Near-infrared Raman spectroscopy of single optically trapped biological cells

Changan Xie, Mumtaz A. Dinno, and Yong-qing Li

Department of Physics, East Carolina University, Greenville, North Carolina 27858-4353

Received Ocrtober 8, 2001

We report on the development and testing of a compact laser tweezers Raman spectroscopy (LTRS) system. The system combines optical trapping and near-infrared Raman spectroscopy for manipulation and identification of single biological cells in solution. A low-power diode laser at 785 nm was used for both trapping and excitation for Raman spectroscopy of the suspended microscopic particles. The design of the LTRS system provides high sensitivity and permits real-time spectroscopic measurements of the biological sample. The system was calibrated by use of polystyrene microbeads and tested on living blood cells and on both living and dead yeast cells. As expected, different images and Raman spectra were observed for the different cells. The LTRS system may provide a valuable tool for the study of fundamental cellular processes and the diagnosis of cellular disorders. © 2002 Optical Society of America

OCIS codes: 170.5660, 170.1530, 120.3890.

Optical tweezers have become a useful tool with which to capture and manipulate biological particles, including cells, bacteria, viruses, and dielectric particles.[1–4] Biomechanical properties of single cells and macromolecules have been extensively studied with laser tweezers.[5,6] Possible photodamage to biological cells in optical traps has been investigated and in some cases identified.[7,8] Recently, researchers have combined a number of spectroscopic techniques, including absorption,[9] fluorescence,[10] and Raman spectroscopy,[11–13] with optical tweezers to characterize molecules contained in single organic microdroplets or microcapsules. Among these, Raman spectroscopy has a great advantage since it can provide information about species, structures, and molecular conformation within the particles. Raman spectroscopy may therefore provide a fingerprint for the identification of biological cells, and thus it can unmask what could be significantly different spectra for normal and abnormal cells such as cancer cells.[14] Another advantage of combining Raman spectroscopy and optical tweezers is that the Brownian motion of the microscopic particles in an aqueous solution can be confined to a small region so that observation of the characteristics of single particles over a long period is allowed. Recently, a Raman tweezers system was developed to study single trapped polystyrene beads with a power of 80 mW from a Ti:sapphire laser for both trapping and excitation.[13] However, obtaining Raman measurements of single trapped living cells is still challenging because the extremely weak Raman scattering from molecules confined to the small cellular space requires high-intensity excitation, which may cause severe photodamage to the living cells. For example, photodamage to single *Eschericia coli* cells in an optical trap was observed after a few minutes of trapping with a cw laser of 100 mW (790–1064 nm).[7]

In this Letter we report on a compact laser tweezers Raman spectroscopy (LTRS) system that combines optical tweezers and near-infrared (NIR) Raman spectroscopy for the manipulation and characterization of a single living cell. In our system, a low-power-diode laser (785 nm) is used both for laser trapping and for Raman excitation. At NIR wavelengths the absorption coefficient of a biological sample is usually low and the fluorescence background is small in comparison with visible excitations. The diode laser is operated in a power-switching scheme. When a cell is trapped, the diode laser is programmed to operate at low power (~2.0 mW at the sample). When a Raman measurement is taken, increasing the laser power (up to 20 mW) for a short period of time (typically 2.0 s for CCD acquisition) ensures high excitation intensity. After the spectrum is taken, the laser returns to low-power operation for trapping. This power-switching scheme meets the requirements for reducing photochemical and (or) thermal damage to the biological samples yet allows sufficient excitation power for Raman spectroscopy. The design of this LTRS system provides high sensitivity, making it possible to obtain Raman spectra from single living red blood cells (RBCs) or yeast cells placed in an optical trap. Spectroscopic differences between a living and a dead yeast cell in an aqueous solution were detectable with our system. To our knowledge, this is the first study of a single biological cell by use of Raman spectroscopy in an optical trap.

