Non-destructive Analysis of the Nuclei of Transgenic Living Cells Using Laser Tweezers and Near-infrared Raman Spectroscopic Technique

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Transgenic cell lines of loblolly pine (Pinus taeda L.) were analyzed by a compact laser-tweezers-Raman-spectroscopy (LTRS) system in this investigation. A low power diode laser at 785 nm was used for both laser optical trapping of single transgenic cells and excitation for near-infrared Raman spectroscopy of the nuclei of synchronized cells, which were treated as single organic particles, at the S-phase of the cell cycle. Transgenic living cells with gfp and uidA genes were used as biological samples to test this LTRS technique. As expected, different Raman spectra were observed from the tested biological samples. This technique provides a high sensitivity and enables real-time spectroscopic measurements of transgenic cell lines. It could be a valuable tool for the study of the fundamental cell and molecular biological process by trapping single nucleus and by providing a wealth of molecular information about the nuclei of cells.

Key words: transgenic cell, laser optical trapping, near-infrared Raman spectroscopy, biological sample

Introduction

Laser trapping, by which a small particle is grabbed by the force of radiation pressure generated from two laser beams, was first reported in 1970 (1, 2). Since its first demonstration, the laser trapping technique has been widely used to capture and manipulate single organic particles in a micrometer range, such as latex beads, aerosol particles, microdroplets, microcapsules, and biological samples including bacteria, blood cells, etc (3–5). At present, the optical tweezers technique, which uses one visible laser beam with a microscope, proves to be a more practical method (6, 7). The optical tweezers using a low-energy near-infrared laser beam with wavelength ranging from 700 to 1100 nm have many advantages. One advantage is that they produce far less sample-damaging photochemical reactions than visible laser light, while another advantage is that they allow the manipulation of not only cells, but also organelles and vesicles within cells (2, 3, 7).

Several spectroscopic techniques, including absorption (8), fluorescence (9), and Raman spectroscopy (10, 11), have been combined with optical tweezers to characterize molecules contained in single organic microdroplets or microcapsules. This combination has provided a powerful tool for identifying chemical composition and revealing molecular behavior in individual particles suspended in complex environments (12). Raman spectroscopy provides high spectral resolution, and it enables one to determine concentrations of environmentally important inorganic ions contained in aerosol particles, such as nitrate and sulfate in single droplets of gases (13–15). Raman spectroscopy using non-invasive near-infrared laser light has become a powerful tool for the microscopic analysis of organic and biological materials (7). Compared to other spectroscopic techniques, Raman spectroscopy has a great advantage as an analytical tool since it can provide information about species, structures, and molecular conformations within the particles (12). Another advantage of combining Raman spectroscopy with optical tweezers is that the Brownian motion of 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 time range is allowed (2, 16, 17). 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 (18).

Recently, a Raman-tweezers system was developed to study single trapped polystyrene beads with a power of 80 mW from a Ti:sapphire laser, both for trapping and excitation (7). However, Raman measurements for single trapped living cells is very challenging because the extremely weak Raman scattering from molecules confined to very small cellular space requires a high intensity excitation, which may cause severe photodamage to the living cells (19). In this study, we report on a compact laser-tweezers-Raman-spectroscopy (LTRS) system used for the manipulation and characterization of single transgenic living cells. The cell lines used in this investigation are just model cell lines with reporter genes.

Results

Apparatus and principle

The experimental schematic of the compact LTRS system is shown in Figure 1. A laser beam from a laser diode (HL7852, Hitachi America, Dallas, USA) was spectrally filtered with an interference filter, and introduced into an inverted microscope to form a single-beam optical trap. The laser spot size at the focus was originally designed at 5 µm to capture the particles in the sample holder. The laser spot size at the focus can be adjusted from 5 to 10 µm during the experiments. A holographic notch filter HNF1 (HNPF-785AR, Kaiser Optical Systems, Ann Arbor, USA) was used as a dichroic beamsplitter that reflected the filtered 785-nm excitation beam at an incident angle of 18 degrees and transmitted the Raman signals. Two notch filters were used to remove the Raleigh scattering light. The beam was then focused onto the entrance slit of an imaging spectrometer (TRIAX 320, Instruments SA, Edison, USA), which is equipped with a liquid-nitrogen-cooled, front-illuminated charged-coupled detector (CCD) with 1024x256 pixels (Spectrum One, Instruments SA). A green-filtered xenon illumination light source and a video CCD camera system were used for the observation and recording of optical images of the trapped cells in real time.

