Application Note

Title

Sophomer F10 as a Synthetic Blocking Agent for Fixed Cells in Immunofluorescence Microscopy

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Introduction and objective

Immunofluorescence (IF) microscopy is a powerful method for visualizing cellular structures with high spatial resolution. To ensure selective and accurate detection of target proteins, non-specific antibody binding must be minimized. Therefore, a blocking agent is routinely applied after fixation to prevent non-specific antibody binding and obtain high-contrast images. However, commonly used animal-derived blockers such as bovine serum albumin (BSA) suffer from lot-to-lot variability and compositional differences between suppliers, which can introduce assay inconsistency [1]. In addition, BSA is an animal-derived product, requiring extensive purification and introducing logistical and ethical considerations. Moreover, BSA solutions (used usually as 1%) must be prepared fresh to prevent aggregation or microbial contamination, adding additional workload and inconsistency to the workflow [2].Therefore, synthetic, animal-free alternatives that provide homogenous blocking at low concentrations are highly desirable.

In typical IF experiments, the protein of interest (POI) is visualised by the combination of specific primary antibody and the secondary antibody with a suitable fluorophore (Alexa dyes). Specific organelles or cellular structures can be visualised to asses the localisation, transport or dynamic changes of the POI [3], [3], [5]. In our lab, we routinely evaluate the organization of microtubules (by tracking α-tubulin) and the state of the cell nucleus (DAPI) [6]. Consistent and homogeneous blocking is essential to obtain high-contrast, reproducible fluorescence signals with minimal background and the fluorescent signal can serve as a blocking performance check.

Sophomer F10 is a fully synthetic polymer designed as an animal-free alternative to BSA, previously validated for use in ELISA and immunoblotting. The objective of this application note is to **evaluate Sophomer F10 for blocking fixed HeLa cells** in immunofluorescence microscopy and assess its compatibility with antibody-based tubulin staining. We tested a range of Sophomer F10 concentrations (0.5%, 0.1%, 0.01%) and compared staining quality, background noise, and microtubule visualization.

Refferences:

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[5] Lichtman JW, Conchello JA. Fluorescence microscopy. Nat Methods. 2005 Dec;2(12):910-9. doi: 10.1038/nmeth817.

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Materials and Equipment

- Product under evaluation: **Sophomer F10**. *SophoMer F10 was obtained from Sophomer s.r.o., Prague, Czechia*
- Cell line**:** HeLa (ATCC) cultivated in cell culture medium: DMEM (Merck)
- Sample supports: 8-well chamber µ-Slide (IBIDI)
- Fixation reagents: Methanol, acetone, formaldehyde (Penta)
- Buffers: PBS, PBS-T (PBS + 1% Tween-20)
- Blocking agents: BSA (Merck), Sophomer F10 (Sophomer)
- Antibodies: Primary antibody: anti-α-tubulin, clone DM1A (Merck), Secondary antibody: goat anti-mouse IgG conjugated with Alexa Fluor 488 (ThermoFisher)
- Nuclear stain: DAPI (Merck)
- Other reagents: Mowiol mounting medium (Merck)
- Equipment: routine cell culture equipment, –20°C freezer, Shaker/rocker, Fluorescence microscope (Olympus IX51, Japan)

Methodology

1. Sample Preparation

HeLa cells were seeded into an 8-well chamber µ-Slide (IBIDI) at a density of 5–10 × 10^3 cells per well in 250 µL DMEM and allowed to adhere for 24 h. The medium was removed and cells were fixed with ice-cold methanol:acetone (1:1 v/v) for 10 minutes at –20 °C. Alternatively, cells were washed with PBS, fixed by 10 %formaldehyde in PBS for 10 minutes at room temperature, followed by multiple washes with PBS. Slides were allowed to dry for 10 minutes and stored sealed at –20 °C until use.