The experimental scheme is shown in Fig. 1. The elliptical beam from a laser diode (HL7852; Hitachi America, Dallas, Tex.) is made circular with an anamorphic prism pair, spectrally filtered, and then introduced into an inverted microscope equipped with an objective (100×; N.A., 1.25), forming a single-beam optical trap. The laser spot size at the focus is approximately 1–2 μm, and the particle is captured and held approximately 10–15 μm above the bottom cover plate of the sample holder. The homemade diode laser is temperature stabilized (within 0.01 °C) so that the linewidth of the single-mode output is maintained within a few tens of megahertz, and the long-term frequency drift is minimized.[15] A holographic notch filter, HNF1 (HNPF-785AR; Kaiser Optical Systems,

0146-9592/02/040249-03$15.00/0

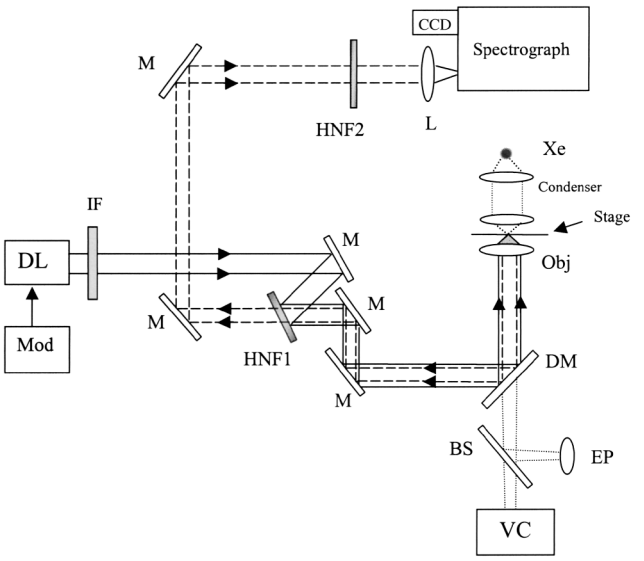

Fig. 1. Experimental setup: DL, diode laser; IF, interference filter; DM, dichroic mirror; HNF1, HNF2, holographic notch filters; M's, reflection mirrors; L, lens; BS, beam splitter; Obj, objective lens; EP, eyepiece; VC, video camera; Mod, modulation current pulses; Xe, green-filtered xenon illumination light.

Ann Arbor, Mich.), is used as a dichroic beam splitter that reflects the 785-nm excitation beam at an incident angle of 18° and transmits the Raman signals above 810 nm. The scattered light from the trapped particle is collimated with the same objective lens and passes through two notch filters that remove most of the Rayleigh scattering light. The beam is then focused onto the entrance slit of an imaging spectrograph. The spectrograph is equipped with a liquid-nitrogen-cooled, front-illuminated CCD (Spectrum One; Instruments SA, Edison, N.J.). A green-filtered xenon illumination light source and a video camera system are used for observation and recording of optical images of the trapped particles or cells. A pulse generator is used to modulate the driving current[15] and thus to increase the excitation power to 20 mW (with an estimated intensity of 0.5 MW/cm^2) during the CCD acquisition interval.

A polystyrene bead of 2.03-μm diameter (Bangs Laboratories) suspended in water was used for calibration and alignment of the LTRS system. Figure 2 shows the Raman spectrum of a trapped polystyrene bead with a CCD integration time of 2.0 s and a 600-g mm^{-1} spectrograph grating. The inset is an image of the trapped polystyrene sphere observed with a video camera. The background noise was taken without the trapped bead under the same acquisition time and height conditions and has been subtracted from the recorded spectra. The observed bands of the Raman signal are identical to those of published spectra,[13] and the observed linewidth of the 1001-cm^{-1} band shows that the Raman shift resolution of our LTRS system is better than 8 cm^{-1}.

Figure 3 depicts the Raman spectra obtained from a trapped, living RBC in saline solution. A fresh blood sample obtained from a healthy volunteer was diluted to 1:10000 with 0.9% saline solution. A cell typically drifts at a speed of a few micrometers per second in the bulk solution before it sticks to the bottom cover plate. Once the cell is captured in the trap, its movement range is less than 300 nm. The inset in Fig. 3 is an image of a RBC that was held ~15 μm above the bottom plate. The Raman spectrum from this cell was recorded with an exposure time of 5.0 s and an excitation of 20 mW, and the trapping power was kept at 2.0 mW. Curve (a) is the spectrum recorded from a trapped RBC, (b) is the background spectrum without an RBC in the trap under the same acquisition conditions, and (c) is the Raman spectrum after background subtraction. Several characteristic bands are observed at 667 cm^{-1} [ν_7 haem], 751 cm^{-1} [ν_{14} haem

