Single bacterial cell detection with nonlinear rotational frequency shifts of driven magnetic microspheres

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Shifts in the nonlinear rotational frequency of magnetic microspheres, driven by an external magnetic field, offer a dynamic approach for the detection of single bacterial cells. We demonstrate this capability by optically measuring such frequency shifts when an *Escherichia coli* attaches to the surface of a 2.0 μm magnetic microsphere, thereby affecting the drag of the system. From this change in drag, the nonlinear rotation rate was reduced, on average, by a factor of 3.8. Sequential bacterial cell attachments were also monitored. © *2007 American Institute of Physics*.
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Magnetic microspheres and nanoparticles have been used for a variety of applications and have even been incorporated into medical diagnostic techniques.[1–4] The translational properties of magnetic particles have proven to be extremely useful in biomedical procedures, such as magnetic separation.[1–3] Other applications include giant magnetoresistive sensors[5] and magnetic tunnel junction sensors.[6] It is possible, however, through standard microscopy techniques, to monitor the rotational behavior of single magnetic particles or small chains of them.[7–11] These small dynamic magnetic systems have been utilized to improve immunoassays,[12] act as micromixers,[8] study microrheology,[9,13] and reduce interfering backgrounds in fluorescent spectroscopy measurements.[7] The sensitive rotational dynamics of these magnetic systems offer potential uses in the detection of single microbiological agents. We report on such an application, demonstrating single and sequential cell detection of *E. coli*.

The method is based on the nonlinear rotational dynamics that a magnetic particle undergoes when driven by a rotating magnetic field and depends both on environmental conditions and on properties of the particle.[11,14,15] At low external driving frequencies, the magnetic particle rotates continuously and synchronously (linear response regime) with the external field. At sufficiently high external driving frequencies, the particle becomes asynchronous (nonlinear) with the driving field. The rotational dynamics of an actively rotated magnetic particle give the following,

$$\langle \dot{\theta} \rangle = \begin{cases} \Omega, & \Omega < \Omega_c \\ \Omega - \sqrt{\Omega^2 - \Omega_c^2}, & \Omega > \Omega_c, \end{cases} \tag{1}$$

where $\langle \dot{\theta} \rangle$ is the particle's average rotation rate and Ω is the driving frequency of the external magnetic field. The critical transition frequency is given by $\Omega_c = mB/\kappa\eta V$, where m is the strength of the magnetic moment of the microsphere, B is the external magnetic field amplitude, κ is the shape factor, η is the dynamic viscosity of the surrounding fluid, and V is the volume of the microsphere. Equation (1) holds for low Reynolds number environments ($\mathrm{Re} \ll 1$) and for our system $\mathrm{Re} \approx 5 \times 10^{-6}$. Rotation for $\Omega > \Omega_c$ is nonlinear.[16,17] We demonstrate that this regime can be used to detect single microbiological agents. For example, when a bacterium attaches to a nonlinearly rotating magnetic microsphere, the volume and shape of the rotating system are drastically changed, increasing the drag and thus slowing the rotation rate (see Fig. 1).

The ability to develop sensitive diagnostic techniques has been a topic of high interest,[18] especially with dynamic systems.[19] One related technology that has demonstrated extreme sensitivity in air and vacuum environments is the nanoelectromechanical systems (NEMS) approach.[20] NEMS have been used to detect a single microbiological agent, such as a virus or a bacterium.[21–24] One NEMS detection scheme utilizes resonant frequency changes when a microbiological agent attaches to a cantilever. However, the sensitivity of such NEMS devices is drastically reduced when operated in fluidic environments.[25] In contrast, the sensitivity of nonlinear rotating magnetic microspheres is based on changes in drag.[14] This allows for single biological agent detection in fluidic environments. Thus, we demonstrate single and sequential bacterial cell detection using frequency shifts in the nonlinear rotation of magnetic microspheres in a fluidic environment.

