INVESTIGATING DNA BINDING KINETICS BY CAMERA-BASED TOTAL INTERNAL REFLECTION FLUORESCENCE CORRELATION SPECTROSCOPY (TIR-FCS)

Jonas Mücksch*,[1], Philipp Blumhardt*,[1], Maximilian Strauss[1,2], Ralf Jungmann[1,2], Petra Schwille[1]

[1] Max Planck Insititute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany
[2] Ludwig Maximilian University, Geschwister-Scholl-Platz 1, 80539 Munich, Germany

*** authors contributed equally**

E-mail: muecksch@biochem.mpg.de

KEY WORDS: Fluorescence Correlation Spectroscopy (FCS), Total Internal Reflection Microscopy (TIRF), DNA hybridization

Fluorescence correlation spectroscopy (FCS) has been used extensively to study the kinetics of various *in vitro* and *in vivo* systems on a molecular level. The vast majority of FCS studies is performed using confocal setups, which feature well-defined detection volumes but suffer from low surface selectivity. Combining FCS with total internal reflection fluorescence (TIRF) illumination drastically enhances the spatial selectivity and enables the investigation of reversible binding of fluorescently labeled ligands to surface-confined receptors. So far, this potential to observe and quantify surface binding using TIR-FCS has been used only to minor extent. Here, we present a versatile optical setup for exploring surface-binding kinetics with TIRF illumination and point- (APD) or camera-based (EMCCD) fluorescence detection. In a first application, our camera-based assay facilitated the investigation of the transient hybridization of fluorescently labeled single-stranded DNA to the complementary handles of a surface-immobilized DNA origami scaffold. We varied the nucleotide overlap, yielding different binding times in the range of milliseconds to seconds. Using this highly tunable system, we systematically explored the parameter space accessible to EMCCD-based TIR-FCS.