Raman sorting and identification of single living micro-organisms with optical tweezers

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We report on a novel technique for sorting and identification of single biological cells and food-borne bacteria based on laser tweezers and Raman spectroscopy (LTRS). With this technique, biological cells of different physiological states in a sample chamber were identified by their Raman spectral signatures and then they were selectively manipulated into a clean collection chamber with optical tweezers through a microchannel. As an example, we sorted the live and dead yeast cells into the collection chamber and validated this with a standard staining technique. We also demonstrated that bacteria existing in spoiled foods could be discriminated from a variety of food particles based on their characteristic Raman spectra and then isolated with laser manipulation. This label-free LTRS sorting technique may find broad applications in microbiology and rapid examination of food-borne diseases. © 2005 Optical Society of America

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Since the first demonstration by Ashkin *et al.* in 1986,[1] optical tweezers have become a powerful tool to manipulate biological particles, such as living cells, bacteria, and viruses.[2] They are now routinely applied to the study of molecular motors at the single-molecule level,[3] the mechanical properties of polymers and biopolymers,[4] colloid and mesoscopic physics,[5,6] and the control of optically trapped structures.[7] Recently, Raman spectroscopy has been combined with optical tweezers to form a laser tweezers Raman spectroscopy (LTRS) or Raman tweezers system, which provides an effective means for both the manipulation and the characterization of single optically trapped particles in an aqueous environment.[8–10] In a LTRS system, a laser beam is tightly focused to form an optical trap, and the same laser beam is used to excite Raman spectra that contain information about the species, structure, and molecular conformation of the trapped particles; therefore the system can provide a fingerprint for identification.[11] Confocal LTRS has been developed and applied for identifying and characterizing single optically trapped nonbiological particles,[9] red blood cells,[8,12] yeast cells and bacterial cells,[10] and liposomal membrane.[13] Real-time Raman spectra from a trapped cell undergoing a heat-denaturation process have been studied.[10] LTRS has been applied for rapid identification of bacterial spores in aqueous solutions.[14]

In this Letter, we report on the use of the LTRS system for label-free sorting and identification of single biological particles. We demonstrate that biological particles in an aqueous solution can not only be identified by their Raman signatures but also can be sorted and collected by the LTRS system; this represents a new category of application. As an example, we demonstrate that live and dead cells existing in a native yeast culture in a sample chamber can be selectively manipulated and sorted into a clean collection chamber based on intrinsic differences in their Raman spectra. As another example, we demonstrate that the bacterial cells existing in spoiled food (milk) can be discriminated and isolated by the LTRS sorting system. It is known that bacterial contamination of foods not only causes food spoilage but also leads to food-borne diseases. LTRS may provide a rapid way to identify and isolate bacteria in food that is of importance to the public health.

This Raman sorting and identification method for single biological particles is different from flow cytometry, which is widely used for cell analysis.[15] In flow cytometry, biological particles are identified and sorted by the properties of particles' size, shape, or fluorescence, rather than by their intrinsic molecular vibration properties as in LTRS. Therefore, extensive sample preparations are needed for flow cytometry, which might have adverse postsorting effects to the cells. In addition, a large cell sample is required for flow cytometry; it may not be applied to a small number of cells. In contrast, in the LTRS sorting method, no sample preparation is required. A single cell in a sample chamber is identified by its Raman spectrum and then optically manipulated to a new chamber for collection. Therefore, the LTRS sorting method is sterile, noninvasive, and suitable for a tiny cell sample.

The principle of LTRS sorting and identification is illustrated in Fig. 1. An aqueous sample mixture that

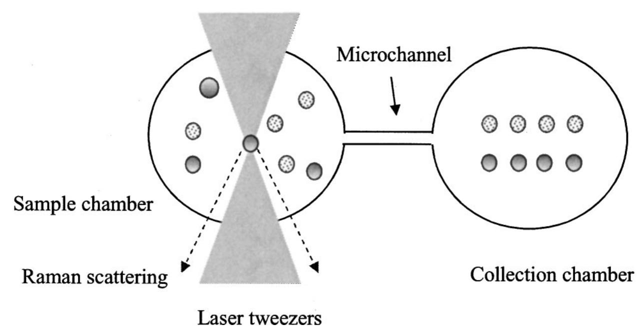

Fig. 1. Schematic of LTRS sorting and identification. A particle in the sample chamber is captured with laser tweezers, identified by Raman spectrum, and then optically manipulated to a clean collection chamber.