Measurement of this rotational frequency is straightforward when utilizing standard microscopy techniques. Magnetic microspheres were prepared by spreading a 20 μl aliquot of stock solution (1% w/v) of 2.0 μm ferromagnetic microspheres functionalized with goat antimouse IgG (Spherotech, Lake Forest, IL) onto a precut microscope slide. The sample was allowed to dry and then coated with ~50 nm of Al. The sample was placed in a uniform mag-

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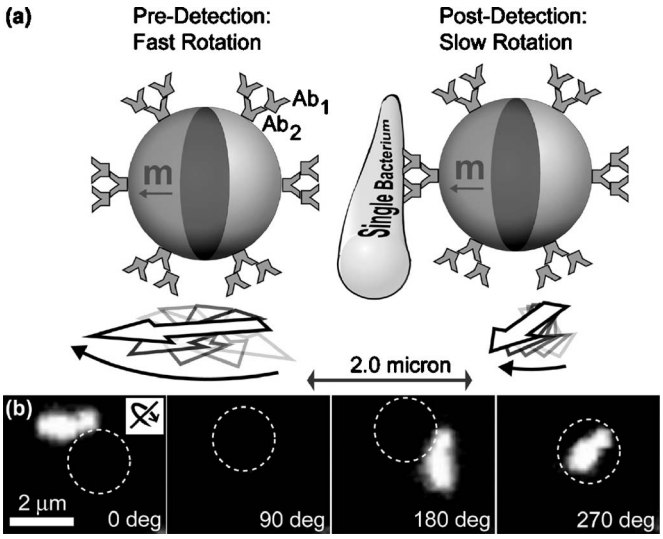

FIG. 1. (a) Schematic of the single cell detection and the nonlinear rotation rate changes, which a magnetic microsphere undergoes when bound to a bacterium. The magnetic microsphere is functionalized with a secondary antibody (goat antimouse IgG Ab_2) and primary antibody (mouse anti-*E.-Coli* IgG Ab_1). (b) Series of successive fluorescence microscopy images of a rotating 2.0 μm magnetic microsphere with an attached *E. Coli* bacterium. The dotted circles indicate the location of the magnetic microsphere, where the axis of rotation lies in the plane of the sample.

netic field of 1.4 kOe to induce magnetization perpendicular to the microscope slide. The spheres were collected and suspended in phosphate buffer solution (PBS) at a *p*H of 7.2. They were then functionalized with anti-*E.-coli* antibodies (Cortex Biochem, San Diego, CA), following the manufacturer procedures. Streptavidin magnetic microspheres functionalized with biotin conjugated anti-*E.-Coli* antibodies were also used. *E. coli* BL21(DE3) were made fluorescent by introducing a DsRed plasmid according to a previously described transformation technique.[26] The bacteria were allowed to grow until the sample reached an optical density of 0.67 at $\lambda=600$ nm, where the magnetic microspheres and bacterial solution were then mixed 1:1 (by volume). The resulting sample had many single microspheres with 1–5 *E. coli* bound to their surface, confirmed using visual analysis (fluorescence microscopy). Rotational frequencies were measured using bright-field, reflection, or fluorescence microscopy, and image analysis techniques reported elsewhere.[11,14]

Measurements were performed in two homemade ~100 μm thick fluidic cells, where experiments were carried out in a pure PBS ($\eta=0.001$ Pa's) or in or in a glycerol-PBS mass fraction of 0.5 ($\eta=0.006$ Pa's), where B=10 Oe. Average nonlinear rotational frequencies were determined using discrete Fourier transform techniques (higher harmonics have been filtered in the resulting power spectrum) to analyze the microspheres' intensity fluctuations. Sequential cell detection was performed by tracking the rotation rate of a magnetic microsphere dimer, where intensity modulations were measured using bright-field microscopy.

Theory and experiments for single magnetic particles, rotating in response to an external driving field, have been demonstrated,[11,14,15] but measurements have not previously been made for the case of a magnetic particle attached to a bacterium. Figure 2(a) shows the average rotation frequency of such a system for increasing external driving frequencies. The data are in good agreement with the fit determined from Eq. (1), and the critical onset of asynchronous rotation Ω_c was found to be 1.27 Hz. Since the rotational dynamics of the magnetic particle were in good agreement with Eq. (1), we assume that any forces resulting from bacterial motility were negligible. This measurement also shows that when a bacterium is bound to the surface of a magnetic microsphere, the system can still be analyzed using previously developed theory.[11,14] Thus, a change in rotational frequency can be used to detect pathogens such as bacteria.

