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Spotlight

Single-cell temporal transcriptomics from tiny cytoplasmic biopsies

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In a recent issue in *Nature*, Chen et al. present Live-seq, a single-cell transcriptomic profiling method using picoliter scale single-cell cytoplasmic biopsies instead of complete cell lysis. Since the cells quickly recover and basically remain unaffected after the cytoplasmic extraction, the authors transform single-cell RNA sequencing (scRNA-seq) from an end point to a temporal analysis platform.

While the physical world is built up from elementary particles with well-defined properties, the building blocks of life are largely heterogeneous. Even the single-cell populations of cell lines are heterogeneous with a relatively wide distribution of properties depending on the actual cell cycle state of individual cells (Nagy et al., 2022). Investigating and understanding cellular heterogeneity and identifying and characterizing possible subpopulations at the tissue and cellular level are becoming increasingly important nowadays. This fundamental knowledge can lead to novel applications and solutions in health and medicine. Thanks to the recent advances of biophysical techniques, the protein, DNA, and RNA composition of individual cells can be precisely investigated with enough throughput to reliably measure the heterogeneity of large cell populations. Similarly, single-cell transcriptomics (single-cell RNA sequencing; scRNA-seq) has become especially important to characterize cellular heterogeneity and to understand biological phenomena at a deeper level (Kolodziejczyk et al., 2015). However, the technique has an important fundamental drawback: it requires the lysis of the targeted cells. Collecting the information damages the cell, making further molecular or functional analyses on the same cell at a later time point impossible (Figure 1, top).

The collaborative project between the groups of Vorholt (ETH) and Deplancke (EPFL), published in a recent issue of *Nature* (Chen et al., 2022) has now solved the above problem in an elegant way. Instead of cell lysis, they employed fluidic force microscopy (FluidFM) to take tiny, picoliter scale single-cell biopsies directly from the cytoplasm of the investigated living cell. Hence, during sample collection, the cell remains alive, quickly recovers, and can continue its life cycle basically unaffected (Chen et al., 2022).

FluidFM is most similar to the widely employed atomic force microscopy (AFM), but it uses hollow, microfabricated cantilevers connected to a liquid reservoir and pressure controller system (Li et al., 2022). The cantilever is force controlled making it ideal to approach individual cells in a gentle and reproducible manner. At the end of the cantilever either a 2–8 μm large opening (microprobe) or a sharp hollow tip (nanosyringe) can be found. The microprobes are mostly used for cell adhesion measurements (Nagy et al., 2022; Sztilkovics et al., 2020), and the nanosyringes find applications in single-cell injections (Guillaume-Gentil et al., 2014) and extractions (Guillaume-Gentil et al., 2016). In the later applications, the sharp hollow needle is thrust into the targeted cell and by employing over- or under-pressure liquid material can be injected into or extracted from the cytoplasm (or even the nucleus). Usually a pyramidal tip is used with a nanometer scale aperture on its side. This geometry allows for smooth piercing of the cell membrane with minimal clogging. Typically, the FluidFM setup is mounted on an inverted optical microscope allowing for straightforward cell selection and targeting.

In an earlier proof of principle study Guillaume-Gentil et al. (2016) had demonstrated that the cells can survive the removal of a relatively large portion of their cytoplasmic content. After extraction of 4 pL cytoplasmic material, 82% of extracted cells were observed to survive. Building on the above results, the Vorholt (ETH) and Deplancke (EPFL) groups introduced Live-seq, a single-cell transcriptome profiling approach using FluidFM cytoplasmic biopsies (Chen et al., 2022; Figure 1, bottom). Since upon RNA extraction the cell preserved its viability, the cell's ground-state transcriptome could be directly and experimentally connected to its downstream molecular or phenotypic behavior for the first time. In a typical cell extraction experiment, the authors collected cytoplasmic biopsies of about 1 pL, containing a sub-picogram to few pictograms of RNA only. Of note, the total RNA content of a mammalian cell is around 1–50 pg, depending on the cell type (Livesey 2003). Collecting and handling such tiny liquid volumes is especially challenging. The authors had to solve and optimize several technical tasks before the actual biological applications of their methodology. First, they optimized their sample collection and transfer protocols to minimize sample loss and degradation. Secondly, they aimed at maximizing the generation of complementary DNA from the extracted mRNA by optimizing the workflow of Smart-seq2 (Picelli et al., 2013), one of the most sensitive RNA-seq methods to detect low amounts of RNA. Third, they needed to develop a FluidFM probe washing process to be able to perform sequential sampling of several cells without cross-contamination.

