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**Feedback stabilized fluorescence microscope integrated with custom atomic force microscope for biological applications.**

While fluorescence imaging and atomic force microscopes (AFMs) are widely used to visualize, track, and manipulate single biomolecules, the resolution of these methods is limited by sample drift. To minimize drift, active feedback methods have recently been used to stabilize single molecule microscopes on the sub-nanometer scale. However, these methods require high intensity lasers which limit their application in single molecule fluorescence measurements. Furthermore, these feedback methods cannot be used to track user-defined regions of the sample, but rather monitor the relative displacement of an unknown point on a fiducial marker. To overcome these limitations, we have developed a novel optical based approach, using low laser intensities that are compatible with single molecule fluorescence measurements, to track the displacement of a user-chosen point on a sample at high frequency and to stabilize the sample with ultra-high accuracy and precision. Our method tracks sample displacement at 8.6 kHz and stabilizes the sample with 15 pm accuracy and 130 pm precision.

While our ultra-stable fluorescence microscope platform can be integrated with an AFM, this requires the design of a highly customizable AFM platform. We therefore developed a custom, Field Programmable Gate Array (FPGA) controlled AFM which is readily integrated into our FPGA controlled sample scanning confocal fluorescence microscope. Our integrated AFM-fluorescence microscope is easily programmed for a variety of unique biological applications. The collimated laser design in our AFM allows for an adjustable sensitivity depending on the range of forces required, and the large range piezo (100µm in all three dimensions) presents the freedom to perform precise measurement and manipulation in a high throughput manner, over a broad range of distances. We demonstrate a high bandwidth of 1MHz with low noise levels of less than 71pN at full bandwidth and below 6pN at a relevant bandwidth of 1kHz.

By integrating this custom AFM into our fluorescence platform, we unlock the capability to perform novel imaging and manipulation experiments. We demonstrate the ability to collect scanned fluorescence images in biological conditions while applying discrete forces to an area of interest by robustly synchronizing the sample stage scanning to lateral AFM tip motion, a brand new way to image cell dynamics under force.