SmartBioSurface® Slides

High-Efficiency Single-Cell Laser Microdissection

of Non-Adherent Cells for Genomic Analysis

The Challenge of Single-Cell Microdissection of Non-Adhering Cells

Single-cell Laser Capture Microdissection (LCM) is a pivotal technology for high-resolution genomic, transcriptomic, and proteomic profiling, enabling the study of cellular heterogeneity.

This task remains technically challenging for **non-adherent cell populations, such as Peripheral Blood Mononuclear Cells (PBMCs), rare cells, or cultured suspension cells**, due to their lack of natural affinity for standard microdissection membrane slides.

High-fidelity single-cell analysis necessitates a balance between **stable immobilization** and **efficient target recovery**. Utilizing robust immobilization on slides, paired with a mechanism for gentle detachment, is critical to mitigate sample loss and preserve the **molecular integrity** required for precise downstream profiling.

The most prevalent technique for this purpose is **cytocentrifugation (cytospin)**, which utilizes centrifugal force to deposit PBMC or non-adherent cells directly onto polyethylene naphthalate (PEN) or polyphenylene sulfide (PPS) membrane-coated slides [1].

Despite its prevalence, this method is limited by **low recovery yields**, which is a critical bottleneck when analyzing rare cell populations. Furthermore, the **mechanical shear** associated with centrifugation can induce structural damage, **potentially compromising the biological integrity** of the sample and the quality of downstream analytical evaluations.

Consequently, **maximizing recovery and preserving morphological integrity** remains a primary technical challenge in the analysis of non-adherent and rare cellular samples; furthermore, enabling **high-efficiency and precise single-cell detachment** from the microdissection support is a critical prerequisite for ensuring that the isolated material remains compatible with high-sensitivity downstream molecular profiling.

The Solution: SmartBioSurface® slides

SmartBioSurface® slides utilize a proprietary nanostructured coating of titanium dioxide (TiO_2) nanoparticles [2].

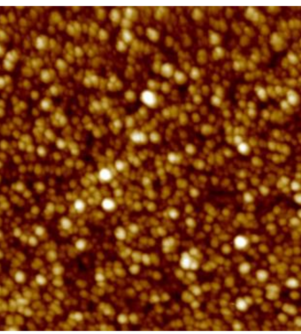

Figure1. SmartBioSurface® slides. Left Image: SmartBioSurface® slides in a different format. Right Image: Atomic Force Microscopy (AFM) of the nanocoating (50 nm thickness)

This surface promotes **spontaneous adhesion of non-adherent cells** [3] while maintaining **intact morphology**, making it an ideal candidate for **precision isolation using LCM** from cytological samples.

We have prepared cytological samples from living cultured suspension cells and PBMC by seeding them on SmartBioSurface® slides: upon fixation and staining, these samples underwent LCM to evaluate the efficiency of the collection of single cells and the compatibility with downstream analysis such as **whole-genome amplification (WGA),** followed by next-generation sequencing (NGS) library preparation and whole-genome sequencing **(WGS)** for the detection of copy number variations **(CNVs)**.

This application note outlines **optimized protocols for the immobilization, fixation, staining, and microdissection** of non-adherent cell populations utilizing the Zeiss PALM MicroBeam LCM (Laser Capture Microdissection) system. We demonstrate a quantitative **100% cell collection efficiency** across diverse cytological preparations, ensuring total sample recovery for sensitive applications.

Downstream genomic analysis was performed on a HER2-amplified model cell line (BT474). Following cell seeding and precise microdissection of individual cells, a comprehensive workflow for **Copy Number Variation (CNV) analysis** was successfully executed, confirming the preservation of genetic integrity post-dissection.

Materials & Methods

1. Sample Preparation and Seeding

PBMC Isolation from a Peripheral Blood Sample

Peripheral blood samples collected from healthy donors [4] in EDTA tubes are processed to isolate leukocytes via red blood cell lysis.

Leukocyte count is determined using an automated hematology analyzer (EmoCue).

Whole blood is incubated with Red Blood Cell Lysis Buffer (RBL) for approximately 5 minutes at room temperature, allowing selective lysis of erythrocytes while preserving leukocytes. Following incubation, the sample is centrifuged at 249 gfor 5 minutes to pellet the leukocyte fraction. The supernatant containing lysed erythrocytes is carefully discarded.

