SmartBioSurface® Slides

Efficient Adhesion of Enriched Circulating Tumor Cells for Downstream Immunofluorescence Analysis

The Challenge: Recovering Rare CTCs After Microfluidic Enrichment

Circulating Tumor Cells (CTCs) are among the most informative biomarkers in liquid biopsy, providing valuable insights into tumor heterogeneity, metastatic dissemination, and treatment response. However, their extremely **low abundance** and **fragile** nature make downstream processing particularly challenging.

Microfluidic enrichment platforms such as the **Parsortix®** system (CelLBx Health) efficiently **isolate CTCs** from whole blood based on their physical properties, such as **size** and **deformability**, independently of epithelial marker expression

Following enrichment, harvested cells are typically deposited onto glass slides by **cytocentrifugation** (Cytospin) or similar methods for cytological or immunofluorescence analysis.

Although widely adopted in routine laboratory practice, cytocentrifugation has been reported to result in substantial **cell loss** during slide preparation. In a validation study of a microfluidic CTC enrichment workflow, **37–51% of harvested cells were not retained on the slide**, highlighting the need for **improved cell deposition** strategies that **maximize recovery** of these extremely rare cells [1].

An ideal imaging substrate should therefore provide efficient immobilization of rare cells while preserving cellular morphology, minimizing background fluorescence, and remaining fully compatible with high-resolution fluorescence microscopy.

The Solution: SmartBioSurface® slides for Secure Immobilization of Parsortix Enriched CTC Samples

SmartBioSurface® (Tethis S.p.A.) is a proprietary **nanostructured titanium-based coating** [2] developed to promote the **spontaneous adhesion** of **non-adherent** and **rare cells** onto optical-grade glass while preserving cellular morphology and structural integrity [3]. The coating consists of a nanostructured titanium layer deposited onto microscopy-grade glass and is available in different thicknesses to support a broad range of fluorescence imaging applications.

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

Unlike conventional adhesion-promoting coatings, SmartBioSurface provides **stable cell immobilization without altering cell morphology**, enabling samples to withstand repeated washing, fixation, and immunostaining procedures while minimizing cell loss. In addition to its excellent adhesive properties, the nanostructured titanium coating exhibits **low autofluorescence** and favourable optical characteristics, making it highly **compatible with fluorescence microscopy** and other demanding imaging workflows.

Following enrichment, rare cells can be **directly deposited** onto SmartBioSurface slides, where they spontaneously adhere to the nanostructured surface. The resulting stable immobilization supports reliable downstream immunofluorescence analysis and high-quality image acquisition, even when only a limited number of cells are available.

In addition to standard fluorescence imaging, titanium-coated substrates have been designed to support **advanced laser-based microscopy** techniques by **minimizing** substrate-related **background** and maintaining compatibility with high-intensity laser illumination.

Materials & Methods

1. Cell Model and Spike-In Sample Preparation

Human T98G glioblastoma cells were used as a surrogate model for circulating tumor cells (CTCs). Cells were thawed, cultured under standard conditions, and harvested prior to the experiment.

Approximately 5,000 T98G cells were spiked into 10 mL of phosphate-buffered saline (PBS) together with a buffy coat obtained from a healthy donor, generating a heterogeneous cell suspension that mimics the cellular composition typically encountered prior to CTC enrichment from peripheral blood.

2. Cell Enrichment Using Parsortix System

The spike-in sample was processed using Parsortix system (CelLBxHealth) a microfluidic CTC enrichment device based on cell size and deformability. This label-free enrichment strategy isolates circulating tumor cells (CTCs) independently of epithelial marker expression, enabling the recovery of heterogeneous tumor cell populations while reducing the number of background blood cells.

Following enrichment, the recovered cell suspension containing T98G cells together with residual leukocytes was deposited onto SmartBioSurface slides to assess cell adhesion, retention during immunofluorescence processing, and compatibility with high-resolution fluorescence microscopy.

3. Cell Deposition and Fixation on SmartBioSurface

To concentrate the recovered cells within a defined area, a reusable CultureWell™ silicone gasket (Grace Bio-Labs) was positioned on the SmartBioSurface slide. The enriched cell suspension was carefully dispensed into the gasket well and incubated for 30 minutes at room temperature, allowing spontaneous adhesion of both tumor cells and residual leukocytes onto the nanostructured titanium coating.

Following cell adhesion, samples were gently washed with PBS and fixed with 4% formaldehyde for 5 minutes at room temperature. After fixation, the slides were rinsed with PBS and processed for immunofluorescence staining. The stable immobilization provided by the SmartBioSurface coating preserved cell morphology and prevented cell loss throughout the washing and fixation steps.

Figure2. Cell deposition on SmartBioSurface. Left: reusable CultureWell™ silicone gasket used to define the deposition area. Right: SmartBioSurface slide showing the gasket-defined deposition area (red arrow), where the enriched cell suspension was seeded, incubated for spontaneous cell adhesion and fixed with formaldehyde 4%.

4. Immunofluorescence Staining

Following fixation, samples were gently washed with PBS and processed using a standard immunofluorescence workflow. Cells were incubated with directly fluorophore-conjugated antibodies in a humid chamber. Tumor cells were identified using Cell Surface Vimentin (CSV) Monoclonal Antibody-PE (Life Technologies), while residual leukocytes were labeled with CD45 Antibody, anti-human, APC REAfinity (Miltenyi Biotec). Following antibody incubation, slides were washed with PBS to remove unbound antibodies, and cell nuclei were counterstained with DAPI. Slides were then prepared for fluorescence imaging according to the standard microscopy workflow.

