SmartBioSurface® Enables High-Quality TIRF and STORM Imaging of Suspension Cells

The Challenge: High-Resolution Imaging of Non-Adherent Cells at the Cell–Substrate Interface

High-resolution fluorescence imaging of non-adherent cells remains challenging, particularly when investigating structures located at or near the cell–substrate interface. Hematological cell lines and primary white blood cells (WBCs) do not naturally adhere to conventional glass surfaces. They can be displaced or lost during fixation, staining, and washing steps, leading to reduced sample retention, increased variability, and inconsistent imaging results [1].

This limitation is especially critical for Total Internal Reflection Fluorescence (TIRF) microscopy, where excitation is confined to a thin optical section adjacent to the substrate [2]. To obtain high-contrast images of membrane-proximal structures, cells must remain stably positioned within the evanescent field throughout sample preparation and imaging. In addition, low substrate-related background fluorescence and optimal optical properties are essential for maximizing signal quality.

Similar challenges apply to Stochastic Optical Reconstruction Microscopy (STORM), where nanoscale image reconstruction depends on precise fluorophore localization and can be adversely affected by background fluorescence, sample drift, or incomplete cell retention [3]. For suspension-derived cells, maintaining stable attachment throughout sample processing and image acquisition is therefore crucial to ensure reliable, reproducible super-resolution data.

Although adhesion-promoting substrates such as Poly-D-Lysine (PDL) are widely used to immobilize suspension cells, their effectiveness can vary depending on the cell type and experimental conditions. As advanced imaging techniques become increasingly important in immunology, hematology, and cell biology research, there is a growing need for substrates that combine robust cell retention, low background fluorescence, and full compatibility with TIRF and STORM workflows.

The Solution: SmartBioSurface® Coating

SmartBioSurface® is a nanostructured titanium-based coating [4] designed to overcome the challenges associated with imaging non-adherent cells. By promoting spontaneous and reliable cell attachment to glass substrates while preserving cellular morphology and structural integrity, the coating enables stable sample preparation and imaging workflows without the need for complex immobilization strategies.

Once attached, cells remain securely positioned throughout fixation, staining, washing, and image acquisition steps, helping to maintain sample consistency and reducing the risk of cell loss during processing.

The coating is deposited on optical-grade glass and is available in different thicknesses, allowing you to optimize the substrate for specific imaging applications (Figure 1). By combining efficient cell retention with excellent optical performance, SmartBioSurface® supports demanding fluorescence imaging techniques that

require stable samples, low background fluorescence, and high image quality, including TIRF and STORM microscopy [5].

Experimental plan

TIRF microscopy was used to evaluate the performance of each substrate at the cell-substrate interface, with particular emphasis on cell positioning within the evanescent field, image contrast, background fluorescence, and overall signal quality. The study aimed to determine whether SmartBioSurface® provides the optical and biological conditions required for high-contrast visualization of membrane-proximal structures.

STORM microscopy was employed to assess substrate suitability for single-molecule localization imaging, focusing on sample stability during acquisition, compatibility with fluorophore blinking and localization processes, and the quality of actin cytoskeleton reconstruction at the nanoscale.

Comparison of the two SmartBioSurface® coatings with PDL controls enabled a direct evaluation of their capability to support robust and reproducible super-resolution imaging of suspension-derived cells under identical experimental conditions.

Figure 1. SmartBioSurface slides. Left Image: SmartBioSurface slides in a different format. Right Image: Atomic Force Microscopy (AFM) of the nanocoating (50 nm thickness)

1. Materials & Methods

Cell Models

Jurkat Clone E6-1 (ATCC® TIB-152™) and U937 (ATCC® CRL-1593.2™) suspension cell lines were used as representative lymphoid and myeloid models, respectively. Primary white blood cells (WBCs) isolated from peripheral blood collected from healthy donors were included as a physiological reference population.

Imaging Platforms and SmartBioSurface® Supports

The compatibility of SmartBioSurface® with TIRF and STORM microscopy was evaluated using titanium-coated chamber slides. Two coating thicknesses were tested: a standard coating (50 nm) and a thin coating (20 nm). Poly-D-Lysine (PDL)-coated chamber slides served as reference controls.

Cells were seeded onto the different substrates and allowed to spontaneously adhere prior to fixation and staining. TIRF and STORM imaging were then performed under identical experimental conditions to compare the performance of SmartBioSurface® and PDL-coated substrates.