To ensure complete removal of red blood cells, the pellet is subjected to two additional lysis cycles. After the final centrifugation step, the leukocyte pellet is resuspended in Dulbecco's Phosphate Buffered Saline 1X without Ca and Mg (DPBS), to achieve the proper cell density of up to approximately 250.000 cells/cm^2.

Cultured Suspension Cells: HeLaS3

Hela S3 cells (ATCC CCL-2) are collected directly from the culture flask by gentle pipetting and transferred into a centrifuge tube. The suspension is centrifuged at approximately 249g for 5 minutes, after which the supernatant is removed, and the cell pellet is resuspended in DPBS 1X.

Cell concentration is determined using a Bürker hemocytometer combined with a viability dye.

The suspension is adjusted to achieve a cellular density up to approximately 250.000 cells/cm^2 before seeding onto SmartBioSurface® slides.

Cultured Adherent Cells: BT-474, HER2 amplified

Cells are collected from the culture flask following enzymatic detachment.

The culture medium is first removed, and the cell monolayer is gently rinsed with DPBS 1X to eliminate residual serum. A gentle rinse with Trypsin-EDTA 1X solution is done to condition the cells' detachment. Trypsin-EDTA solution 1X is then added to cover the cell layer, and the flask is incubated at 37 °C for approximately 3–5 minutes until cells detach. Gentle tapping may be applied to facilitate detachment.

Once detached, complete culture medium is added to neutralize trypsin activity. The cell suspension is transferred into a centrifuge tube and centrifuged at approximately 249g for 5 minutes. The supernatant is discarded, and the cell pellet is resuspended in DPBS 1X.

Cell concentration is determined using a Bürker haemocytometer combined with a viability dye.

The suspension is adjusted to achieve a cellular density up to approximately 250.000 cells/cm^2 before seeding onto SmartBioSurface® slides.

Cell Seeding on SmartBioSurface® Slides

Cell suspensions prepared as described above are dispensed directly onto SmartBioSurface® slides.

The slides are left undisturbed for approximately 20 minutes at room temperature, allowing cells to settle and spontaneously adhere to the nanostructured surface.

This step enables the immobilization of cells while preserving cell morphology and maintaining compatibility with downstream staining procedures and laser microdissection.

After adhesion, slides are gently washed with DPBS 1X and can be optionally fixed or left unfixed, depending on the experimental requirements, and then processed according to the analytical condition (Figure 2).

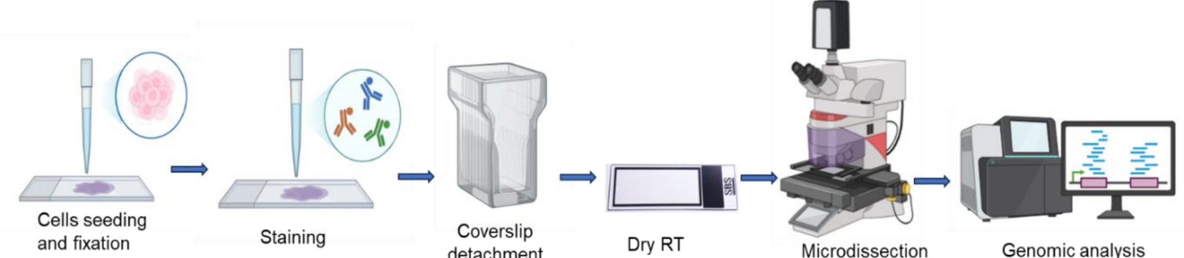

Figure 2. Schematic description of the analytical workflow starting from cell seeding on SmartBioSurface® slides to single-cell genomic analysis.

2. Fixation

Following cell seeding and adhesion, samples can be fixed using Paraformaldehyde (PFA), 2 or 4%, according to the following experimental settings, for 20 minutes at room temperature.

Slides are briefly rinsed with DPBS 1X and then with distilled water and allowed to air dry completely.

The slides are then either stored at −80 °C or processed immediately for microdissection experiments.

