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Localized Heating of Single Nucleic Acids using Infrared Light
DAVID NESBITT, JILA/NIST/University of Colorado, ERIK HOLMSTROM, JILA/University of Colorado — To alleviate many of the shortcomings associated with bulk sample heating at the objective of a microscope, we have developed a system that uses a cw IR laser (1455 nm) to locally heat a small volume of water via absorption in the second overtone of the OH stretch. By focusing the IR laser onto the confocal volume of a single-molecule fluorescence microscope, we can rapidly and locally adjust the sample temperature. A resonantly absorbing thin water cell placed after the sample but before our detection system removes residual transmitted IR light, allowing for continuous observation of fluorescent molecules while heating. With time-correlated single-photon counting and the known temperature-dependent lifetime of Rhodamine B, we have calibrated the range of temperature achievable by IR laser heating to be from room temperature to greater than 90 $^{\circ}$C. We apply this system to the melting of duplex nucleic acids tethered to a glass surface. Additionally, we demonstrate that the localized IR laser can function as a static heat source for temperature-dependent kinetic studies of RNA folding, which prove to be in excellent agreement with previous measurements using bulk stage heating.

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