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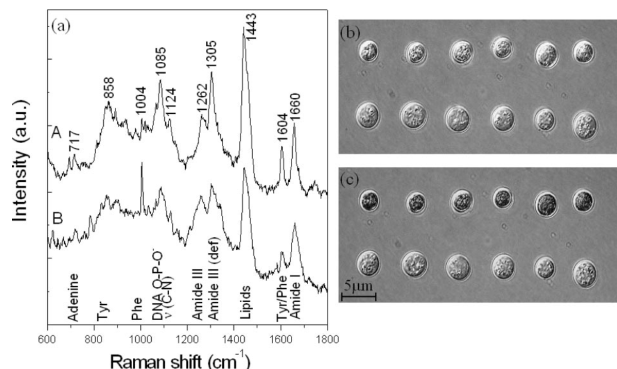

Fig. 2. (a) Near-infrared Raman spectra of single live yeast cells (curve A) and dead yeast cells (curve B) in a batch culture. The acquisition time was 20 s with a laser power of ~17 mw at 785 nm. Tyr, tyrosine; phe, phenylalanine; def, deformed. (b) Image of the sorted yeast cells in the collection chamber. Top row, dead yeast cells; bottom row, live yeast cells. (c) Image of the sorted yeast cells stained with 2% eosin solution.

contains both wanted and unwanted cells is loaded in the sample chamber. An unknown cell is first captured by the focused laser beam, and its Raman spectrum is then taken so that the cell is identified by a computer-aided algorithm as wanted or unwanted. After identification, the trapped cell is manipulated to a collection chamber through a microchannel (~10 μm wide and ~20 μm deep) by the same laser tweezers. The wanted cells can be placed in a specific row and unwanted cells in the other row. The sorted cells will adhere to the cover glass surface in the collection chamber with the use of 1% sodium chloride solution. The possibility of a cell migrating from one chamber to another one by Brownian motion is very low. However, a selected cell can be transported through a microchannel with the optical tweezers.

The experimental setup is similar to that in our previous report.[10] A laser beam from a wavelength-stabilized diode laser at 785-nm is circularized with a prism pair, spatially filtered, and then introduced into an inverted microscope (Nikon TE2000, Marietta, Georgia) equipped with an objective (100 ×, NA=1.30) to form a single-beam optical trap. A biological particle in an aqueous medium can be trapped with the gradient force yielded by the focused beam. The same laser beam excites Raman scattering from the trapped particle. The collimated backwards Raman-scattering light, after it is spatially and spectrally filtered, is focused onto the entrance slit of a spectrograph and detected by a liquid-nitrogen-cooled CCD (Symphony CCD, Jobin-Yvon, Edison, New Jersey). The image of the trapped particle can be viewed through a video camera system illuminated with a green-filtered lamp. The spectral resolution of the Raman system is estimated to be ~6 cm^{-1}.

Figure 2 shows that biological cells in different physiological states in a native culture sample can be identified and sorted by LTRS. In this experiment, yeast cells (Hubbard Scientific, Chippewa Falls, Wisconsin) were cultured in a 2% glucose medium for 12 h at 37 °C. The majority of the cells were alive, and there was a small portion of dead cells. Under a light microscope, the live and dead yeast cells show very similar images, and therefore it is difficult to discriminate the dead cells from the live cells without staining. However, it has been shown that the live cells generate very distinct Raman spectra from the dead cells,[10] and this intrinsic difference in spectra can be used to identify the physiological states of the cells in a batch culture. Figure 2(a) shows the Raman spectra of single live yeast cells (curve A) and dead yeast cells (curve B), in which the phenylalanine band at 1004 cm^{-1} shows much higher intensity in the dead cell spectrum because of protein denaturation.[10] In our LTRS sorting experiment, the cultured yeast cells were first loaded into the sample chamber, and the collection chamber was then filled with 1% NaCl solution to enhance cell adhesion to the cover glass. Then, a cell in the sample chamber was randomly chosen with the laser tweezers, and the Raman spectrum of the trapped cell was acquired. The acquired Raman spectrum was then compared with the spectra shown in Fig. 2(a). If the acquired spectrum was coincident with curve A, the trapped cell was identified as the live cell; on the other hand, if the acquired spectrum was coincident with curve B, the trapped cell was identified as a dead cell. Once the cell was identified, it was held by the trapping laser and then transported through the microchannel to a specified location of the collection chamber by moving the microscope stage. The above identification and sorting process was repeated for another randomly trapped cell. As the result, Fig. 2(b) shows the images of six sorted live cells (bottom row) and six dead cells (top row). From the cell images, it is difficult to identify the live and dead cells.

