A high-throughput optical biosensor platform for *in vitro* monitoring DNA conformation and DNA-protein interaction

A high-throughput platform combining Spectral Self-Interference Fluorescence Microscopy (SSFM) [1] and DNA microarray technology is introduced as a novel tool to study surface-immobilized DNA conformation [2] and DNA-protein interactions [3]. SSFM maps the spectral oscillations emitted by fluorophores located above a reflecting surface into a precise height determination relative to the surface. In contrast to earlier fluorescence interference microscopy techniques that rely on intensity variation of total emission, SSFM utilizes the spectral information and provides sub-nanometer accuracy with a single measurement. Recently, we have upgraded SSFM into dual-color modality to be able to accurately determine two axial positions at the same location. Also, SSFM is combined with label-free quantification to enable simultaneous monitoring both conformation and molecular mass density during an interaction in real-time. Using monolayers of proteins as well as single and double-stranded DNA we have demonstrated sub-nanometer axial height determination for thin layers of fluorophores [1]. SSFM has also been used to estimate the conformation of single-stranded DNA and the average orientation of double-stranded DNA of different lengths, and the amount of hybridization [2]. By applying a smart polymeric surface, we can manipulate surface-immobilized double-stranded DNA orientation and quantify with SSFM [4]. Understanding the conformation of the surface-immobilized probes and characterizing the binding affinities will aid in optimizing biosensing surfaces. With SSFM, we are developing a nano-engineered molecular probe surface utilizing programmable DNA linkers to optimize the surface capture efficiency of viruses at low concentrations. The quantification of DNA conformations and conformational changes, when combined with new surface functionalization techniques and label-free quantification of biomass density on surfaces, provides critical information for studying DNA-protein interactions forwarding DNA microarray technology into a new realm of applications.

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