Single cell isolation with cellenONE®

for sequencing applications

with integrated nanoliter dispenser

Low Volume, High Precision & Recovery Single Cell Isolation
For Sequencing... and Much More
cellenONE, an automated single cell dispensing system based on patented piezo-acoustic technology, allows precise cell deposition on a wide range of microplates (96, 384, 1536) and microwell substrates.

Most dispensing and microfluidic technologies follow Poisson distribution, which leads to multiple cells per position, low efficiency and biased data. cellenONE uses software-integrated visual feedback to ensure only single cells are deposited in every position.

Each drop generated with cellenONE can contain:

(A) no cell

(B) one cell,

(C) multiple cells.

cellenONE only dispenses single-cell containing drops (B) directly into microplates or microwell chips of your choice.

All remaining drops are dispensed into a recovery tube, resulting in none of the cells being lost and leaving the possibility to reprocess those non-isolated cells later.

Samples are analyzed live and pictures recorded for post processing analysis.
cellenONE can now be ordered with optional fluorescence detection setup which can be used for:

- Isolation of live single cells (Live/Dead)
  - Remove dead cells from scRNA-seq libraries
- Isolation of very rare single cells
  - Only isolate single cells of interests
- Cloning best expressers
  - Boost clonal recovery and expressers levels

Optional fluorescence detection comes in two levels:

<table>
<thead>
<tr>
<th>One channel</th>
<th>FITC</th>
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<tr>
<td>Four channels</td>
<td>DAPI</td>
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A mixture of four cell populations were labeled with Hoechst 33342 (DAPI channel), CellTracker Green (FITC channel), CellTracker Orange (Cy3 channel) and CellTracker Deep Red (Cy5 channel).

25 single cells from each cell population were successfully isolated on microscope slide with the fluorescence detection module of cellenONE.
Outstanding accuracy

Results from 5x100 positions filled with single cells from four different cells samples. Single cells rate is indicated in pink, up to 99% for A549 cells; in white are empty positions and in grey, multiple cells positions.

Unlike other technologies (microdroplet, microfluidic traps), doublets can be completely excluded and only single cells are dispensed.

Open and versatile platform

Thanks to high definition optics, cells are individually identified and isolated. These are selected according to their size, geometry and now potentially using fluorescent markers (see page 3). Once selected, cells are dispensed into microplates such as 96, 384, 1536wp or into microwells such as ICELL8™ chip.

Successfully isolated cells and particles so far:
- **cell lines** such as CHO, hybridoma, HEK293T, HeLa, A549, PC3, H1975, HepaRG, Jurkat
- **primary cells** such as PBMC (including B- and T- cell fractions), fibroblasts, keratinocytes, melanocytes, cardiomyocytes, HUVEC, neural stem cells
- **nuclei** from cell line, fresh frozen (FF) and formalin-fixed paraffin-embedded (FFPE) tissue slices
- **microbeads** like PMMA microbeads from 2 to 30µm diameters, TOYOPEARL®, PS microbeads

Key Features

- **No doublets**
- **Unseen recovery**
- **Suitable for most cell types**

Cells and particles from 2 to 70 µm
System Set up Workflow

1. **Cell Sample Preparation**
   - Place sample in holder
   - Aspirate sample
   - Prepare a minimum volume of 2 µL, ideally around 10 µL
   - Optimal cell concentration below 200 cells/µL

2. **Cell Mapping**
   - Automated cell tracking and report generation
   - Choose isolation parameters
   - Select type of target (96, 384, 1536 well. ICELL8™ chip)
   - System setup only takes a few minutes
   - Users can walk away during isolation process

3. **Automated Isolation**
   - Up to 2 microplates (or equivalent) per run
   - 100 cells in 4 min or 1000 cells in less than 45 min*
   - Walk-away and easy to operate system
   - Temperature control to avoid RNA degradation

* at optimal cell concentration
cellenONE is an open platform providing both single cell isolation and nanoliter dispensing.

