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Looking for steps of individual enzymes moving along DNA

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Understanding the molecular mechanism of any motor activity involves determining the elementary step size with which it moves. RecBCD is a processive, DNA-based motor with both helicase and nuclease activities. To directly measure RecBCD's putative step size of 4 base pairs (1.4 nm), several technical advances were incorporated in a new high-resolution optical trapping instrument capable of resolving 0.1 nm motion. First, mechanical drift was eliminated by developing a differential measurement system based upon improved laser beam-pointing stability and the introduction of a fiducial mark attached to a microscope coverslip (e.g., a stuck bead). To generalize this technique to measure subnanometer vertical motion, we intensity stabilized the detection laser and differentially amplified the vertical signal. We further enhanced this process by actively stabilizing the sample in 3D. In the presence of substantial thermal heating, 3D differential measurements with active stabilization achieved short term (1 s) stabilities of 0.13, 0.08 and 0.22 nm (RMS) in x, y, and z, respectively. Positional stability, as demonstrated by our differential subtraction, does not guarantee subnanometer resolution of an optically trapped bead *under load*. We therefore intensity stabilized our trapping laser to $\sim$0.1% at 100 Hz. Finally, our technique requires a DNA tether to be within a small distance (3 μm) of a fiducial mark. If the stuck bead is too close or directly along one of the primary axes of the stage motion, it interferes with the measurement. Since the location of stuck beads and DNA tethers is random, this leads to only a few stuck bead/DNA tether pairs that can be successfully used. To overcome these limitations, we developed a regular grid of nanoposts. We will present our progress on integrating these technological advances to measure individual steps of RecBCD.