3. Staining

Immunofluorescence Staining

Slides are first allowed to thaw at room temperature for at least 30 min. Cells are permeabilized in ice-cold methanol for 5 min under a chemical hood and air dried for at least 15 min until complete drying. Slides are then rehydrated with DPBS 1X for 1 min in a humid chamber.
Cells are permeabilized with 0.1% Tergitol in DPBS 1X for 10 min at room temperature, followed by three washes in DPBS 1X for 1 min each. To minimize nonspecific binding, slides are incubated with a blocking solution, 5% normal goat serum (NGS) in DPBS 1X, for 1 hour at room temperature.
Directly conjugated antibodies against Alexa Fluor® 647 anti-human CD45 Recombinant Antibody (BioLegend 393406) and Alexa Fluor® 647 anti-human CD66b Antibody (BioLegend 392912) are applied in blocking solution and incubated for 2 hours at 37 °C in a humid chamber. Slides are then washed three times with DPBS 1X for 5 min each at room temperature.
Cell nuclei are stained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) (1 µg/mL in DPBS 1X) for 10 min at room temperature, followed by a 5 min wash in DPBS 1X and a brief rinse in MilliQ water.
Slides are air-dried under a biological hood for at least 15 min, protected from light, and mounted using a glycerol-based fluorescence mounting medium (ProLong P36984 ThermoFisher) with a 24 × 50 mm coverslip. Mounted slides are left at room temperature for at least 30 min before image acquisition.

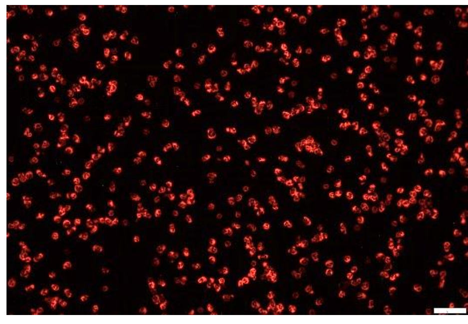

Figure 3. Example of WBC stained in the 647 channel with Alexa Fluor® 647 anti-CD45 and anti-CD66b antibodies.

Brightfield Staining: haematoxylin and eosin

Slides are first allowed to thaw at room temperature. Then are hydrated in distilled water for 30 seconds and incubated with Mayer's hematoxylin for 4 min to stain cell nuclei. Slides are then rinsed twice in running water for 30 seconds, followed by incubation in bluing solution (CP423, DiaPath) for 30 seconds and a final rinse in distilled water for 30 seconds. Slides are dehydrated in 95% ethanol for 30 seconds and counterstained with alcoholic eosin for 1 min to stain cytoplasmic and extracellular components.
After eosin staining, slides are rinsed in 95% ethanol for 20 seconds and 99% ethanol for 10 seconds, then cleared in Ottix Plus (X0076 DiaPath) with two consecutive incubations of at least 1 min under a chemical hood.
Slides are mounted using permanent mounting medium (Micromount 060200 DiaPath) by applying two drops onto the coverslip, allowing excess Ottix Plus to drain, and carefully placing the slide on the coverslip while avoiding air bubbles.
Slides are left to dry under a chemical hood before further processing.

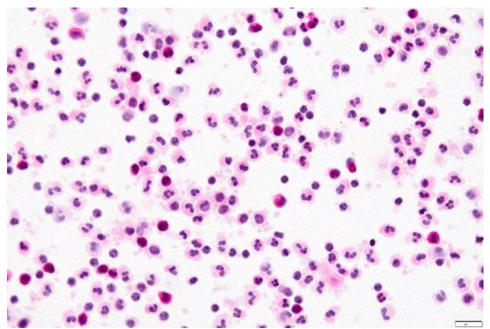

Figure 4. WBC stained with hematoxylin and eosin (H&E) and observed under light microscopy.

4. Laser Microdissection

Slide preparation for microdissection

Before laser microdissection, coverslips are removed by soaking the slides overnight at room temperature in the appropriate solution: slides mounted with glycerol-based mounting media (e.g., Prolong) are incubated in DPBS 1x, while slides mounted with permanent mounting media (e.g., Micromount) are incubated in Ottix to allow coverslip detachment.