2. Rehydration and Blocking

Before staining, cells were rehydrated with PBS-T for 3 minutes at room temperature. Different blocking conditions were tested:

- Traditional control: 1% BSA in PBS-T (1 h at RT)
- Tested polymer: Sophomer F10 at 0.5%, 0.1%, 0.01% in PBS-T (1 h at RT)

3. Immunostaining Workflow

1. Wash 1× in PBS-T (3 min).
2. Apply primary antibody anti-α-tubulin (DM1A) diluted 1:100 in blocking solution (BSA or Sophomer F10). Incubate 1 h at RT on a rocker.
3. Wash 2× in PBS-T (3 min each).
4. Apply secondary antibody Alexa Fluor 488 goat anti-mouse IgG diluted 1:500 in blocking solution. Incubate 1 h at RT.
5. Wash 2× in PBS-T (3 min), then 1× in PBS (3 min).
6. Stain nuclei with DAPI **(**1 µg/mL in PBS) for 10 minutes.
7. Wash 2× in PBS.
8. Mount in Mowiol and examine on an Olympus IX51 fluorescence microscope.

4. Evaluation Parameters

- Homogeneity of fluorescence signal (visibility and continuity of microtubule filaments)
- Background fluorescence
- Specificity and intensity of microtubule staining, detection of microtubular changes upon treatment
- Nuclear staining clarity

- Comparison with 1% BSA-blocked samples

Results

Across all tested concentrations, Sophomer F10 provided efficient and homogeneous blocking of fixed cells, preventing non-specific binding and allowing high-contrast visualization of microtubules and nuclei.

Microtubule Staining Quality

Cells blocked with Sophomer F10 at 0.5%, 0.1%, and 0.01% exhibited:

- Clear visualization of α-tubulin filaments, well-resolved microtubule networks with minimal background, detection of the microtubular changes in the treatment
- Comparable or enhanced fluorescence intensity relative to the 1% BSA control
- No visible disruption or collapse of microtubule architecture in control cells

Lower concentrations (0.1% and 0.01%) still provided excellent blocking, demonstrating that substantially lower polymer amounts are sufficient.

Background Signal and Noise

Sophomer F10-blocked samples showed:

- Same or lower autofluorescence compared to BSA
- Same/better resolution and contrast between microtubules and surrounding structures

DAPI staining was unaffected and consistently bright across all tested conditions.