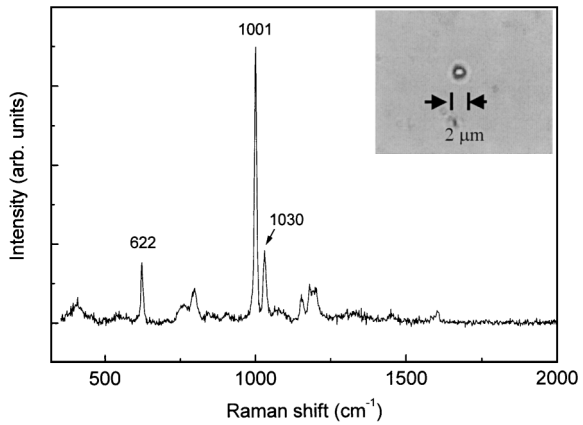

Fig. 2. Raman spectrum and (inset) image of a single polystyrene latex bead of 2.03-μm diameter in an optical trap. The trapping power is 2.0 mW and the CCD acquisition time is 2.0 s with 20-mW excitation.

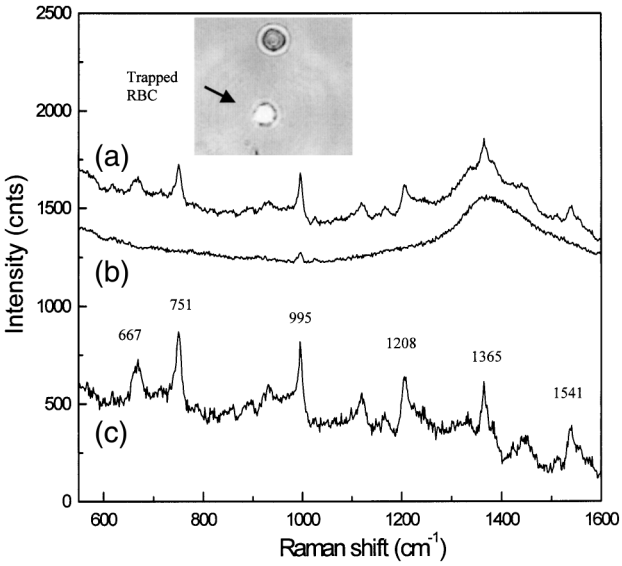

Fig. 3. NIR Raman spectra and image of a single RBC (erythrocyte) in saline solution. The acquisition time is 5.0 s with 20-mW excitation. Curve (a) is the spectrum recorded when a RBC is trapped, curve (b) is the background without a RBC in the trap, and curve (c) is the subtraction between (a) and (b), magnified by 2 for display.

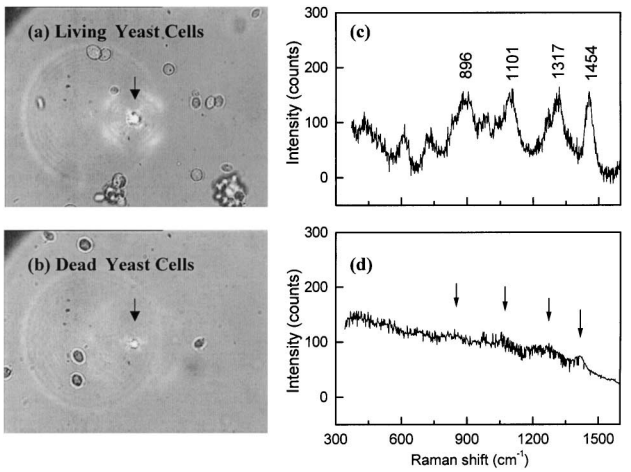

Fig. 4. NIR Raman spectra and images of a single living yeast cell and a dead yeast cell in solution. A significant difference can be seen in the Raman spectra of the living and the dead cells.

vibration], 995 cm^{-1} [$\delta(C_\beta C_1)$], 1365 cm^{-1} [$\nu_4(C_\alpha N)$], and 1541 cm^{-1} [$\nu_{11}(C_\beta C_\beta)$] and are identical to those of assigned and published bands.[16] Note that, since the excitation size (~2 μm) is smaller than the cell size (~7 μm), the spectra collected from the trapped RBC cell come from a part of the cell rather than the entire cell. The characteristic bands from the trapped RBC did not change significantly during 30 min. However, if the cell was trapped and excited with a cw laser of 20 mW, the characteristic bands disappeared after ~15 min because of the photodamage effect (not shown).

