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# Biomolecule Interaction Studies Using Pattened Surfaces and Single-Molecule Fluorescence Microscopy

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Over the last 20 years, single-molecule (SM) fluorescence microscopy has evolved significantly, becoming a powerful tool for addressing complex biological questions. These methods enable the monitoring of dynamic interactions, conformational fluctuations, and biomolecular movements in both *in vitro* and *in vivo* systems. Among next-generation SM biophysics approaches, the DNA Curtains technique stands out as an effective method for studying protein-DNA interactions at the single-molecule level [1]. In our laboratory, we have developed a nanoscale platform based on this technique, where long DNA molecules are stretched along a surface using buffer flow [2]. Biotin and digoxigenin (dig)-labeled DNA molecules are immobilized on nanoscale lines composed of streptavidin or traptavidin proteins, which are patterned on silanized and PEGylated glass coverslips. The immobilized DNA molecules are then fluorescently labeled for analysis.

In addition to studies *in vitro*, SM fluorescence microscopy is also applied in live biological systems, allowing the investigation of biomolecular behavior under natural conditions. In our laboratory we aim to develop methods for protein-DNA studies in *Schizosaccharomyces pombe*, which serves as an excellent eukaryotic model organism. Ensuring the positional stability of *S. pombe* yeast cells during long-term SM studies requires precise surface structuring of proteins, for example, lectin that interact with cell surface molecules.

Both *in vitro* and *in vivo* SM studies demand precise glass surface preparation.This can be achieved by micro-contact printing (μCP) soft lithography technique, which utilizes a PDMS stamp [3]. Here, we present the surface patterning methods employed in our laboratory, highlighting their applications in DNA-protein interaction studies and live-cell imaging. In addition to these actual aplications, the aforementioned approaches, combined with quantum sensing of NV/SiV-containing sub-micron size diamonds, show potential for studying biomolecule interactions at the molecular level while simultaneously monitoring multiple environmental parameters.

REFERENCES

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