Fluorescence images were acquired using an Olympus FV3000 Confocal Laser Scanning Microscope. Multichannel confocal imaging was performed to evaluate cell retention on the SmartBioSurface coating, preservation of cellular morphology, staining specificity, fluorescence signal quality, and substrate-related background fluorescence. Particular attention was given to the identification of $CSV^{+}/CD45^{-}$ tumor cells within the residual leukocyte population, demonstrating the suitability of SmartBioSurface for downstream immunofluorescence analysis of enriched cell samples.

Results

1. SmartBioSurface Provides Stable Adhesion of Enriched Cells Throughout Sample Processing

Following enrichment and deposition onto SmartBioSurface, both T98G cells and residual leukocytes efficiently adhered to the nanostructured titanium coating after a 30-minute incubation. Cells remained firmly immobilized throughout the subsequent washing, fixation, and immunofluorescence staining procedures while preserving their morphology and structural integrity.

The stable adhesion provided by SmartBioSurface enabled reliable downstream sample processing with no evident cell detachment during the experimental workflow, supporting the analysis of rare enriched cell populations.

2. Specific Immunofluorescence Identification of Tumor Cells

Direct immunofluorescence staining enabled clear visualization and identification of the enriched cell populations on SmartBioSurface slides. T98G tumor cells exhibited strong Cell Surface Vimentin (CSV) staining together with well-preserved nuclear morphology, while residual leukocytes were identified by CD45 staining.

Representative confocal images acquired from different fields of view demonstrated consistent fluorescence labeling and excellent preservation of cell morphology following enrichment and deposition. The low background fluorescence of the SmartBioSurface coating allowed clear visualization of fluorescent signals, facilitating the identification of target tumor cells within the remaining leukocyte population (Figures 1–3).

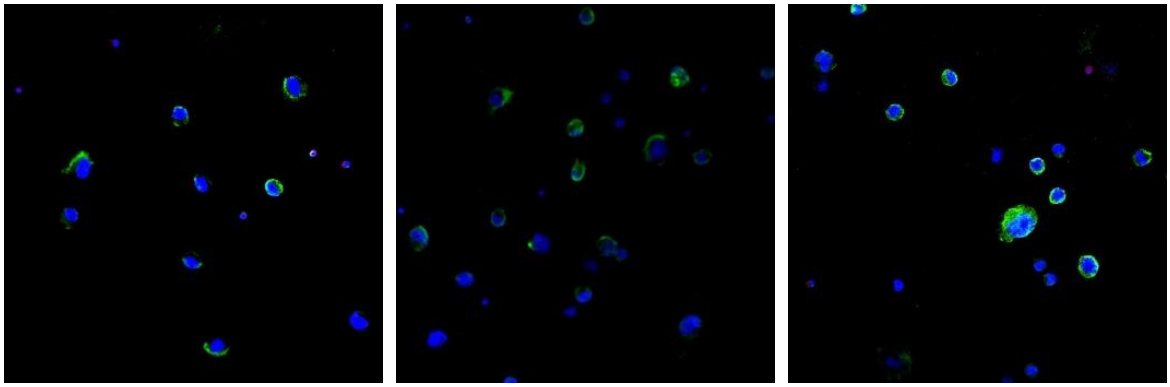

Figure 3 Immunofluorescence imaging of enriched T98G tumor cells and background lymphocytes on SmartBioSurface slides. Target tumor cells show specific surface expression for Cell-Surface Vimentin (CSV-PE, green), while background leukocytes are identified by CD45-APC (red). Nuclei are counterstained with DAPI (blue). Images were acquired using an Olympus FV3000 confocal microscope equipped with a 20× objective (NA 0.8).

3. Compatibility with Confocal Fluorescence Imaging

Confocal microscopy confirmed the excellent compatibility of SmartBioSurface with multicolor immunofluorescence imaging of enriched cell samples. The nanostructured titanium coating provided a clean optical background while maintaining stable cell immobilization throughout sample preparation.

High-quality fluorescence images enabled clear visualization of cellular morphology and fluorescent biomarkers without detectable substrate-related imaging artifacts. These findings demonstrate that SmartBioSurface is well suited for downstream fluorescence characterization of enriched circulating tumor cell samples following microfluidic enrichment workflows.

Conclusions

- **SmartBioSurface** enables stable **adhesion** of **Parsortix-enriched circulating tumor cells**, **preserving cell morphology** throughout fixation and immunofluorescence staining.
- The nanostructured titanium coating provides **low background fluorescence** and excellent compatibility with **confocal** microscopy, allowing clear visualization of tumor cells within residual leukocyte populations.
- The combined Parsortix–SmartBioSurface workflow provides a **simple and reliable approach** for downstream immunofluorescence characterization of **enriched circulating tumor cells**.
- These results demonstrate that SmartBioSurface provides an effective substrate for downstream imaging and immunofluorescence characterization of enriched CTC samples.

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SmartBioSurface®

Maximizing Cell Recovery for Reliable Downstream Analysis

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