The following day, once coverslips had detached, slides are allowed to air dry completely at room temperature, making the samples accessible for laser microdissection.

Laser Capture Microdissection

Microdissection was performed on a Zeiss PALM system using Laser Pressure Catapulting (LPC). In laser pressure catapulting systems, the UV laser is used both to isolate the selected cell and to propel it into a collection device.
The slide is placed on the microscope stage, and the target cell is identified. The laser is focused directly on the area of interest, and one or more laser pulses are applied to detach the cell from the surface.
A catapulting pulse is applied, generating sufficient energy to propel the isolated cell upward into a collection cap positioned above the slide.

The following parameters were used for microdissection: LPC Energy: 56–78; Focus: 33–38; objective: 40X. In case of samples undergoing multiplexed staining, parameters can be further tuned and adjusted to ensure efficient detachment and clean catapulting

This method enables efficient recovery of individual cells with minimal contamination from surrounding material.

5. Molecular analysis

Following laser microdissection, isolated single BT-474 cells are subjected to WGA to obtain sufficient DNA for downstream analysis.

WGA is performed using the Menarini Ampli1™ WGA Kit, following the manufacturer's instructions for single-cell genomic DNA amplification. Amplified DNA is subsequently purified and quantified to assess yield and fragment size distribution.

Sequencing libraries are prepared using the Menarini Ampli1™ LowPass Kit, according to the manufacturer's protocol, which is specifically optimized for Ampli1-amplified DNA. Libraries are indexed, amplified, and purified before sequencing.

Prepared libraries are sequenced on an Illumina platform to generate WGS data.

Sequencing data are processed using standard bioinformatic pipelines to assess genome-wide CNVs, enabling the identification of chromosomal gains and losses at single-cell resolution.

Results

1. Single-cell microdissection and capture confirmation: an easy task

The laser microdissection workflow using Laser Pressure Catapulting on SmartBioSurface® slides yields an easy "clean-cut" result.

The slide is placed on the microscope holder, and the operator sets all parameters in the PalmRobo software for microdissection.

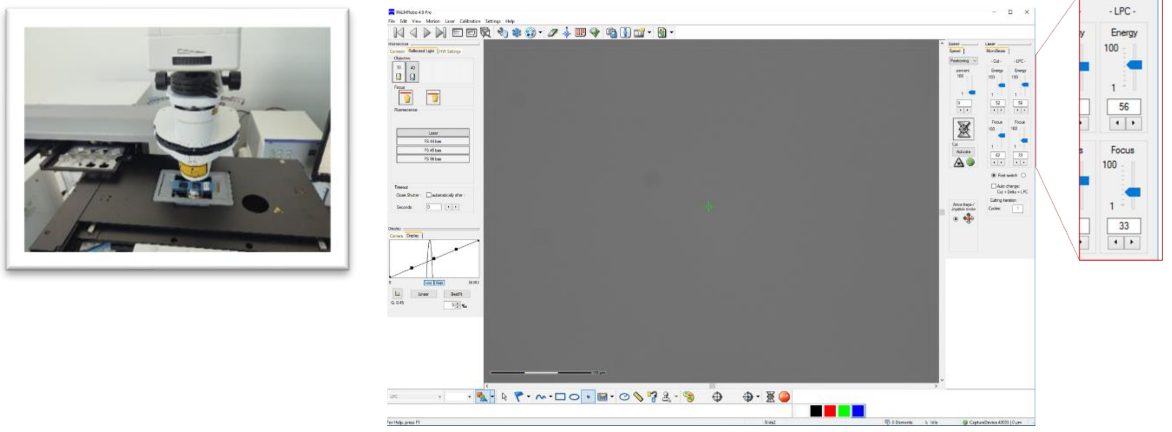

Figure 5. Left: representative image of the SmartBioSurface® slide placed on the microscope holder for laser microdissection. Right: scheme of the PalmRobo Software Interface.

Using brightfield microscopy at 40X magnification on the microdissector, cells are clearly identified: upon selecting the specific target, the cell is promptly detached and recovered onto the collection cap (Figure 6).