Production, confirmation, and synchronization of transgenic living cells

Calli derived from loblolly pine embryos were used to establish cell cultures. Cell cultures, consisting of single cells (Figure 2A), were infected with the Agrobacterium tumefaciens strain GV3850 containing pBINm-gfp5-ER, which carries a chimeric neomycin phosphotransferase gene and a modified green fluorescent protein (gfp) gene with an endoplasmic reticulum targeting sequence (20, 21), or the strain GV3101 containing pPCV6NFHygGUSINT, which carries a hygromycin phosphotransferase gene and a modified β-glucuronidase gene (uidA) with an intron, as described in Tang et al (22, 23). Transgenic callus lines derived from the A. tumefaciens-mediated transformation were isolated following a selection on callus induction media, supplemented with kanamycin or hygromycin, and used to establish cell suspension cultures (22, 23). Expression of the uidA gene in transgenic cells was confirmed by green fluorescence microscopy with blue light excitation and Southern blot analysis (Figure 2, B and C). Expression of the gfp gene in transgenic cells was confirmed by green fluorescence microscopy with blue light excitation and Southern blot analysis (Figure 2, D, E, and F). Transgenic cell suspension cultures were sub-cultured weekly and filtered with a 0.2 mm pore size filter. Cell suspension cultures comprised of single cells were treated with 0.2 µM aphidicolin to obtain synchronization. After 24 h of treatment, aphidicolin was removed by washing 15 times in 30 min with liquid medium. Living cells at the S-phase of the cell cycle (1 h after aphidicolin removal) were selected for near-infrared Raman spectroscopic characterization.

Raman spectroscopy of trapped biological particles

The LTRS system was calibrated and aligned by using a polystyrene bead of 2.03-µm diameter (Bangs Laboratories, Inc., Fishers, USA) suspended in water. The bead was trapped at the focus of the laser beam while the CCD integration was collected. The Raman spectra of a trapped polystyrene bead were available with a CCD integration time of 2.0 s and a 600 g/mm⁻¹ spectrograph grating. The image of the trapped polystyrene sphere was recorded with the video camera and exported to a computer. The background noise was taken without the trapped bead under the same acquisition time and height and was subtracted from the recorded spectra. The observed
**Fig. 1** The LTRS system and experimental setup. A diode laser (DL) beam passes through an interference filter (IF) and a holographic notch filter (HNF1), then is introduced in an inverted microscope through a dichroic mirror (DM) to form an optical trap. The Raman scattered light of the trapped particles is collected with the same objective, passes through two holographic notch filters (HNF1 and HNF2), and is then focused on to the entrance of an imaging spectrograph equipped with a CCD detector. M: reflection mirror; L: lens; BS: beam splitter; Obj: objective lens; EP: eyepiece; VC: video camera; Mod: modulation current pulses; Xe lamp: Green-filtered xenon illumination light. An image shows a transgenic cell in the focused laser beam of the Raman microscope. The arrow indicates the nucleus of cell that is the target of laser beam.

**Fig. 2** Transgenic cell lines. **A.** Control living cell; **B.** uidA transgenic cell; **C.** Southern blot of uidA transgenic cells; **D.** gfp transgenic cell transmitted light reference image; **E.** gfp transgenic cell in blue light; **F.** Southern blot of gfp transgenic cells.
bands of the Raman signal are identical to the published spectra by Ajito and Torimitsu (7).

With the LTRS system calibrated and aligned by a polystyrene bead, we have tested different biological samples in previous studies, such as a living red blood cell, a living yeast cell, an unstained dead yeast cell (25), Escherichia coli, Enterobacter aerogenes, bovine serum albumin protein, and phenylalanine (26). Raman spectra obtained from trapped biological samples varied with the differences of biological molecules of the samples. Differences in Raman spectra of living biological cells were mostly obtained from Raman shift in wavenumber 500 to 1,700 cm\(^{-1}\). The Raman spectra from these cells were recorded with an exposure time of 2.0–5.0 s and an excitation of 20 mW while the trapping power was kept at 2.0 mW. Based on the data of Raman spectra from yeast (25), E. coli (26), E. aerogenes (26), Clostridium acetobutylicum ATTC 824 (27), human eosinophilic granulocyte (28), polytene chromosome II of Chironomus thummi thummi (28), neutrophilic granulocytes (29), and human coronary atherosclerosis (30), we concluded with a tentative assignment for the observed Raman spectra of living biological cells as shown in Figure 3. Characteristic bands were observed at 667 cm\(^{-1}\) \([v_7\text{ haem vibration}]\), 751 cm\(^{-1}\) \([v_{14}\text{ haem vibration}]\), 1,365 cm\(^{-1}\) \([v_4(C-CN)]\), and 1,541 cm\(^{-1}\) \([v_{11}(C\beta-C\beta)]\), which are identical to those assigned and published bands from biological cell samples (31).

