

Application Note

Isolation of Circulating Tumor Cells: A Model Workflow for Single-Cell Analysis

Efficient Single-Cell Picking for Mutation Analysis

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Abstract

This application note presents a model workflow simulating the identification and isolation of circulating tumor cells (CTCs) for subsequent single-cell mutation analysis. Using the HCT-116 colon cancer cell line mixed with healthy leukocytes, cells were loaded into SIEVEWELL™ nanowell arrays, semi-automatically detected with the Evident scanR imaging system, and individually collected with the MMI CellEctor cell picker. This gentle, microcapillary-based isolation preserved cell integrity and enabled successful DNA extraction and whole-genome amplification (WGA) with subsequent detection of chromosomal aneuploidies and copy number aberrations (CNAs). The workflow demonstrates a reliable and reproducible approach for studying rare target cells at the single-cell level, combining semi-automated image-based detection, visual control, and gentle isolation.

Introduction

CTCs are rare malignant cells that detach from primary or metastatic tumors and enter the bloodstream, where their presence correlates with cancer progression, metastatic potential, and poor prognosis [1, 2]. As a minimally invasive “liquid biopsy,” CTCs provide real-time insights into tumor heterogeneity, therapeutic response, and clonal evolution [3, 4]. Single-cell molecular characterization further enables high-resolution genetic analysis to support personalized oncology and treatment decisions [5].

CTCs found in peripheral blood are considered attractive biomarkers for monitoring the metastatic activity of solid malignancies. Compared to conventional tissue biopsies and

imaging methods, liquid biopsy approaches offer key advantages, including minimal invasiveness, serial repeatability, and a comprehensive representation of the tumor's molecular landscape. These properties make CTCs particularly suitable for real-time clinical therapy monitoring and longitudinal disease assessment.

Resolving Tumor Heterogeneity

Quantitative CTC analysis provides valuable information about residual tumor burden and treatment response following surgical removal of a primary tumor. However, a deeper understanding of cancer biology requires molecular characterization at the single-cell level. Solid tumors typically consist of multiple subclones that differ in their genetic and phenotypic features—an underlying cause of therapeutic resistance and disease relapse. Conventional bulk assays, which average signals across mixed CTC populations, risk overlooking small, but clinically relevant subpopulations such as tumor cells with stem cell-like properties which drive metastasis. Precision oncology therefore aims to resolve tumor heterogeneity, both spatially and temporally, through single-cell molecular profiling, enabling the development of personalized therapeutic strategies. Such analyses may include multi-omics investigations at the DNA (genome), RNA (transcriptome), and protein (proteome) levels [6, 7], as well as functional assays such as cell culture and drug testing, [8]. A prerequisite for these studies is the use of suitable technologies for efficient and gentle single-cell isolation.

Challenges and Solutions in CTC Isolation

Isolating individual CTCs for downstream analysis requires specialized techniques, each with unique strengths and cell

limitations. Fluorescence-activated cell sorting (FACS) allows high-throughput processing of thousands of cells per second and is highly automated. However, it generally demands large cell suspensions (millions of cells), provides limited visual confirmation, and can compromise CTC integrity due to electrical charges and shear forces. All of this potentially affects subsequent analysis. Alternative approaches, such as micromanipulation or microfluidic-based capture, often suffer from low throughput, limited automation, or reduced flexibility.

An ideal single-cell isolation technology should combine high efficiency, purity, and cell viability with speed, and visual confirmation. A gentle yet effective solution can be achieved through a microwell-based cell picking approach. In this setup, target cells are loaded into miniaturized wells of micrometer dimensions, semi-automatically detected using dedicated imaging software with AI-assisted pattern recognition, and subsequently isolated with a microcapillary-based picking system. This strategy ensures gentle handling to preserve cell integrity for high-quality downstream analyses, provides full visual control, reduces processing time, and is particularly suitable for rare or small cell populations.

HCT-116 as a Model System for CTC Isolation

The HCT-116 colorectal cancer cell line can be used as a model system for CTCs studies. While not actual CTCs, these cells are widely employed in spiking experiments due to their well-characterized epithelial features and clear marker expression. This makes them ideal for validating single-cell isolation workflows and assessing the efficiency of CTC isolation technologies.