While the entire range of frequencies for magnetic particles, with and without bacteria, could be scanned, as was done in Fig. 2(a), it is much faster and more straightforward to only measure the value of the nonlinear rotation frequency, $\langle\dot{\theta}\rangle$, at a given external driving frequency of Ω, where $\Omega>\Omega_c$. Figure 2(b) shows the result of such measurements in a fluidic cell for the rotation rates of 20 particles with bacteria and for 20 particles without bacteria. The presence of a bacterium on the surface of the magnetic microspheres caused a measurable change in the average rotation frequency, namely, the average frequency of the particles at a driving frequency of 4.0 Hz changed from $\langle\dot{\theta}_1\rangle=0.72$ Hz to $\langle\dot{\theta}_2\rangle=0.19$ Hz, by a factor of ~3.8. This change in rotation frequency is similar in value to our previous measurements on a 1.0 μm particle that was attached to a single 1.9 μm ferromagnetic microsphere.[14] Sequential attachment of bac-

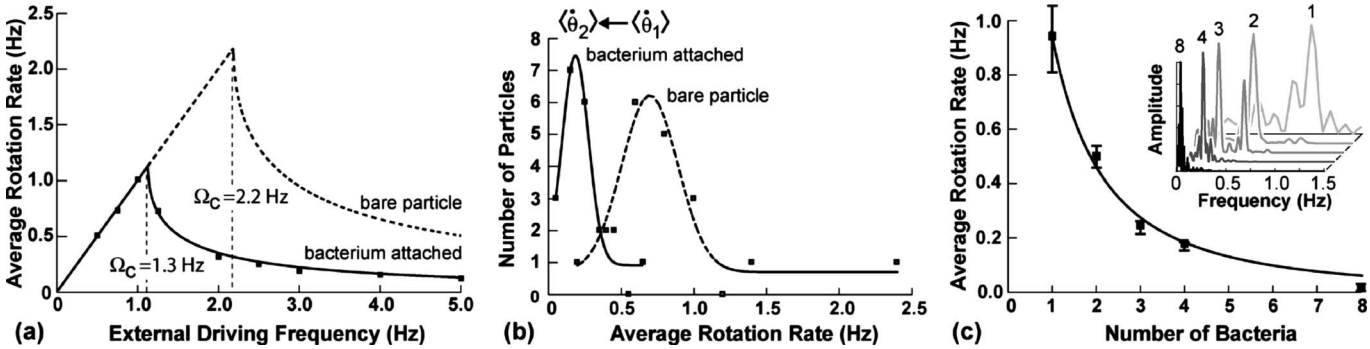

FIG. 2. (a) The rotational response of a single magnetic microsphere with an attached bacterium at various external driving frequencies, where the squares are experimental data and the line is a theoretical fit from Eq. (1) (the dotted line is an approximated curve for a microsphere without a bacterium). (b) The average nonlinear rotation frequency of 20 particles in a fluidic cell incubated with bacteria (solid curve) and a fluidic cell without bacteria (dashed curve). The magnetic microspheres with one bacterium attached rotated an average of 3.8 times slower than those without. (c) Average rotation rate of a magnetic microsphere dimer driven at 3.75 Hz, where 1, 2, 3, 4, and 8 bacterial cells were sequentially attached. The fit corresponds to an expected change in the nonlinear frequency, determined from Eq. (1) for incremental additions of volume. The inset shows the normalized power spectral density of the intensity fluctuations of the dimer with 1, 2, 3, 4, and 8 cells attached sequentially.

terial cells was also observed, on a dimer of magnetic microspheres [Figure 2(c)], showing single and multiple cell detection capabilities.

Once a bacterium is attached to a magnetic microsphere, this technique could also be used to monitor single bacterial cell growth. Indeed, preliminary experiments (to be published) have shown that shifts in the nonlinear rotational frequency are very sensitive to the growth of bacteria over several minutes. Changes in the nonlinear rotation frequency could therefore have further applications for the study of single bacterium growth dynamics and for rapid antibiotic susceptibility measurements.

In summary, optically monitored shifts in the nonlinear rotational frequency of driven magnetic microspheres were used to detect single bacterial cells as well as their sequential attachment, demonstrating the versatility of this dynamic approach. The nonlinear rotational frequencies of 2.0 μm magnetic microspheres were reduced, on average, by a factor of 3.8 (i.e., the rotational period increased by 280%), demonstrating single cell sensitivity in a fluidic environment. An additional feature of this method is its ability to detect single label-free bacterial cells.

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