

TOWARD ABSOLUTE MOLECULAR NUMBERS IN DNA-PAINT

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Single-molecule localization microscopy (SMLM) has revolutionized optical microscopy, extending resolution down to the level of individual molecules. However, the actual counting of molecules relies on preliminary knowledge of the blinking behavior of individual targets or on a calibration to a reference. Particularly for biological applications, great care has to be taken, since a plethora of factors influence the quality and applicability of calibration-dependent approaches to count targets in localization clusters particularly in SMLM data obtained from heterogeneous samples. Here, we present localization-based Fluorescence Correlation Spectroscopy¹ (lbFCS) as the first absolute molecular counting approach for DNA-PAINT microscopy and, to our knowledge, for SMLM in general. We demonstrate that lbFCS overcomes the limitation of previous DNA-PAINT counting and allows the quantification of target molecules independent of the localization cluster density. In accordance with the promising results of our systematic proof-of-principle study on DNA origami structures as idealized targets, lbFCS could potentially also provide quantitative access to more challenging biological targets featuring heterogeneous cluster sizes in the future.

Reference:

- (1) Stein, J.; Stehr, F.; Schueler, P.; Blumhardt, P.; Schueder, F.; Mücksch, J.; Jungmann, R.; Schwille, P. Toward Absolute Molecular Numbers in DNA-PAINT. *Nano Lett.* **2019**.