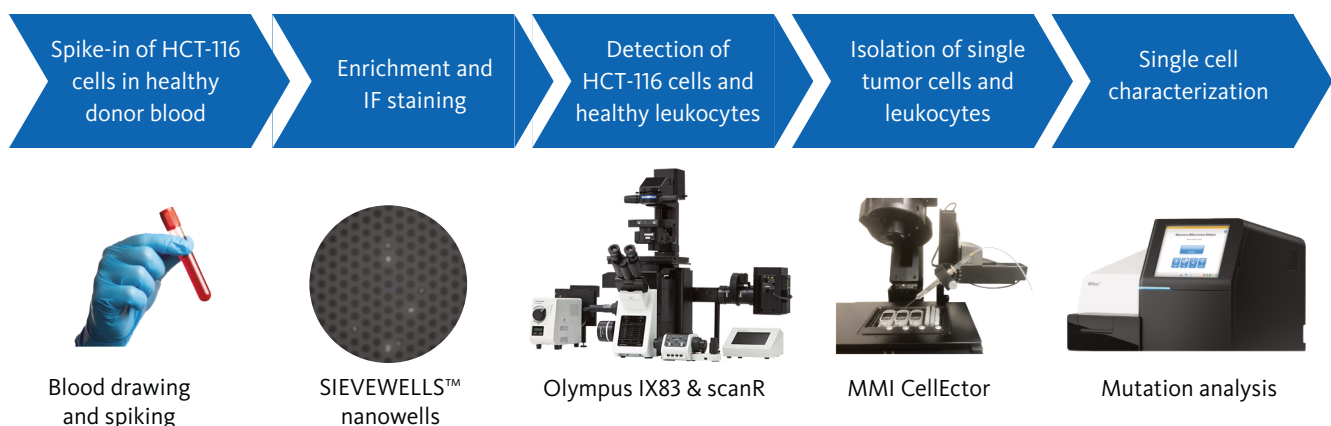


Fig. 1: Workflow used in this study for the detection and isolation of tumor cells, followed by subsequent mutational analysis.



Scope of This Application Note

In this Application Note, we present a robust, complete workflow for the identification, isolation, and single-cell mutation analysis of CTCs. Using the HCT-116 colon cancer cell line as a model system, we simulated CTC isolation by spiking HCT-116 cells into blood of healthy donors, followed by immunocytochemical staining. The cells were loaded into SIEVEWELL™ nanowell arrays and semi-automatically detected with the Evident scanR system based on staining patterns. Individual cells were then isolated using the MMI CellEctor, a precision cell picker that enables non-destructive isolation under full visual control. This workflow preserved cell integrity, enabling successful DNA extraction and whole-genome amplification on a single cell level, thereby demonstrating its suitability for downstream CTCs genomic applications.

Material and Methods

Cultivation of HCT-116 cells

According to [9], suspensions of HCT-116 tumor cells were cryopreserved in ~1 ml aliquots using liquid nitrogen and thawed in a 37°C water bath before use. Thawed cells were transferred into a 15 ml conical centrifuge tube containing 9 ml of pre-warmed culture medium and centrifuged at 125 g for 6 min. After discarding the supernatant, the cell pellet was resuspended in 15 ml of fresh medium, seeded into 75 cm² flasks, and incubated at 37°C with 5% CO₂.

At ~80% confluence, cells were subcultured. The medium was removed, and adherent cells were washed twice with 5 ml of pre-warmed PBS. To detach the cells, 2 ml of pre-warmed trypsin-EDTA was added and incubated for 3 min at room temperature. Detached cells were transferred to a 15 ml conical centrifuge tube containing 8 ml of culture medium, centrifuged at 200 g for 5 min, and resuspended in 10 ml of fresh medium. One milliliter of this suspension was transferred to a new culture flask and supplemented with 14 ml of fresh medium, then incubated until the next passage.

The remaining cells were centrifuged (200 g, 5 min) and the supernatant was removed. The pellet was resuspended in an equal volume of PBS wash buffer and used within 5 h at room temperature. After subculturing, a cell concentration of approximately 5×10^6 cells/mL with >95% viability was achieved. The concentration was determined using an automated cell counter [9].

Donor Blood Collection and Spiking with HCT-116 Cells

Venous blood was collected from voluntary healthy donors into standard EDTA tubes. Samples were anonymized and stored for a maximum of 2 days at +4°C until further use. HCT-116 cells were added to the donor blood at ratios typically ranging from ~1:2 to 1:100 (tumor cells to leukocytes).

Erythrocyte Lysis

Selective lysis of erythrocytes was performed by treating 1 ml of spiked blood with 9 ml of ammonium chloride. The mixture was gently mixed and incubated for 10 min at 4°C. Samples were then centrifuged for 10 min at 700 g and 4°C, and the supernatant was removed. The pellet was resuspended in 10 ml of PBS to achieve a final concentration of approximately 500,000 leukocytes per ml.