Preparation of Primary White Blood Cells from peripheral blood

Peripheral blood samples collected from healthy donors [6] 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 g for 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 1× without Ca and Mg (DPBS), to achieve the proper cell density of up to approximately 250.000 cells/cm^2.

Preparation of Jurkat and U937 Cells

Jurkat and U937 cells were harvested directly from suspension cultures, washed in DPBS, and adjusted to a final density of approximately 250,000 cells/cm^2. Cell concentration and viability were verified before seeding onto the different substrate conditions.

Cell Seeding and Fixation

Cell suspensions were dispensed directly into chamber slides and incubated for approximately 20 minutes at room temperature to allow spontaneous attachment to the substrate.

Following adhesion, samples were fixed with 4% paraformaldehyde (PFA) for 20 minutes, washed with DPBS, and processed for immunofluorescence staining (Figure 2).

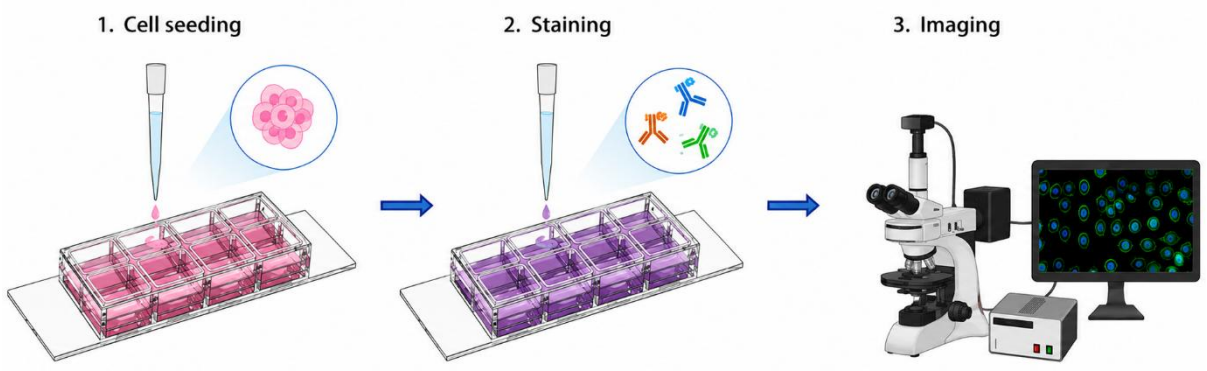

Figure 2. Schematic overview of the experimental workflow for fluorescence imaging, including cell seeding on SmartBioSurface chamber slides, immunofluorescence staining, and image acquisition.

Immunofluorescence Staining

Following fixation, chamber slides were maintained in DPBS 1X and processed directly for immunofluorescence staining. Cells were permeabilized with 0.1% Tergitol™ in DPBS 1× for 10 min at room temperature, followed by three washes with DPBS 1× for 1 min each.

To minimize non-specific binding, samples were incubated with a blocking solution consisting of 5% Normal Goat Serum (NGS; Cell Signaling Technology) in DPBS 1× for 1 h at room temperature.

Samples were stained with Phalloidin-iFluor™ 647 (Abcam ab176759) in blocking solution and incubated for 2 h at 37°C in a humid chamber to visualize the actin cytoskeleton. Following staining, samples were washed three times with DPBS 1× for 1 min each at room temperature. For TIRF imaging, DAPI-only samples were prepared separately as negative controls for background fluorescence assessment

Samples were maintained in PBS after staining and imaged directly in the chamber slides without mounting medium, according to the requirements of TIRF and STORM microscopy.

2. Results

SmartBioSurface Improves TIRF Imaging Performance

TIRF microscopy provided a highly sensitive assessment of substrate performance, as excitation is confined to a thin optical region immediately adjacent to the glass surface. Under identical acquisition and visualization settings, both titanium-coated substrates (50 nm and 20 nm) consistently produced higher-contrast fluorescence images than PDL-coated controls across Jurkat cells, U937 cells, and primary WBCs (Figure 3).

Actin-associated fluorescence was more clearly resolved from background signal on titanium-coated surfaces, resulting in improved image quality and a higher apparent signal-to-noise ratio. This enhancement was consistently observed across all tested cell types and for both coating thicknesses, with the most evident differences detected in the regions closest to the substrate.