Representative Fluorescence Images

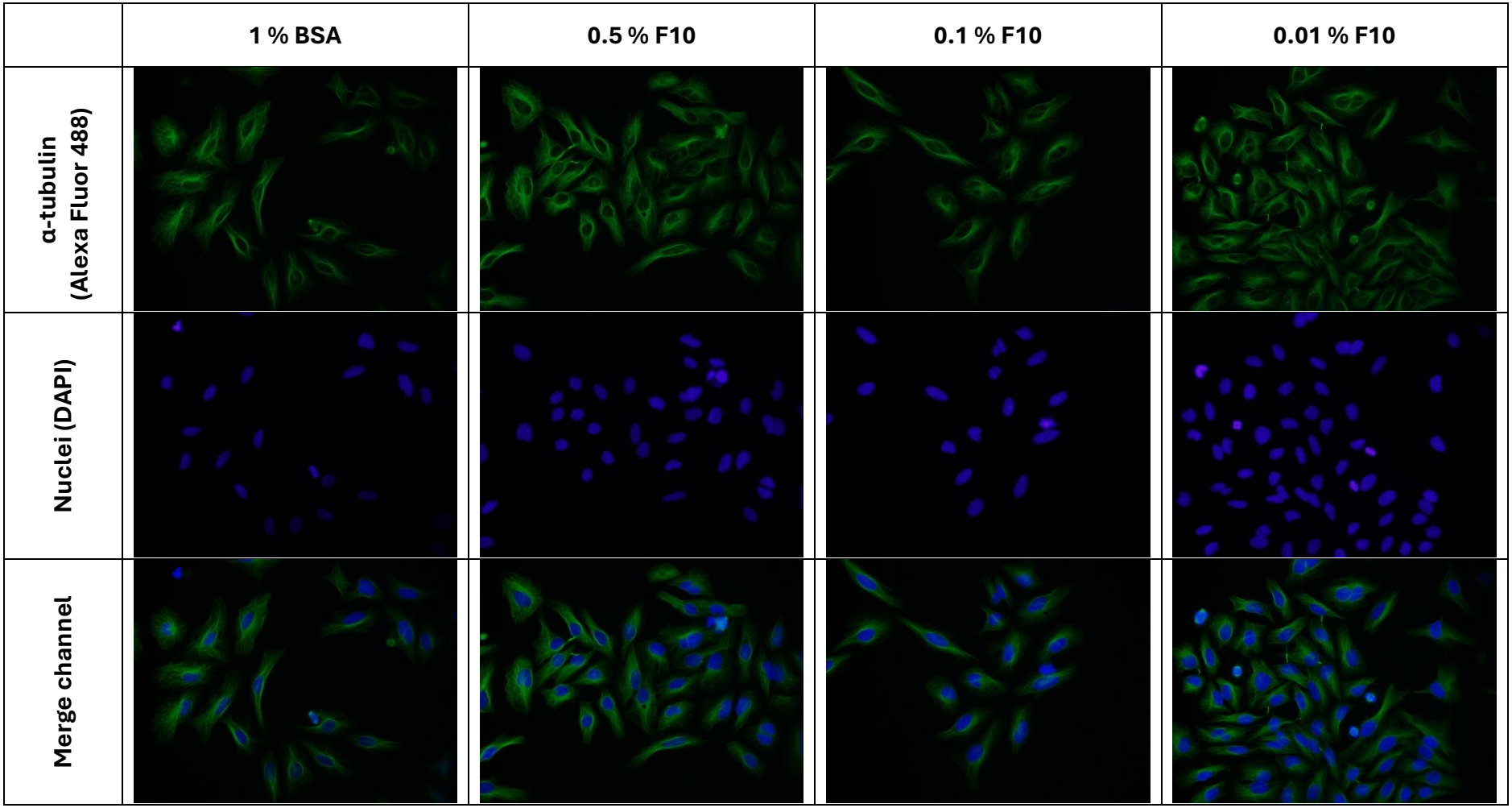

Figure 1 *Immunofluorescence staining of α-tubulin (Alexa Fluor 488, green) and nuclei (DAPI, blue) in untreated HeLa cells fixed by methanol:acetone (1:1* v/v*) and blocked with varying concentrations of Sophomer F10 or 1% BSA. Magnification 400x.*

