

Colloidal lenses allow high-temperature single-molecule imaging and improve fluorophore photostability

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Although single-molecule fluorescence spectroscopy was first demonstrated at near-absolute zero temperatures (1.8 K)¹, the field has since advanced to include room-temperature observations², largely owing to the use of objective lenses with high numerical aperture, brighter fluorophores and more sensitive detectors. This has opened the door for many chemical and biological systems to be studied at native temperatures at the single-molecule level both *in vitro*^{3–4} and *in vivo*^{5–6}. However, it is difficult to study systems and phenomena at temperatures above 37 °C, because the index-matching fluids used with high-numerical-aperture objective lenses can conduct heat from the sample to the lens, and sustained exposure to high temperatures can cause the lens to fail. Here, we report that TiO₂ colloids with diameters of 2 μm and a high refractive index can act as lenses that are capable of single-molecule imaging at 70 °C when placed in immediate proximity to an emitting molecule. The optical system is completed by a low-numerical-aperture optic that can have a long working distance and an air interface, which allows the sample to be independently heated. Colloidal lenses were used for parallel imaging of surface-immobilized single fluorophores and for real-time single-molecule measurements of mesophilic and thermophilic enzymes at 70 °C. Fluorophores in close proximity to TiO₂ also showed a 40% increase in photostability due to a reduction of the excited-state lifetime.

Integrated optical elements such as colloidal lenses have been used to focus or collect light in a variety of applications. For example, hemispherical solid immersion lenses⁷ and spherical colloidal lenses have been used to construct a rotational sensor^{8,9}, to create an optomagnetic dimmer¹⁰, to manipulate a light beam¹¹, to generate two-dimensional micropatterns and nanopatterns^{12–14} and to optically couple one-dimensional arrays of colloidal particles¹⁵. Wenger and colleagues¹⁶ recently demonstrated that a latex microsphere in conjunction with a low numerical-aperture (NA) objective lens can be used for fluorescence-correlation spectroscopy with near single-molecule sensitivity. An efficient nanolens system based on gold nanosphere aggregates was shown to have a strong electromagnetic surface-enhanced Raman scattering (SERS) enhancement, demonstrating the potential of nanolenses for single-molecule SERS¹⁷. However, these techniques have not yet achieved true single-molecule sensitivity. Single-molecule detection in droplets¹⁸ and flowing sample streams¹⁹ has been demonstrated with long working distance and low-NA optics, but these approaches do not combine parallelization with the observation of time traces and have not been demonstrated at high temperatures. In one non-fluorescent single-molecule study, the temperature dependence of rotation and hydrolysis of the F1-ATPase was

monitored by attaching a polystyrene bead to the rotor subunit. The rotation rate of the motor was inferred by observing the bead motion in conditions ranging from 4 to 50 °C (ref. 20).

Geometric optics can be used to estimate the collection efficiency of a micrometre-sized colloidal lens. The configuration is illustrated in Fig. 1a and has been previously described for the lensing of a smaller fluorescent microsphere⁸. By selecting spherical colloids composed of a high-index refraction material such as TiO₂ ($n_2 \geq 2.0$), the absolute value of the exit angle in water ($n_1 = 1.33$) is always less than 25°, even for very large angles of incidence (Fig. 1b). If θ_0 is the semi-aperture of an external microscope objective, any exit ray with $\phi' - \theta' < \theta_0$ will be collected. TiO₂ colloidal lenses should therefore improve the effective NA of a low-NA air objective (0.5 NA, $\theta_0 = 30^\circ$) to $NA_{\text{eff}} = (2.0)\sin(84^\circ) = 1.99$. Higher values of θ will probably cause the light to undergo total internal reflection (TIR) within the colloid.

Finite-difference time-domain (FDTD) simulations were performed with the Meep software package (see Supplementary Information) to more rigorously determine how key parameters of the system affect the collection efficiency²¹. A point source was positioned next to a spherical colloid and the power P transmitted through a plane on the opposite side of the lens was calculated (Fig. 1c). The power was normalized by dividing it by the total power emitted by the point source in all directions without the colloid present. Different plane dimensions were chosen to represent the semi-aperture light collection of 14 different microscope objectives ($1^\circ \leq \theta_0 \leq 65^\circ$). The relationship between the index n_2 of the colloid and P is shown in Fig. 1d, where high-index colloids are shown to provide the greatest relative enhancement for objectives with small semi-apertures. For example, the collection efficiency of an $n_2 = 2.4$ colloidal lens-aided 0.3 NA air objective was improved nearly eightfold compared to that obtained using the objective alone. Even high-NA objectives had a modest approximately twofold improvement with the use of $n_2 = 1.6$ – 1.8 colloids. Simulations were also run to determine how P is influenced by changing the distance between the fluorophore and the colloid, the radius of the colloid, and the wavelength of the emitted light (Supplementary Fig. S1).

The simulations predict that $n_2 = 2.0$ colloids should allow a 0.5 NA air objective ($\theta_0 = 30^\circ$) to achieve a 44% improvement in light collection efficiency compared to a 1.4 NA oil-immersion objective ($\theta_0 = 67^\circ$). The simulations therefore predict an effective NA of 1.68, which is slightly lower than the 1.99 effective NA predicted by ray optics. We first tested this by imaging individual quantum dot nanoparticles and found that 2.0 μm TiO₂ colloidal lenses had an effective NA of ~ 1.44 . This substantially exceeds the performance of conventional air-gap objectives and matches all but the most exotic oil-immersion objectives in use today.

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