![Fig. 3 Raman spectra of single biological cells. Peaks are marked with the wavenumber of the Raman shift, and tentative attributions of the bands are given. A: adenine; C: cytosine; T: thymine; G: guanine; Phe: phenylalanine; Tyr: tyrosine; Trp: tryptophan.](image)

**Raman spectroscopy of trapped transgenic living cells**

Near-infrared Raman spectroscopy has been used to identify single living cells as mentioned above. Here we demonstrate its application in identifying transgenic living cells with different reporter genes. Three living cell samples, including one uidA transgenic cell line, one gfp transgenic cell line, and the control cell line, were used for Raman spectroscopy. These samples were derived from the same cell suspension cultures originated from one single callus clone. Cell suspension cultures were synchronized with aphidicolin. Living cells at the S-phase of the cell cycle (1 h after aphidicolin removal) were used for near-infrared Raman spectroscopic characterization. These cells had the same genetic background and the same level of growth, development, and metabolism within the cell when they were used for Raman spectroscopic characterization. Therefore, the differences in Raman spectra represent the differences in molecular information of nuclei that were caused by the introduced transgene.

From GUS (β-glucuronidase) histological assays (24; Figure 2B) and green fluorescence microscope images excited by blue light (Figure 2E) in transgenic cells, we can clearly distinguish the transgenic uidA, transgenic gfp, and control cultures. However, these
Tang et al. did not provide us with the molecular information arising from the transgene in nuclei. The Raman spectra showed a significant difference among these living cells with a difference in only one gene. An example of the Raman spectra from living cells selected from three samples is shown in Figure 4, with tentative attributions of some major Raman bands. Figure 4 shows the Raman spectra of living cells with four characteristic bands at 631, 729, 1,004, and 1,186 cm$^{-1}$. The spectral features for these three cell samples are similar (Figure 4, A and B). Compared to the control (C1), the main difference for uidA transgenic cells (S1) seems to be the decrease of tyrosine at 631 cm$^{-1}$ and the increase of intensity between 1,300 and 1,400 cm$^{-1}$, which represents the molecular information derived from adenine, guanine, and tyrosine (Figure 3). The main difference between gfp transgenic cells (D1) and the control cells (C1) seems to be the decrease of the tyrosine at 631 cm$^{-1}$, the increase of the adenine at 729 cm$^{-1}$, the increase of the phenylalanine at 1,004 cm$^{-1}$, and the decrease of intensity between wavenumbers 1,300 and 1,400 cm$^{-1}$. The molecular information represents the changes in gene activities in nuclei of different transgenic living cells. These results were confirmed by the hierarchical cluster analysis of Raman spectra of three cell lines (Figure 4C).

Fig. 4 Near-infrared Raman spectra (A) of control living cells (C1), uidA transgenic cells (S1), and gfp transgenic cells (D1) and their differences (D1-C1 and D1-S1) (B) among individual cell lines. For each cell line, 30-50 cells were observed. C. Dendrogram from hierarchical cluster analysis of Raman spectra of three cell lines. Ten cells were randomly selected from each cell line. The dendrogram was generated from the four PC scores by using Ward’s clustering algorithm and the squared Euclidean distance.
Discussion

Raman spectroscopy using visible laser light has been widely used for the microscopic analysis of organic particles, however, it is rare in the analysis of biological cell samples because of its sample damaging photochemical effects (7). The LTRS system used in our experiments uses a low power diode laser (785 nm) for laser trapping and Raman excitation. The diode laser is operated in a power-switching scheme. When a cell is trapped and manipulated, the diode laser is programmed to operate at a low power (such as 2.0 mW at the sample). When a Raman measurement is taken, the laser power is increased (up to 20 mW) for a short period of time (typically 2.0 s for CCD integration) to ensure high excitation intensity for Raman spectroscopy. After the spectrum is taken, the laser returns to its previous low power operation for optical trapping. This power-switching scheme meets the requirements for reducing photochemical and thermal damage to the biological cell samples, as well as allows sufficient excitation power for Raman spectroscopy. The design of this LTRS system provides high sensitivity to obtain Raman spectra from single living red blood cells or yeast cells placed in an optical trap. Spectroscopic differences between a living and a damaged yeast cell in an aqueous solution are detectable with this system (25).