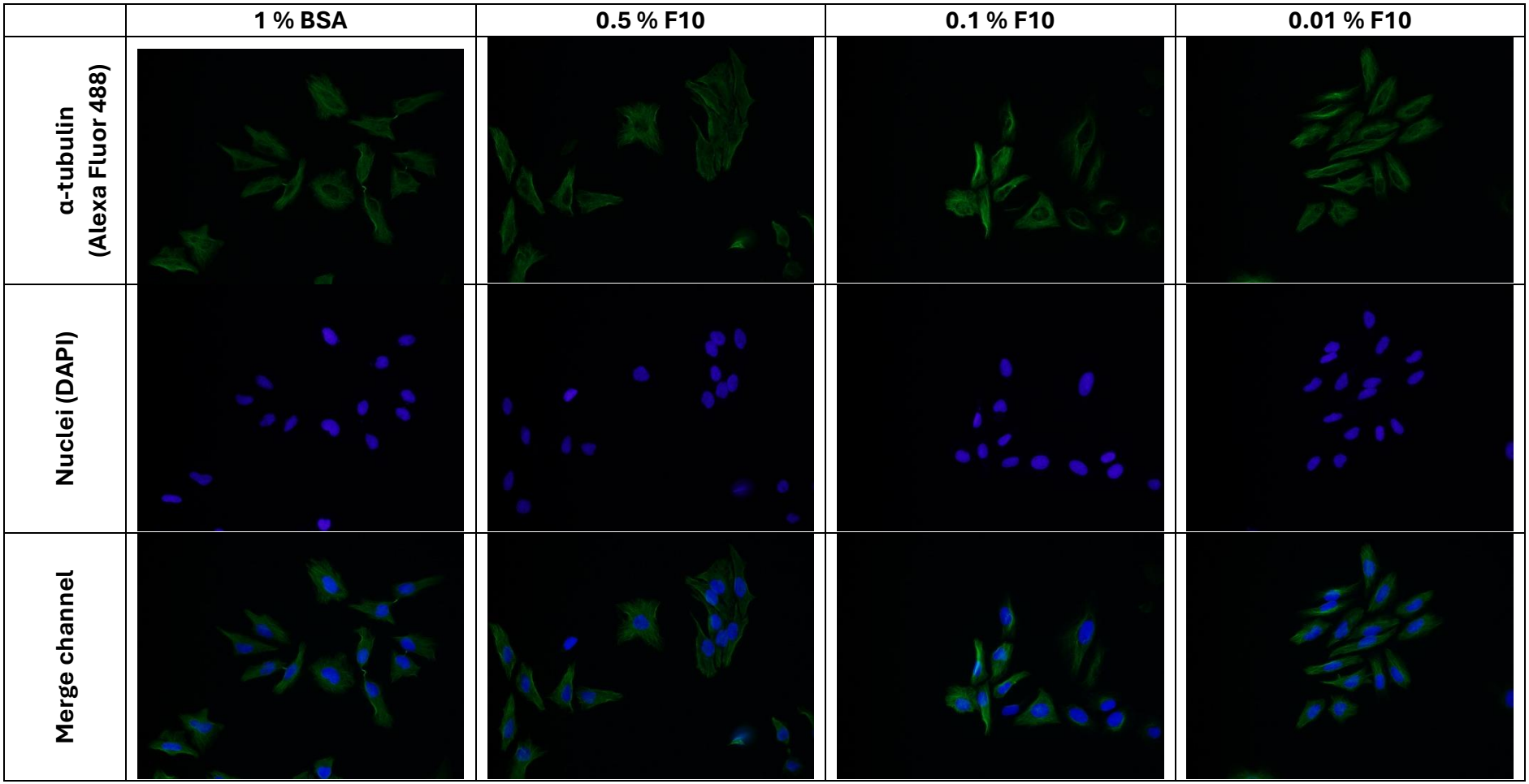

Figure 2 *Immunofluorescence staining of α-tubulin (Alexa Fluor 488, green) and nuclei (DAPI, blue) in untreated HeLa cells fixed by 10% formaldehyde and blocked with varying concentrations of Sophomer F10 or 1% BSA. Magnification 400x.*

Conclusion

Sophomer F10 proved to be a reliable and highly effective synthetic blocking agent for immunofluorescence microscopy of fixed cells. At concentrations as low as 0.01%, it provided homogeneous blocking comparable to or better than traditional 1% BSA while reducing background fluorescence. Importantly, upon the Sophomer blocking, the resolution and integrity of the filaments were comparable to—or in some cases even better than—those observed with BSA, confirming excellent compatibility with cytoskeletal structures.

Key advantages observed include:

- **Animal-free, fully synthetic reagent**
- **Lower required concentrations than BSA**
- **Consistent batch-to-batch performance**
- **Excellent compatibility with primary and secondary antibodies used for IF**
- **Reduced background, enabling clearer microtubule visualization**

These results demonstrate that Sophomer F10 is a robust, user-friendly alternative to BSA for immunofluorescence experiments and can significantly improve workflow reproducibility while maintaining high-quality filament resolution.

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