Step 1 Target Identification	Step 2 Post-Dissection	Step 3 Capture Confirmation
Target cell identified on SmartBioSurface® slide	*Excision Site*	*Cell recovered in the collection cap*

Figure 6. Target cell identification, laser microdissection, and successful cell capture in the collection cap: the white arrow indicates the target undergoing microdissection.

2. Efficiency of cell microdissection

Across more than 200 **individual targets,** the SmartBioSurface® slide provided a **100% success rate**, with most cells requiring only a single laser shot.

Distribution of Laser Shots per Cell (n=216)

- **1 Shot:** 86.6%
- **2 Shots:** 1.9%
- **3+ Shots:** 6.9%

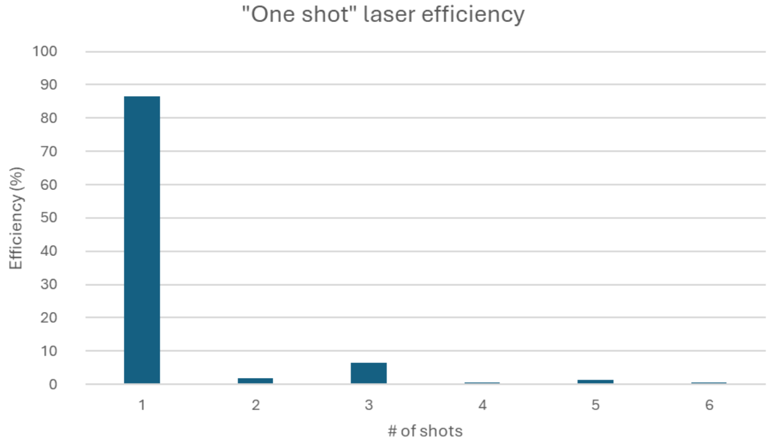

Figure 7. Efficiency of microdissection in relation to the number of "laser shots".

The high percentage of "1-shot" successes (nearly 90%) demonstrates the optimal balance of adhesion and releasability offered by the SmartBioSurface® slide.
Microdissected cells were collected from different fixation conditions (0%, 2%, and 4% PFA), with most cells fixed at 4% PFA (n = 149), followed by 2% PFA (n = 51) and 0% PFA (n = 16)
High microdissection efficiency was maintained across all fixation conditions, indicating that PFA treatment does not impair cell detachment and recovery.

3. Molecular analysis of BT474 for Her2 amplification

WGS analysis of single BT-474 cells microdissected from SmartBioSurface® slides demonstrates that increasing PFA fixation (0%, 2%, and 4%) results in a progressive increase in background noise in copy number profiles, reflected by increased signal variability (Figure 8). In line with this, fixation is associated with a higher level of noise in the genomic profiles; however, strong copy number alterations remain clearly detectable. Recurrent CNV patterns are also observed across analyzed cells, including a consistent deletion

on chromosome 9 detected across fixation conditions (Figure 8), a genomic alteration frequently reported in cancer cells and therefore likely reflecting a clonal feature of the tumor-derived model rather than a technical artifact.

High-amplitude events such as the characteristic amplification on chromosome 17, including the ERBB2 (HER2) locus, are robustly and consistently identified across all samples regardless of the PFA percentage (Figure 9), confirming the reliability of the approach in detecting clinically relevant genomic alterations.

Overall, these results indicate that, although fixation increases noise, it does not compromise the detection of biologically relevant CNVs, confirming the suitability of this workflow for single-cell CNV analysis from fixed samples.