A global approach to analyze the composition of the whole cell is the use of vibrational spectroscopy. On the single-cell level, infrared spectroscopy can only be used to analyze the relatively large cells of higher organism because of the limitation of spatial resolution (27). Raman spectroscopy, like infrared spectroscopy, is based on molecular vibration. Interaction between a photon and a molecule results in the excitation of vibrations (29). Raman spectroscopy can be used to obtain information about chemical composition, bonding situation, symmetry, structures, and physical parameters such as the length of bonds. The combination of Raman spectroscopy, microscopy, and a laser optical trap provides us opportunities to analyze single biological cell samples (29, 32, 33). In this investigation, we first demonstrated the application of near-infrared Raman spectroscopy combined with both laser optical trapping and a shifted excitation difference technique in characterizing synchronized transgenic living cells at the level of nucleus. Molecular information derived from the nuclei of different transgenic living cells were detected and, in return, the information can be used to identify different cell lines at the same genetic background and developmental stage. Some of the Raman bands in the spectrum could be attributed to relevant molecules or cell components according to the literature on Raman spectroscopy of biological cell samples (27).

Comparing to the traditional methods in single cell analysis, such as flow cytometry (34) and image cytometry (35), LTRS has several advantages. One advantage is that optical tweezers can hold living cells suspended in liquid medium and the information from spectra is obtained directly in a non-destructive and non-invasive way, without performing any chemical reactions; another advantage is that a better signal-to-noise ratio can be obtained since the cell is maintained at the focus of the excitation beam, which permits optimum excitation and collection of Raman scattering. In addition, the acquired data set is multi-dimensional, several hundred wavenumbers are recorded in a spectrum and for each of them there is a separate signal. Compared to flow cytometry, more information is obtained from each single cell. In this study, differences in molecular information from the nuclei of different transgenic living cells had been demonstrated in their Raman spectra. This information may be useful for the understanding of fundamental cell processes and for genetic identification of biological samples. Our technique combines the advantages of near-infrared Raman spectroscopy and optical tweezers for the characterization of transgenic living cells using the nuclei of cells as targets. To our knowledge, this is the first report of studying single transgenic cells by Raman spectroscopy in an optical trap.

Like the widely used Laser Scanning Microscopy (LSM) (Confocal) and the recently developed Laser Capture Microdissection (LCM; ref. 36), our LTRS system uses laser as a tool, too. The difference of our system is that a low power diode laser at 785 nm is used for both laser optical trapping of the nuclei of single transgenic cells and excitation for near-infrared Raman spectroscopy of the nuclei of synchronized cells. LCM uses laser to trap and select specific tissues or cells, which provides a rapid means of isolating pure cellular preparations directly from heterogeneous tissues, based on conventional histological identification, for the analysis of DNA, RNA, and protein. Compared to the lasers used in LSM and LCM, the laser used in our system is different only in power and wavelength. In our system, the laser spot size at the focus is designed at 5 µm to capture the nuclei in the sample holder. The diode laser is operated in a
power-switching scheme as described above. This design allows us to trap the nuclei and avoid producing any damage to the nuclei.

In this study, the Raman spectra reflect the chemical composition, bonding situation, symmetry, structures, and physical parameters, such as the length of bonds and molecule folding in the nuclei of cells, not the whole cell, because the diameter of plant cells used in this work is 50–100 µm. The spot size of our laser on the sample is 5 µm, which is unable to capture molecular information from the whole cell. But it is enough to capture molecular information from the nuclei at the specific stage of the cell cycle by scanning the nuclei because the diameter of the nuclei at the S-phase of the cell cycle is about 10 µm or smaller. That is why we use aphidicolin to synchronize transgenic cells. We think that synchronization of transgenic cells helps us not only in obtaining the Raman spectra of the nuclei of cells, but also in comparing the molecular information among different cell lines, because we put the nuclei of cells at the same environment, the same physiological status, and the same phase of the cell cycle. Differences in Raman spectra should be from different chemical composition, bonding situation, symmetry, structures, and physical parameters such as the length of bonds and molecule folding in the nuclei of cells. We only measure the nuclei of cells and the Raman spectra are from the nuclei of cells only. We think that the Raman spectra of the nuclei of cells are very similar to the fingerprints of people.