Such versatility allows users to automate many of the crucial steps involved in the ever growing number of single cell library preparation methods available today. Moreover, ability to work in nanovolumes and microwells allows a drastic reduction in reagents consumption and associated costs.
In collaboration with the Department of Neurology at Weill Institute in San Francisco, a study performed aiming to isolate single immune cells in multiple sclerosis patient’s cerebrospinal fluid (CSF) samples for subsequent single cell RNA sequencing. The main challenge associated with such sample is the very low number of cells present in these samples which can vary from only 20 to 500 cells per 20mL of patient’s CSF.

To demonstrate achievable cellenONE recovery rates, a proof of concept experiment was undertaken with low concentration PBMC samples (~25 cells/µL). Three samples of 30µL were prepared, for each sample, 4 aliquots of 5 µL were pipetted into 4 wells of a 1536 wp and the exact number of cells was counted under microscope to calculate starting cell concentrations (average input or \( \bar{x} \)). Finally, for each sample, a 5µL aliquot was processed using cellenONE and as many as possible single cells were isolated into a microwell chip and subsequently counted by microscopy (output or \( \bar{y} \)).

On average, recovery reached 95%, meaning most of the cells present in each sample were successfully isolated as single cells (number of single cells successfully isolated with cellenONE over average cell number for each sample).

**Benefits**

- From minute samples (from just 2µL)
- Recovery up to 95%
- No dead volume
Single cell WGS study performed jointly at UBC and BCCRC aimed to analyze genome heterogeneity, mutational process and clonal evolution over tens of thousands of single cells or single nuclei from a variety of tissues. The author developed DLP+, a fully integrated pipeline from wet bench to analysis platform.

**Direct Library Preparation (DLP+)** is a new single cell tagmentation-based WGS method without preamplification which includes an analysis pipeline and allows clustering of thousands of cells at once.

The platform was successfully used to study lineages and tissue heterogeneity according to parameters such as genome state, ploidy, copy number alteration and single nucleotide variant.
“cellenONE technology enables the work we are doing: isolating cell and then dispensing a few nanoliters from various reagents. The system is spatially and volumetrically accurate and is able to spot each cell, one after another, into each well.”

Dr. Robin Coope,
British Columbia Cancer Genome Sciences Centre

DLP+ for efficient Whole Genome Sequencing

Direct Library Preparation Method

Benefits

cellenONE®

Low volume & reagent costs
0.27 US$/cell

High quality library

Versatile: cell and nuclei from 5 to 80µm

Open and high throughput
1000’s cell/experiments

Results

• Compatible with cell line, dissociated mouse and human tissues and nuclei from FF human breast cancer PDX tissue
• Optimized WGS protocol for nanovolume library preparation
• Open-source cloud computing pipeline for single-cell genome analytics (http://www.cellmine.org)
• Pseudobulk sequence from assembled single cell genomes successfully used as reference for clustering the same single cells
• Level genome ploidies, cell specific mitotic error rates, and cell specific replication status were successfully obtained from single cell data
**Technical information**

- Dispensing technology: piezo acoustic drop-on-demand
- Dispense volume: 50-800 pL per drop
- Linear drives for X/Y and spindle drive for Z
- Resolution: 1 µm
- Accuracy (Absolute Position): < 10 µm
- Precision (Repeat Position): < 3 µm
- HD camera for detection of cells or particles from 2µm
- Max. speed: 100 cm/sec
- Isolation area (mm): x=180; y=120 (2 microtiter plates)
- Dimensions LxWxH (mm): 650 x 700 x 1590
  - -> including monitor’s arm L = 1300 mm
  - -> with door open H = 2050 mm
- Weight: 205 kg
- Voltage: 110 V; 220 V

**Options & Software**

- Temperature, humidity and dew point control
- 2nd channel for nanoliter dispensing
- Fluorescence module up to 4 channels (DAPI, FITC, Cy3 or Cy5)
- Customized holders for microwells chips
- Fiducial recognition and automated target alignment

**Related Products & Services**

- cellenBEADS for calibration
- cellenWASH for sterilization
- cellenVIALS for recovery
- cellenSERVICEs for application support