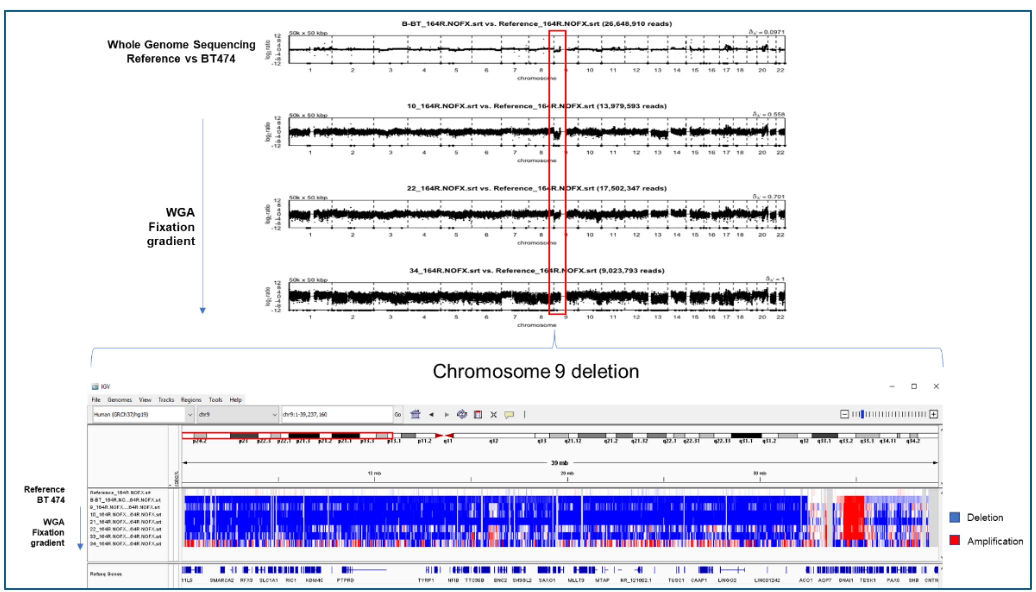

Figure 8. Effect of PFA fixation on single-cell CNV profiles.

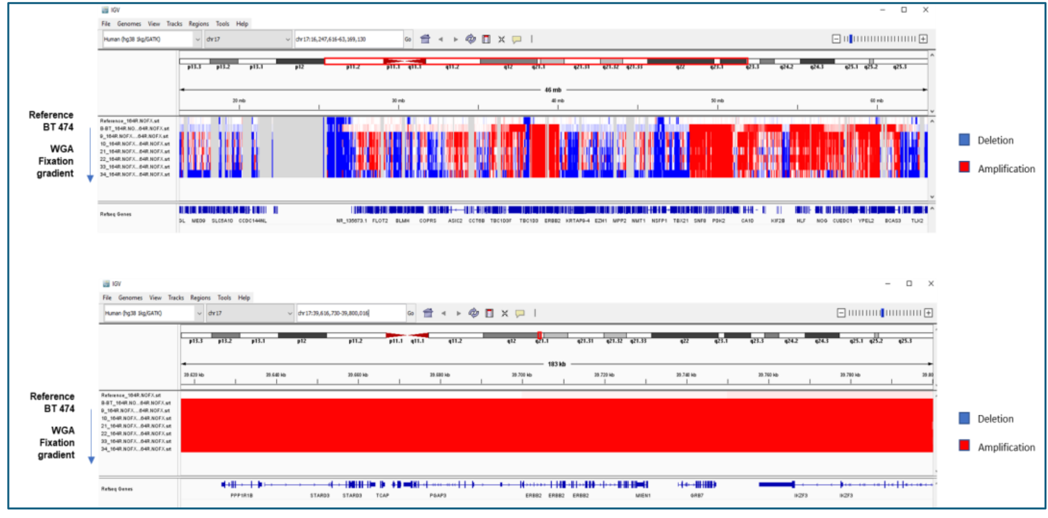

Figure 9. Detection of high-amplitude CNVs is preserved across fixation conditions.

4. Cross-Platform Versatility

Independent research [5] has shown SmartBioSurface® slide compatibility with gravity-assisted systems, such as the Leica Microsystems LMD6500, confirming that the nanostructured coating is a solution for commercial LCM platforms.

Conclusion

SmartBioSurface® slides represent a unique technological tool for single-cell research.

By providing a "ready-to-use" adhesive surface that remains compatible with downstream molecular analysis and multiple laser capture technologies, it enables:

- **Streamlined pre-analytical and analytical workflows:** no specialized slide preparation needed
- **Efficient cell adhesion of non-adherent cells,** including rare cells (6)
- **Multi-modal imaging:** full compatibility with brightfield and immunofluorescence staining
- **High Efficiency microdissection:** 100% single cell recovery
- **Compatibility with downstream molecular analysis (e.g., CNV)**

References

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