The measurements to the changes in specific components in cells are very sensitive and are able to identify the changes from single molecules in cells. Fluorescence and force spectroscopy have been used to measure the behavior of individual biomacromolecules to characterize the complex dynamics of biological processes involving in the protein and RNA folding problems (37–39). With the help of dye molecules attached to specific molecules in plant cells, we are surely able to measure single molecules in the living cells, including DNA, RNA, protein, enzyme, and carbohydrates, by Raman spectra. We can also do quantitative analyses of biomacromolecules in living cells by comparing the Raman spectra, just like the quantitative analysis of GFP fluorescence.

Just as using a GFP or a GUS reporter in transgenic plants, we use GFP and GUS as reporters to show the differences of the cells where the nuclei are used to produce Raman spectra. The Raman spectra are not directly from the GFP or GUS proteins, but from the nuclei of cells, where the only difference is one with gfp gene and another with uidA gene. This ensures us to use Raman spectra to identify different cell lines. We think that the changes in signals assigned to Tyr or Phe (and other molecules) represent signals emanating from the nuclei of cells where the transgene was inserted into the genome, not directly from the transgene itself or the reporter protein. However, the changes in Raman spectra of the nuclei of cells are caused by the insertion of gfp gene or uidA gene. At present, we are working on developing techniques to directly measure single molecules in living cells.

We focus on the nuclei of cells at S-phase by using aphidicolin to synchronize transgenic cells. Information provided by Raman spectra represents the molecular activities in the nuclei of cells at S-phase. Because of the differentially transcriptional activities in the nuclei of cells with gfp gene or uidA gene, signatures in the spectrum of A, C, or T are captured by Raman spectra and they are gross changes.

Cells in culture are subject to genetic drift and are heterogeneous, but the cells we measured are at the specific S-phase. It is a relatively stable phase for the nuclei in shape. During the period of culture, the cells have undergone somaclonal variation, and this would result in the differences in Raman spectra. However, the results will be based on the number of cells that had been measured. We just measured one control cell line, one gfp transgenic cell line, and one uidA transgenic cell line, but did not measure and compare independent lines carrying the same reporter. From each line, we measured a limited number of cells instead of all of the cells in cell suspension cultures. We did not detect the somaclonal variation in the three cell lines tested because of its low frequency. If we check more nuclei of cells (such as 10,000), the somaclonal variation in the three cell lines should be captured by Raman spectra. Although we have measured many cells from each cell line, no significant difference in Raman spectra was found among the cells from the same cell line. That is why we think that Raman spectra may be useful in plant molecular biology.

We think that this technology would be useful in resolving problems in plant molecular biology or biochemistry, such as DNA replication, transcription, signal transduction inside the nuclei of cells, and molecular activities in the cell cycle at this moment. It will be also valuable in gene function and single molecule analyses in single living cells, but it largely depends on the improvement of this technology. In
our current projects, the proposed effort is to identify unknown biological and chemical substances, to verify the effective disinfection of known contaminated environments, and to diagnose military and civil personnel for contraction of a disease after exposure to a bio-agent. One of the duties is to develop this technology for plant biologists. However, we think two things need to do right now before this technology can be widely used in plant molecular biology. One is to develop this technology to identify single molecules in living cells, such as using the technique of dye molecules attached to another specific molecules; the other is to improve the data processing system and to make Raman measurement more sensitive. Our other is to improve the data processing system and to make Raman measurement more sensitive. Our manuscript is probably the first one to use Raman spectra to analyze transgenic plant cells. We speculate that the spectral changes are due to the changes of the nucleus because of the insertion of the transgene. GFP and GUS proteins in the protein extract solutions did not show significant difference in the Raman spectra.

Materials and Methods

LTRS system

The experimental schematic is shown in Figure 1. The experimental setup of LTRS was described in detail previously ((25, 26, 40, 41)).