Cell Loading into SIEVEWELLS™ Nanowells

A total of 400 µl of the cell suspension was loaded onto a SIEVEWELL™ nanowell slide (SWS 2001-5, 370,000 nanowells, width 20 µm). The total cell number was kept below 200,000 to prevent clogging. This procedure followed the workflow outlined in the SIEVEWELL™ Slide User Guide [10].

Immunofluorescent Labeling

HCT-116 cells were labeled with a PE-conjugated anti-cytokeratin antibody (REA831, Miltenyi Biotec) at a 1:50 dilution, while leukocytes were stained with an APC-conjugated anti-CD45 antibody (REA747, Miltenyi Biotec) at the same dilution. In addition, cell nuclei were stained with Hoechst 33342 (5 µg/ml), which is essential for the identification of nucleated cells during segmentation.

HCT-116 and Leukocyte Detection with Evident scanR

HCT-116 cells and healthy leukocytes were detected using the Evident scanR screening station, a high-content imaging and analysis platform designed for automated and flexible fluorescence screening.

The following steps were taken to prepare for cell detection:

- I. The plate layout for the SIEVEWELLS™ nanowells was defined in the plate manager.
- II. An appropriate scan range was selected to ensure optimal autofocus during image acquisition.
- III. The required color channels were chosen: brightfield (BF), Cy5 for CD45-positive leukocytes, Cy3 for cytokeratin-positive HCT-116 cells, and DAPI for cell nuclei.

HCT-116 cells and leukocytes were identified as described below: (Fig. 2):

- I. Recognition and segmentation of cell nuclei in the DAPI channel.
- II. Setting appropriate thresholds for the Cy3 (CK-PE) and Cy5 (CD45-APC) channels to exclude background noise and low-intensity artifacts.
- III. Definition of regions for CD45-positive (leukocytes) and CK-positive (HCT-116) cells.
- IV. Manual review of the detected leukocytes and HCT-116 cells, followed by importing their positions into the MMI CellTools software for the cell-picking procedure.

Image acquisition was carried out using a 10× X-Line air objective (UPLXAPO). Further details on working with the scanR system are available in the Evident scanR manual [11].

Single-Cell Picking Using MMI CellEctor

The coordinates of the identified HCT-116 cells and leukocytes were first imported into the CellTools software of the MMI CellEctor, using the stage layout shown in Fig. 3. To prepare for the single-cell picking procedure, the hydraulic system was filled with dyed mineral oil (Sudan Blue), and a 30 µm diameter capillary was mounted. The oil was manually dispensed until it emerged from the tip. An inlay containing 100 µl reaction tubes (two strips of eight tubes each) was then inserted. The capillary with tubing was subsequently filled with approximately 45 µl of washing solution consisting of 0.05% Tween in PBS. The experimental setup is shown in Fig. 4.

For cell picking, the capillary was positioned at the coordinates of the target cells on the SIEVEWELL™ slide, and 25 nl was

aspirated. Cell picking was performed in the BF channel. The appearance of the HCT-116 cells in both BF and DAPI prior to picking is shown in Fig. 5. The cell was then deposited into a 100 µl reaction tube along with approximately 1 µl of solution (Fig. 6). Detailed instructions on operating the MMI CellEctor can be found in the corresponding manual [12].

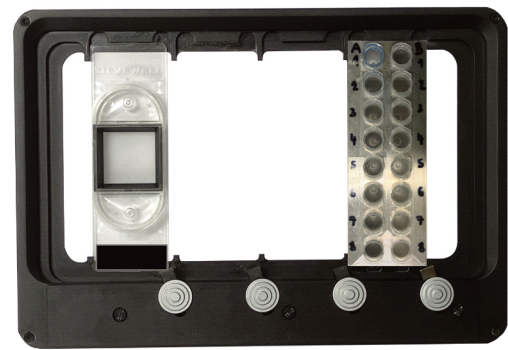


Fig. 3: Stage layout for the isolation of HCT-116 cells and leukocytes. Left: SIEVEWELL™ nanowell. Right: inlay with two strips of 100 µl reaction tubes.

Single-Cell Mutation Analysis

Single-cell analysis of the collected cells was performed using the Ampli™ WGA Plus Kit and the Ampli™ LowPass Kit from Menarini. The analysis of the genome of individual cells begins with whole genome amplification (WGA) and concludes with the detection of chromosomal aneuploidies and copy number aberrations (CNAs) via low-pass whole genome sequencing.

Detailed protocols for performing the analysis using the Ampli™ WGA Plus Kit and LowPass Kit are provided in the respective user manuals [13, 14].