The improved visualization of membrane-proximal structures suggests that the nanostructured titanium surface provides favorable conditions for TIRF imaging while maintaining efficient retention of suspension-derived cells during sample processing and image acquisition.

Overall, these results demonstrate that SmartBioSurface® -coated substrates are fully compatible with TIRF microscopy and support high-contrast imaging at the cell-surface interface compared with conventional PDL-coated controls.

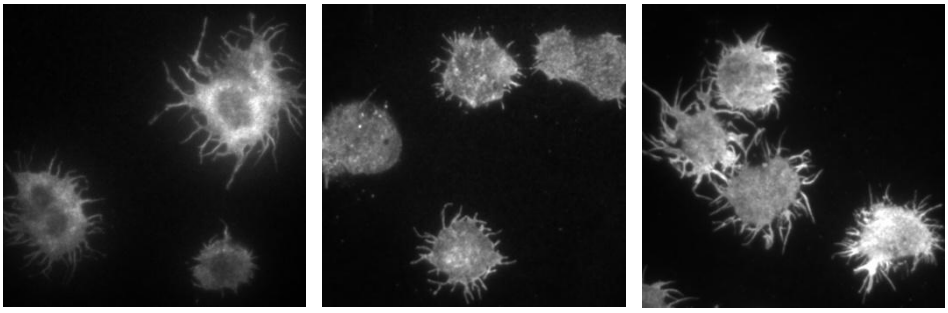

Figure 3. Total internal reflection fluorescence (TIRF) microscopy images of Jurkat cells prepared under three different conditions (Poly-D-Lysine, SmartBioSurface thin, and SmartBioSurface std, from left to right). Cells were stained with Phalloidin (Alexa Fluor 647, white). Images were acquired using a 100×/1.49 NA oil immersion objective (512 × 512 pixels; single-plane acquisition) using identical TIRF imaging parameters and are shown as representative images of the cell adhesion plane.

Thin Titanium Coating Enhances STORM Super-Resolution Imaging

STORM microscopy successfully generated super-resolution reconstructions of actin structures on all tested substrates, confirming the compatibility of titanium-coated chamber slides with single-molecule localization imaging. High-quality reconstructions were obtained for Jurkat cells, U937 cells, and primary WBCs (Figure 4).

Differences between substrate conditions became more pronounced under STORM imaging than under conventional fluorescence microscopy. Among the tested surfaces, the 20 nm titanium coating consistently delivered the best reconstruction quality, characterized by lower background fluorescence, improved localization contrast, and more homogeneous visualization of fine actin structures.

In comparison, PDL-coated controls generally showed reduced image quality and higher background contribution, while the 50 nm titanium coating occasionally generated reconstructions with greater spatial variability. Despite these differences, both titanium-coated substrates supported efficient fluorophore blinking and accurate localization, demonstrating full compatibility with STORM imaging workflows.

Overall, the results identify the 20 nm SmartBioSurface coating as the most effective configuration for super-resolution imaging, providing the optimal balance between cell immobilization, low background signal, and high-quality nanoscale reconstruction.

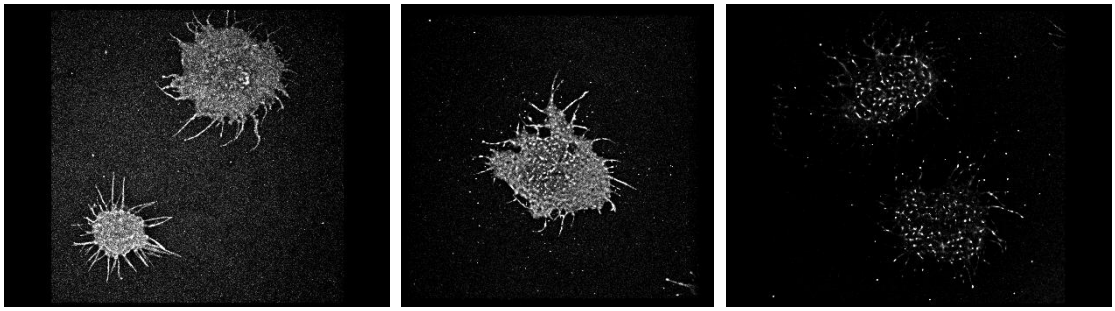

Figure 4. Stochastic optical reconstruction microscopy (STORM) images of Jurkat cells prepared under three different conditions (Poly-D-Lysine, SmartBioSurface thin, and SmartBioSurface std, from left to right). Cells were stained with Phalloidin (Alexa Fluor 647, white). Images were acquired using a 100×/1.49 NA oil immersion objective (256 × 256 pixels; single-plane acquisition; 20,000 frames per reconstruction) using identical STORM imaging parameters and are shown as representative images of the cell adhesion plane.