Some questions naturally arose about the effectiveness and reliability of

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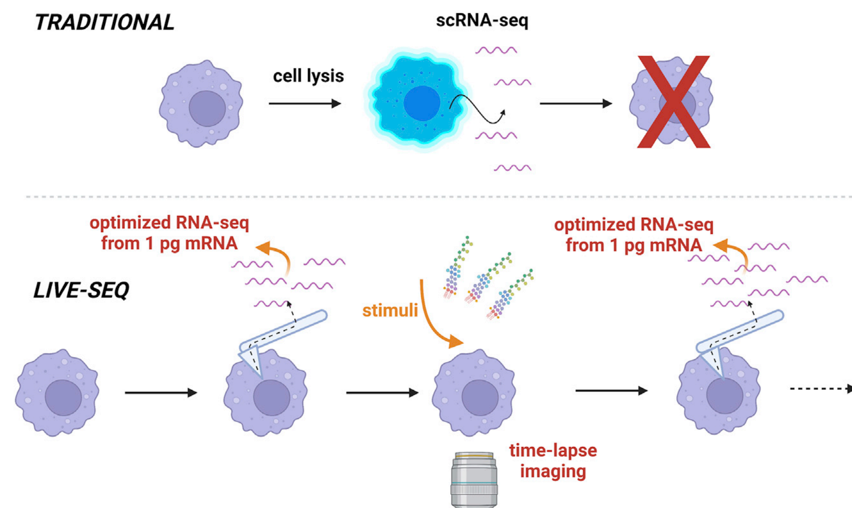

Figure 1. Schematic comparison of traditional scRNA-seq and Live-seq
After mRNA collection by FluidFM nanoextraction of single-cell biopsy, the cell remains viable and its life cycle can be followed further. Its response to external stimuli can be monitored and a next extraction with mRNA analysis can be performed in a straightforward manner. In contrast, cell lysis damages the cell in traditional scRNA-seq, making further investigation on the same cell impossible. Figure created using BioRender (https://biorender.com/)

Live-seq in relation to traditional single-cell sequencing methods and whether their optimized protocols have any impact on cellular functions. To this end, they performed parallel experiments with fully lysed cells and compared the results in detail. The authors found that cells of the same type and state highly correlated regardless of the sampling method. The results demonstrated that Live-seq enables the stratification of cell types and states similar to conventional scRNA-seq from fully lysed cells. However, slightly more cells were misassigned in Live-seq, potentially due to its lower sensitivity compared to scRNA-seq.

To study the impact of the method on cellular functions, the authors then probed the effect of Live-seq sample collection on cell viability, cell growth, cell cycle progression, and gene expression and concluded that Live-seq enables transcriptome profiling of single cells without major perturbations on the above cellular functions. However, even if they proved that the cells quickly recover their volume after taking the cytoplasmic biopsy and progress through their cell cycle, they could not rule out whether Live-seq introduces a small cell cycle delay.

In order to demonstrate Live-seq's ability to record a cell's molecular signature before and after cell state transitioning, a rapid (lipopolysaccharide [LPS] stimuli) and slow (differentiation) state transition model were employed. For the rapid model, the authors sampled RAW (macrophage-like) cells, stimulated them with LPS, and sampled the same cells a second time. All together, 24 cells were investigated with a 4 h sampling delay. Using scRNA-seq data, they proved that Live-seq correctly showed the transition from a basal to an LPS stimulated state, providing a direct, transcriptome-wide readout of a cell's trajectory. Similar, positive results were obtained in the slow state transitional model, where the second extraction was delayed by 2–7 days. Therefore, Live-seq allows the recording of transcriptomic dynamics from the same cell, providing a direct readout of cell state transitions. While macrophages (RAW cells) respond to LPS heterogeneously, no systematic, genome-wide analysis possibly driving this heterogeneity was performed. However, using the introduced method, the authors found Nfkbia expression as an important driver of LPS response heterogeneity. Moreover, using Live-seq combined with a cell-cycle indicator (Fucci), the authors discovered that RAW cells tend to respond weaker to LPS stimuli when they are in the S cell cycle phase.

In summary, Live-seq can address a broad range of biological questions by transforming scRNA-seq from an end point to a temporal analysis workflow. The presented results might help to initiate future research answering outstanding questions in cell dynamics and cellular phenotypic variation. Moreover, the authors predict that Live-seq will most probably transfer other omics technologies (proteomics, metabolomics) from the present end-point-type assay into a temporal analysis platform. Live-seq, therefore, complements other single-cell technologies available today well. However, its biggest weakness is its scalability, especially using a standard FluidFM platform where most of the processes are manually performed and strongly depend on the skills of the user. It is important to note that incubator-equipped robotic FluidFM setups are already available where targeting the cells at mm-cm scale areas in a largely automated manner is straightforward. The most advanced robotic FluidFM setup can even handle several petri dishes at the same time with the possibility of automatic probe washing and exchange. While mainly demonstrated in cell-adhesion studies (Nagy et al., 2022; Sztilkovics et al., 2020) and surface printing of functionalized polymers (Saftics et al., 2019), these automatized and robust platforms are expected to upgrade Live-seq and other related single-cell injection/extraction studies to a next level and open up novel directions in the biological applications of FluidFM.

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DECLARATION OF INTERESTS

The author declares no competing interests.

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