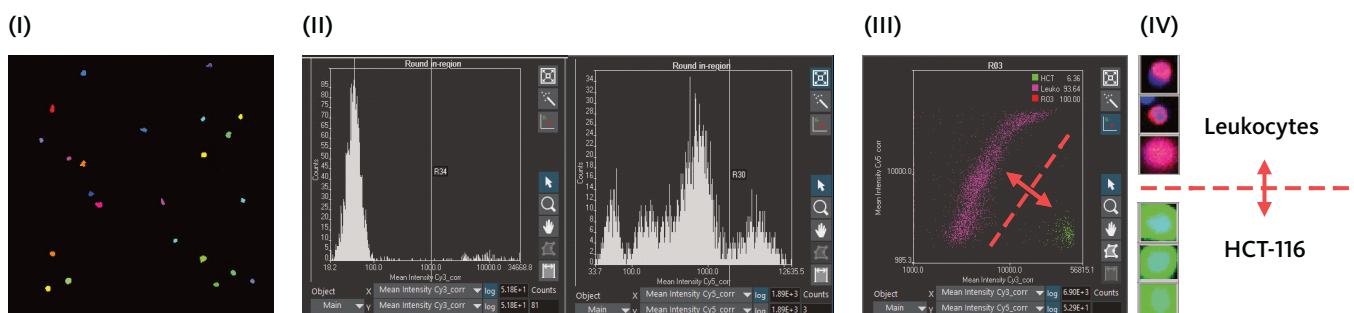


Fig. 2: Workflow for HCT-116 cell identification. (I) Recognition of cell nuclei in the DAPI channel; (II) Setting thresholds for the Cy3 (CK-PE) and Cy5 (CD45-APC) channels; (III) Definition of regions for CD45-positive leukocytes and CK-positive HCT-116 cells; (IV) Manual review of the detected leukocytes and HCT-116 cells.

Results

Detection of HCT-116 Cells and Leukocytes

The detection of healthy leukocytes and spiked HCT-116 cells using the Evident scanR system worked efficiently. Distinct regions for the two cell types were clearly identified through staining with CK-PE for HCT-116 cells and CD45-APC for leukocytes. The additional DAPI staining enabled reliable recognition of nuclei, which facilitated the seamless import of cell positions into the MMI CellTools software for subsequent cell picking.

The use of SIEVEWELL™ nanowells ensured that each target cell was precisely located in a single nanowell, preventing the presence of multiple cells in one well. This setup simplified the transfer of cell positions to the CellEctor cell picker and ensured that the cells remained stable within the wells during the isolation process.

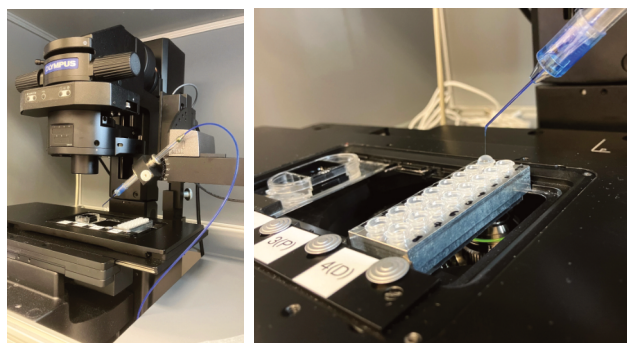


Fig. 4: Experimental setup for single-cell picking. HCT-116 cells and leukocytes were gently picked using a 30 µm capillary with the MMI CellEctor mounted on an Evident Olympus IX83 inverted microscope.

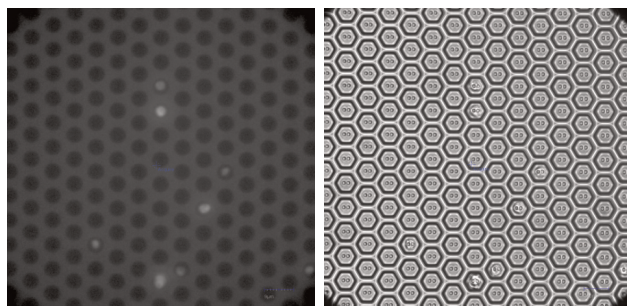


Fig. 5: Stained HCT-116 cells in the nanowells of the SIEVEWELL™ slide (40x magnification). Left: DAPI channel – imaging mode required for the identification of nucleated cells for cell segmentation. Right: Brightfield imaging mode used for cell picking.

