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Absolute calibration of forces in optical tweezers

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Optical tweezers are highly versatile laser traps for neutral microparticles, with fundamental applications in physics and in single molecule cell biology. Force measurements are performed by converting the stiffness response to displacement of trapped transparent microspheres, employed as force transducers. Usually, calibration is indirect, by comparison with fluid drag forces. This can lead to discrepancies by sizable factors. Progress achieved in a program aiming at absolute calibration, conducted over the past 15 years, is briefly reviewed. Here we overcome its last major obstacle, a theoretical overestimation of the peak stiffness, within the most employed range for applications, and we perform experimental validation. The discrepancy is traced to the effect of primary aberrations of the optical system, which are now included in the theory. All required experimental parameters are readily accessible. Astigmatism, the dominant effect, is measured by analyzing reflected images of the focused laser spot, adapting frequently employed video microscopy techniques. Combined with interface spherical aberration, it reveals a previously unknown window of instability for trapping. Comparison with experimental data leads to an overall agreement within error bars, with no fitting, for a broad range of microsphere radii, from the Rayleigh regime to the ray optics one, for different polarizations and trapping heights, including all commonly employed parameter domains. Besides signaling full first-principles theoretical understanding of optical tweezers operation, the results may lead to improved instrument design and control over experiments, as well as to an extended domain of applicability, allowing reliable force measurements, in principle, from femtonewtons to nanonewtons.