Transgenic living cells

Callus induction and establishment of cell suspension cultures from single callus clone of loblolly pine (Pinus taeda L.) were described in Tang et al (22, 23). The binary expression vector pHINm-gfp5-ER was obtained from C. N. Stewart (University of North Carolina, Greensboro, USA) and J. Haseloff (MRC Laboratory of Molecular Biology, Cambridge, UK) and was introduced into A. tumefaciens strains GV3850 by electroporation. After electroporation, the chamber was rinsed with 0.8 mL YEP medium (42), and the cells were collected in a sterile tube. Cells were incubated with shaking (250 rpm) for 1 h at 28°C and plated on YEP agar medium containing 100 µg/L kanamycin. After incubation at 28°C for 36 h, colonies were picked, and DNA extracted from randomly selected clones was analyzed using restriction enzymes. Expression of gfp gene was observed with a stereo dissecting microscope equipped with a fluorescence module consisting of a 100-W mercury lamp and GFP plus excitation and emission filters (Leica, Heerbrugg, Switzerland). This system (excitation filter 480/40 nm; dichroic mirror 505 nm LP; barrier filter 510 nm LP) permits visualization of GFP following excitation by blue light. The fluorescence images were recorded with an Olympus exposure control unit (PM-20) and a 35-mm camera (PM-C35). Preparation of uidA transgenic living cells was described in Tang et al (22, 23). Both uidA and gfp transgenic living cells were prepared synchronously, then used for aphidicolin treatment at the same time with the control.

DNA isolation and Southern blot

Genomic DNA was isolated from 5 g fresh tissues of control and putative transgenic cells according to the methods of Wagner et al (42). Solutions for Southern blot analysis, including a TAE electrophoresis buffer, a hybridization solution, and SSC solutions for final washes, were prepared according to Sambrook et al (42). Twenty µg of DNA from gfp transgenic cells were digested overnight with the restriction enzymes BamHI and SstI (Boehringer Mannheim Corp., Indianapolis, USA) at 37°C. Twenty µg of DNA from uidA transgenic cells were digested overnight with the restriction enzymes BstEII and NcoI (Boehringer Mannheim) at 37°C. The digested DNAs were electrophoretically separated on a 0.8% TAE-agarose gel in TAE buffer and denatured with 0.5 NaOH, then capillary-blotted onto a nylon membrane (Magna; Micron Separations Inc., Westborough, USA) using an alkali transfer buffer. According to standard protocols (42), the DNA fixed on membranes was hybridized separately in hybridization solution at 65°C with a probe (816 bp fragment of the uidA gene) labeled with β-[32P]dCTP (Ready-to-Go Labeling Beads; Pharmacia, Piscataway, USA). Membranes were washed twice in 2×SSC, 0.1% SDS at 65°C for 5 min each; once in 0.5×SSC, 0.1% SDS at 62°C for 15 min; and once in 0.1×SSC, 0.1% SDS at room temperature for 30 min. The hybridization of the probe DNAs to the blot was recorded by exposing to Kodak X-Omat-AR films (Sigma, St. Louis, USA) at −80°C for 1–3 days.

Synchronization of transgenic cells

After the uidA and gfp transgenic cell cultures were established, cell suspension cultures were sub-cultured weekly in fresh liquid medium with the same composition. The synchronization experiments of cul-
tured cells were performed according to Nagata et al. In brief, cells were harvested by centrifugation at 5,000 rpm for 10 min, rinsed with fresh liquid medium for 3 times, then were re-suspended in fresh liquid medium supplemented with 0.2 μM aphidicolin (Sigma) and cultured in incubator shaker at 150 rpm for 24 h. Aphidicolin was removed by washing 5 times in 30 min with fresh liquid medium. Living cells at the S-phase of the cell cycle (1 h after aphidicolin removal) were used for near-infrared Raman spectroscopic characterization.

Raman spectroscopy of transgenic living cells

Synchronized uidA and gfp transgenic cell cultures were rinsed 5 times in 30 min with fresh liquid medium to remove aphidicolin. Each time, cell cultures were centrifuged at 3,000 rpm for 5 min and the harvested cells were re-suspended in fresh liquid medium. After washing 5 times, cells cultures were cultured in fresh liquid medium for 30 min at 150 rpm shaker, and the living cells were used as biological samples and transferred to the microscope sample holder. A laser beam from a low-power laser diode at near 785 nm was used to form a single-beam optical trap to capture the nuclei of cells. An illumination lamp and a video camera system were used to observe the image of the trapped nuclei of the cells. The spectrographs were obtained by a liquid-nitrogen-cooled, front-illuminated CCD with 1024×256 pixels (Spectrum One).

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