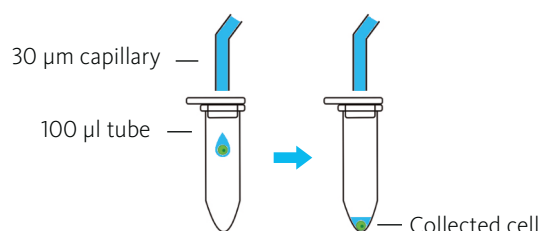


Fig. 6: For deposition, single cells were isolated directly into a 100 µl tube along with ~1 µl of solution.

Single Cell Picking

The picking procedure using the MMI CellEctor proceeded smoothly following the transfer of cell positions. Individual cells (both HCT-116 and healthy leukocytes) were successfully isolated into 100 µL tubes with a collection efficiency of nearly 100%. The gentle isolation process preserved cell viability, making the cells suitable for subsequent single-cell mutation analysis.

Copy Number Alterations in Leukocytes and HCT-116 Cells

Whole genome amplification using the Ampli™ WGA Plus Kit was successfully performed on picked healthy leukocytes and HCT-116 cells, followed by low-pass whole genome sequencing to assess chromosomal aneuploidies and CNAs. Healthy leukocytes exhibited predominantly diploid copy number profiles, with only a few low-amplitude deviations, consistent with genomic stability. In contrast, HCT-116 cells displayed widespread copy number variability across the genome. Multiple regions showed markedly increased or decreased copy number, indicating both gains and losses of chromosomal material. Notably, chromosomes 8, 10, and 17 exhibited elevated copy number estimates, while the X chromosome showed a pronounced reduction, reflecting substantial chromosomal instability (Fig. 7).

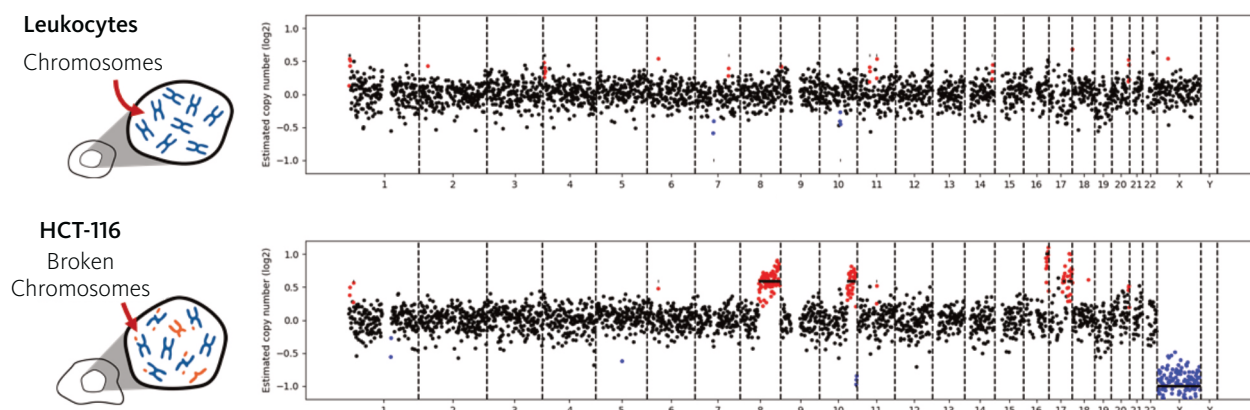


Fig. 7: Comparison of copy number profiles between healthy leukocytes and HCT-116 cells. Leukocytes display mostly diploid copy numbers with few minor deviations, whereas HCT-116 cells show widespread gains and losses across multiple chromosomes, indicating pronounced chromosomal instability.

Conclusion

The detection of healthy leukocytes and HCT-116 cells using the scanR platform worked reliably. The SIEVEWELL™ nanowells proved highly suitable for confining single cells. The transfer of coordinates for cell picking with the MMI CellEctor was smooth. The subsequent cell picking achieved high collection efficiency. Single-cell CNA analysis revealed clear differences between the picked leukocytes and HCT-116 cells, demonstrating that the CellEctor picking process minimally affects cell viability and thus enables downstream single-cell applications. Overall, this model study with HCT-116 tumor cells shows that the combined detection and picking workflow is robust and can be readily applied to the detection and isolation of CTCs for single-cell analyses.

Key Findings

- HCT-116 cells are a suitable model system for the isolation of CTCs.
- The MMI CellEctor cell picker is highly effective for CTC/HCT-116 cell isolation due to its gentle handling and high collection efficiency.
- Picked cells are ideally suited for single-cell downstream applications, such as mutation analysis, producing reproducible and meaningful results.

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