To The Associate Editor PRL

## Dear Editor

We would like to submit our paper "Direct verification of the fluctuation-dissipation relation in viscously coupled oscillators" for publication in Physical Review Letters. In this paper, we provide a direct experimental verification of the fluctuation-dissipation relation in a system of two hydrodynamically coupled colloidal particles trapped in separate optical tweezers in a viscous medium (water). In addition, we identify a resonance in the response function, which is a surprise given the overdamped nature of the dynamics.

The fluctuation-response relation is a pillar iof non-equilibrium statistical physics, relating the intrinsic fluctuations in thermal equilibrium to the systematic (linear) response to forces applied externally. The theorem has been widely used in microrheology applications to determine the characteristics of simple as well as complex, viscoelastic fluids. However, most research conducted in non-equilibrium statistical physics tacitly assumes the validity of the fluctuation-dissipation relation, and there exist few direct experimental verifications of the result, especially in systems composed of multiple Brownian particles that, additionally, are coupled hydrodynamically.

In this work, we use optical tweezers to trap two colloidal particles in separate traps in a viscous medium, and experimentally demonstrate that fluctuationdissipation relation holds. In addition, we observe that when one of the trapped particles is driven sinusoidally, the other displays an amplitude resonance at a particular drive frequency. This observation is facilitated by the fact that the particles are trapped extremely close to each other, with a surface-surface separation that is less than the particle radius. This necessarily complicates the experiment since we need to ensure that there is no cross-talk in both the trapping and detection schemes, so that the response of one particle does not spill into that of the other. We check all such systematics carefully, and finally demonstrate a clear amplitude resonance in the motion of the driven bead the first result of this kind in optical tweezers, where the viscous nature of the trapping medium damps out all inertial effects, thus precluding the observation of motional resonances. The width and center frequency of the resonance can be tuned, so that it can be used as a useful tool for accurate two-point microrheology. We specifically envisage this approach for measuring the viscosity of liquids using the trapped colloidal particles as probes. All our experimental results are also validated by a theoretical model of the system using appropriate fluid-mediated interactions represented by the mobility tensors.

We believe that this direct verification of the fluctuation-dissipation relation will be of interest to the entire readership of the journal, while the more specific aspects of the resonance and its use in microrheology will attract the attention of readers working in soft matter physics and fluid mechanics. We hope you will seek a peer review to determine the suitability of this manuscript for publication in Physical Review Letters.

Yours sincerely,

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