HiLo-STORM Reveals Reduced Substrate-Dependent Effects

To further investigate the differences observed during TIRF-STORM imaging, additional acquisitions were performed in HiLo-STORM mode. Under these conditions, reconstruction quality became more comparable across all tested substrates, and the differences observed under TIRF-STORM were substantially reduced (Figure 5).

These results indicate that the substrate-dependent effects are primarily localized at the cell-surface interface, where TIRF excitation is most sensitive to cell positioning and background signal.

The findings further support the conclusion that titanium-coated substrates mainly influence imaging performance in the region closest to the imaging surface.

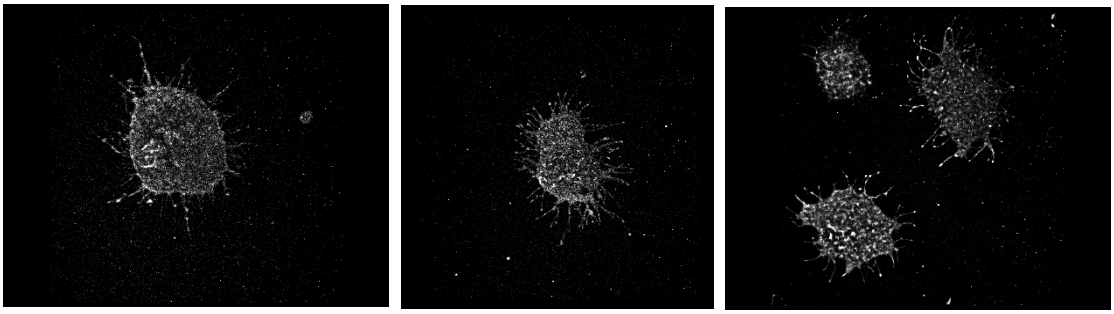

Figure 5. STORM images acquired using highly inclined and laminated optical sheet (HILO) illumination of Jurkat cells prepared under three different conditions (Poly-D-Lysine, SmartBioSurface thin, and SmartBioSurface std, from left to right). Cells were stained with Phalloidin (Alexa Fluor 647, white). Images were acquired using a 100×/1.49 NA oil immersion objective (256 × 256 pixels; single-plane acquisition; 20,000 frames per reconstruction) using identical HILO-STORM imaging parameters and are shown as representative images of the cell adhesion plane.

3. Conclusion

The results demonstrate that SmartBioSurface® titanium-coated chamber slides provide an effective platform for high-resolution fluorescence imaging of non-adherent cells using both TIRF and STORM microscopy. Across all tested cell models, the coating supported reliable cell retention throughout sample preparation and imaging, while remaining fully compatible with standard immunofluorescence workflows.

Key benefits of SmartBioSurface® include:

- Efficient immobilization of non-adherent cells on imaging-grade glass surfaces.
- Full compatibility with fixation and immunofluorescence staining protocols.
- Reduced background fluorescence and improved image contrast at the imaging interface.

- Enhanced TIRF imaging performance compared with PDL-coated controls, resulting in clearer visualization of membrane-proximal structures.
- Full compatibility with STORM super-resolution microscopy, supporting robust fluorophore localization and high-quality image reconstruction.
- Superior STORM performance with the 20 nm titanium coating, which consistently provided the best reconstruction quality, localization contrast, and overall image definition among the tested conditions.

Overall, SmartBioSurface® enabled robust and reproducible imaging of suspension-derived cells under both TIRF and STORM conditions. Its benefits were most evident at the cell-substrate interface, where reduced background fluorescence and improved cell positioning enhanced image quality. Among the tested configurations, the 20 nm coating provided the best overall performance and represents the preferred option for demanding super-resolution imaging applications.

These findings demonstrate that SmartBioSurface® simplifies advanced imaging workflows for non-adherent cells by combining efficient cell immobilization with the optical performance required for high-contrast TIRF imaging and high-quality STORM reconstruction.

References

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SmartBioSurface®

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