To validate our sorting results, the sorted cells in the collection chamber were stained with eosin dye of

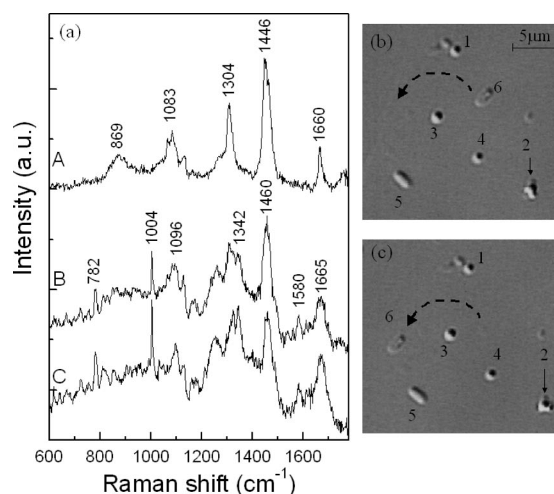

Fig. 3. (a) Near-infrared Raman spectra of three types of microparticles found in a spoiled milk sample. Curve A, spherical particles 1 and 2; curve B, spherical particles 3 and 4; curve C, rodlike particles 5 and 6. The acquisition time was 20 s for curve A and 60 s for curves B and C with a power of 20 mW. (b) Image of the microparticles in an aqueous spoiled milk sample. (c) Image of an isolated bacterial cell (particle 6) that was optically manipulated to a new location.

2% concentration. The eosin dye molecules entered the cell body only if the cell was dead. Therefore, the dead cells were stained and became colored. However, the live cells were still clear, since the eosin dye molecules could not pass through their cell walls. Figure 2(c) shows images of the sorted cells after staining. It can be seen that after staining all the cells in the top row turned red and the cells in the bottom row remained unchanged. This clearly indicates that the sorted cells in the top row are dead and those in the bottom row are alive. This verifies the correctness and effectiveness of our sorting results that base on intrinsic Raman signatures. In general, the LTRS sorting method can be applied to sort cells in the other physiological states, not just limited to cells in live and dead states. By using a motorized stage and a galvanomirror steering beam, the sorting speed could be increased substantially.

The LTRS sorting system can also be used to discriminate and isolate bacteria in spoiled foods, such as milk and hamburgers. In food samples, bacterial cells show similarity in size and shape with some tiny food particles under a light microscope, which could be spherical, cylindrical, or irregular shapes in general. However, bacteria may have very different Raman spectra from those of the food particles and this signature can be used for bacterial identification and isolation. Figure 3 depicts the collected Raman spectra and microscope images of different particles in spoiled milk. In our experiment, a sample of 20 μL of spoiled milk was diluted with 1% sodium chloride solution and loaded in the sample chamber. Figure 3(b) shows the typical images of microscopic particles observed in this aqueous sample. Some of them are spherical (e.g., particles 1–4), some are rod shaped (e.g., particles 5 and 6), and the size of the particles varies within a few micrometers. In general, it is difficult to discriminate the bacteria from the lipid particles in the spoiled milk sample by their images. Based on the collected Raman spectra, we found that particles 1 and 2 generated similar spectra (curve A), as shown in Fig. 3(a). Particles 3 and 4 generated similar spectra (curve B), and particles 5 and 6 generated similar spectra (curve C). Curve A is the Raman spectrum of lipid particles in milk,[16] and curves B and C are the Raman spectra of bacteria in the spoiled milk.[11] The results shown in Fig. 3(a) demonstrate that there are obvious differences in Raman spectra between the food particles and bacterial cells. Food particles contain mainly starch, lipid, protein, and other materials, and the nucleic acid bands (782, 1096, and 1580 cm^{-1}) usually do not appear in the spectra of the food particles. Based on this signature, the LTRS sorting system allows isolation or collection of the suspected bacteria for further identification by optical manipulation. Figure 3(c) shows the image of a bacterium (particle 6) that was manipulated to a new position. In general, these identified bacteria can be moved to a clean collection chamber and thus are isolated for additional molecular analyses. Although we cannot determine the species of bacteria in this work, the LTRS still provides an effective tool for food inspection of bacterial contamination and for the isolation of suspected food-borne micro-organisms.

In summary, we have demonstrated a novel technique for label-free sorting and identification of single micro-organisms based on laser tweezers and Raman spectroscopy. In our scheme, a biological particle in an aqueous sample chamber is first captured by a trapping laser beam, identified by Raman-scattering spectrum, and then selectively sorted into a clean collection chamber by the trapping beam through a microchannel. Both live and dead yeast cells in a batch culture have been successfully sorted and validated. We have also demonstrated that bacterial cells that spoil the foods (milk) could be spectrally discriminated and isolated from the other food particles. This label-free Raman tweezers sorting technique may find broad applications in microbiology and rapid examination of food-borne diseases.

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