSI-FL Day 5 colony images for the same plate confirming outgrowths of the single cells dispensed.

Results

A

Instrument details	Single Cell efficiency (%)	Colony out-growth (%)	Impedance accuracy (%)
Instrument 12	83	97	89
Instrument 39	80	90	88
Instrument 37	63	100	77
Combined	75	96	85

B



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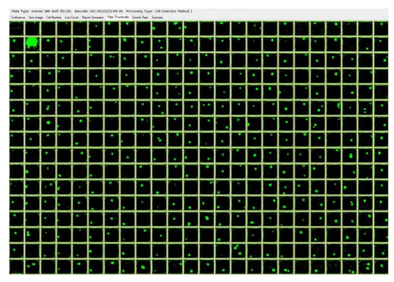

Figure 9. A. Averages of single cell efficiency and Impedance accuracies of 384-well plates of individual instruments and an average of the instruments. B. Graphical representation of averages of all three instruments data of 384-well plates. C. CSI-FL Day 5 colony images for the same plate confirm outgrowths of the single cells dispensed.

HEK-293 colony outgrowth and screening

HEK-293 cells were dispensed using DispenCell and their Day 0 images were obtained with a fluorescence channel. The cell size is slightly higher (11–15 µm) than CHOs (~10µm) and therefore needed threshold optimization based on the sample size population range.

A

B

Figure 10. A. Representation of the impedance peak data of the HEK-293 cells plate after dispense and analysis which was later imaged on CSI-FL on the very same day to get an image-based count. B. Day 0 monoclonality report generated by CSI-FL multichannel florescence imaging.

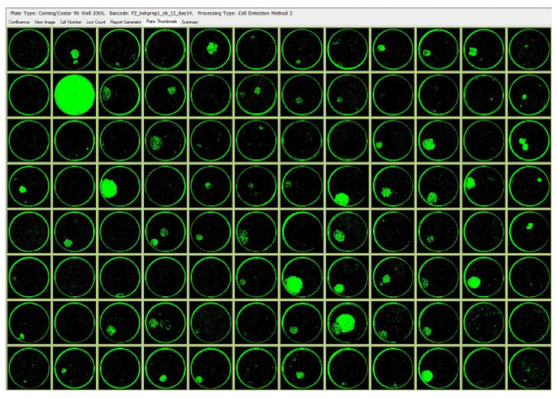

Figure 11. CSI-FL Day 14 colony images of HEK-293s confirming outgrowths of the single cells dispensed.

Limited dilution and DispenCell

We demonstrated a workflow for increasing the throughput of single-cell isolation procedures and clonal outgrowth compared to the limiting dilution method. The efficiency of single-cell dispensing by limited dilutions was at most 37%, while the efficiency of single-cell dispensing by DispenCell was greater than 60% and could go up to 90% with optimized sample preparation.

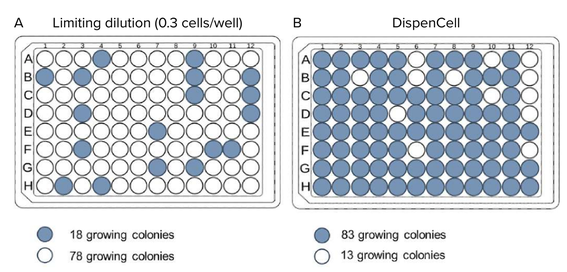

Figure 12. Comparison between the traditional method (LD) of single cell isolation with that of DispenCell. A. The single cell colonies obtained by limited dilution, B. DispenCell and C. DispenCell with an added feature of impedance-based prediction.

Conclusions and future directions

- This combination of the single-cell dispenser and CSI-FL imager workflow provides an innovative and simple impedance-based method for the isolation of intact single cells and imaging.
- The single-cell dispenser provides high efficiency and high viability over limiting dilution.
- The DispenCell and the CSI-FL can be integrated into an automated workflow using a liquid handler, an automated incubator, and a collaborative robot.
- Its incorporation into an automated platform can increase in dispensing throughput and reliability and has the potential to facilitate gene editing.
- In addition, it can generate multiple engineered cell lines that carry specific indel and can facilitate the development of several bioprocess developments or drug screening studies.

MOLECULAR DEVICES