Detection and Correction of Optical Aberrations in Co-localization Single-Molecule Spectroscopy Experiments of DNA Replication

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Replication of DNA, being a fundamental biological process that is universal to all forms of life, is a scientific topic that has been studied for many decades. Still, however, there are essential mechanisms involved in this highly complex biochemical reaction of which the details are unknown, and we need technological innovation to be able to study them in more detail. Some of these mechanisms, such as the party-by-part loading of the multi-protein replisome and the inheritance of histones can be studied well using single-molecule microscopy techniques.

Here, we present experimental and computational approches to quantify various kinds of optical aberrations in Co-localization Single-Molecule Spectroscopy (COSMOS) experiments of DNA replication and use that information to enhance the precision and accuracy of the imaging technique. First, we have developed algorithms that can fit various kinds of optical aberrations to simulated grid images. Second, we designed and performed an experiment with quantum dots and piezo stages to capture a grid image in TIRF mode. Third, we show that our algorithms are able to detect and correct a mixture model of optical aberrations in our data. Lastly, we present our plans to develop an image correction pipeline that allows us to report on super-resolved co-localization of various replisome components.