NIR Raman spectroscopy can also be used to identify the physiological states of single cells in an optical trap without the need for staining. Figures 4(a) and 4(b) show microscope images of living and dead yeast cells in the optical trap, respectively. The living yeast cells (Hubbard Scientific) were cultured in a yeast solution (prepared at a concentration of 40 g/L) at room temperature. We prepared the dead yeast cells by sinking a tube of living yeast cell solution in a boiling water bath for 10 min and then gradually cooling the tube to room temperature. From the images in Figs. 4(a) and 4(b), one cannot clearly distinguish the living cells from the dead cells when they are unstained, although the living cells appear a little more transparent than the dead cells. However, the Raman spectrum show a significant difference between the living and the dead cells. Figure 4(c) shows Raman spectra of a living yeast cell with four characteristic bands: 896, 1101, 1317, and 1454 cm^{-1}. Figure 4(d) shows the spectrum obtained in the same exposure (5.0-s) and excitation (20-mW) conditions for a trapped dead yeast cell: The characteristic bands disappeared and the scattering background at low-frequency shifts increased. These results show that the boiling water killed the yeast cells and changed their histology and molecular configuration. Note that a trapped cell remains alive for more than 30 min at this low trapping power.

In summary, we have developed a compact LTRS system that combines the advantages of NIR Raman spectroscopy and optical tweezers for the characterization of single biological cells with a low-power semiconductor laser. The laser power-switching technique allows low-power trapping (~2.0 mW) and high-power Raman excitation (~20 mW) of a biological cell. Single red blood cells and yeast cells were trapped and Raman spectra from them were recorded with our LTRS system. Identification of differences in the Raman spectra of living and dead yeast cells was demonstrated. Using the LTRS system, one may obtain molecular information from *in vitro* single biological cells that may be useful for the understanding of fundamental cell processes and for diagnosis of cellular disorders. To our knowledge, this is the first report of a LTRS study of living cells.

Y.-Q. Li acknowledges support from Oak Ridge Associated Universities through the Ralph E. Powe Junior Faculty Enhancement Award program and a College Research Award from East Carolina University. Y.-Q. Li's e-mail address is liy@mail.ecu.edu.

References

1. A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and S. Chu, Opt. Lett. **11,** 288 (1986).
2. A. Ashkin, K. M. Dziedzic, and T. Yamane, Nature **330,** 769 (1987).
3. K. Svoboda and S. M. Block, Annu. Rev. Biophys. Biomol. Struct. **23,** 247 (1994).
4. M. P. Sheetz, ed., *Laser Tweezers in Cell Biology*, Vol. 55 of Methods in Cell Biology (Academic, San Diego, Calif., 1998).
5. J. Guck, R. Ananthakrishnan, T. J. Moon, C. C. Cunningham, and J. Kas, Phys. Rev. Lett. **84,** 5451 (2000).
6. K. Visscher, M. J. Schnitzer, and S. M. Block, Nature **400,** 184 (1999).
7. K. Neuman, E. H. Chadd, G. F. Liou, K. Bergman, and S. M. Block, Biophys. J. **77,** 2656 (1999).
8. K. Konig, H. Liang, M. W. Berns, and B. J. Tromberg, Nature **377,** 20 (1995).
9. N. Kikatani, H.-B. Kim, S. Habuchi, and M. Chiba, Trends Anal. Chem. **18,** 675 (1999).
10. Y. Liu, G. J. Sonek, M. W. Berns, K. Konig, and B. J. Tromberg, Opt. Lett. **20,** 2246 (1995).
11. M. Lankers, J. Popp, and W. Kiefer, Appl. Spectrosc. **48,** 1166 (1994).
12. K. Ajito, Appl. Spectrosc. **52,** 339 (1998).
13. K. Ajito and K. Torimitsu, Trends Anal. Chem. **20,** 255 (2001).
14. S. R. Hawi, W. B. Campbell, A. Kajdacsy-Balla, R. Murphy, F. Adar, and K. Nithipatikom, Cancer Lett. **110,** 35 (1996).
15. Y. Q. Li, W. H. Burkett, and M. Xiao, Opt. Lett. **21,** 982 (1996).
16. B. R. Wood, B. Tait, and D. McNaughton, Biochim. Biophys. Acta **1539,** 58 (2001).