Lens-scanning Raman microspectroscopy system using compact disc optical pickup technology

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Abstract: We propose and demonstrate the adoption of the compact disc optical pickup technology for Raman microspectroscopy. We utilize both the focusing and the 2-dimensional lateral scanning capabilities of the optical pickup for implementing a miniaturized microspectroscopy system. The resolution of this pickup-based system is characterized by scanning polystyrene microspheres. We test the completed microspectroscopy system by obtaining Raman images of Adenine microstructures. This system will be particularly useful for table-top biological analyzers and other remote medicine applications.

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OCIS Code: (170.3890) Medical optics instrumentation, (170.5660) Raman spectroscopy, (170.5810) Scanning microscopy

Reference and Links

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1. Introduction

The Raman spectroscopic technique is currently finding increasing applications in areas such as molecular biology, drug discovery, and analytical chemistry due mainly to its capability to provide the characteristic information of the samples under study in a nondestructive fashion. The label-free nature of the technique renders the sample preparation minimized and facilitates rapid analyses. In setting up of Raman spectroscopy systems, the confocal microscopy scheme has often been adopted for its ability to excite and monitor a very limited volume of the sample and such incorporation gives rise to a rapidly expanding field of confocal Raman microspectroscopy [1].

Since a spectroscopic image covering the whole extent of a sample can only be generated by combining the data from each limited probing volume, the scanning is the essential

#7275 - $15.00 USD Received 26 April 2005; revised 7 June 2005; accepted 7 June 2005

technique in confocal Raman microspectroscopy, which often referred to as the scanning confocal Raman microspectroscopy [2]. The two widely adopted scanning techniques are the sample-scanning and the beam-scanning. The former technique translates the sample while the latter steers the beam. The focusing optics is kept stationary in both techniques. The sample-scanning is usually accomplished by piezoelectric actuators. For the beam-scanning, rotating mirror scanners are mainly adopted. Acousto-optic beam deflectors are also used for a faster scanning. The above two scanning methods tend to be large and exhibit little potential to satisfy the growing demand for miniature, table-top analysis instruments.

Recently the lens-scanning has gained its ground as a beam steering technique due mainly to its suitability for miniaturized systems. For example, Toshiyoshi *et al* proposed and demonstrated a MEMS scanner for fiber-optic switches [3] and Kwon *et al* utilized the same scheme for implementing a MEMS scanning confocal microscope that occupied less than 2 mm^3 [4]. While the micro-optic polymer lenses in the MEMS devices performed satisfactorily for the beam-deflection and reflective microscopy, their optical characteristics are not adequate for Raman microspectroscopy which requires a larger diameter for more efficient collection of scattered light and a higher lens quality for a tight focusing. Since MEMS actuators cannot accommodate lenses with diameter exceeding a few hundred microns, a new scanning method is required for adopting the lens-scanning scheme for miniature scanning Raman microspectroscopy systems.

In this paper, we propose and demonstrate the utilization of a compact disc optical pickup as a 2-dimensional scanning solution for our miniature Raman microspectroscopy system. The adoption of optical pickup technology is a well-established practice in various areas of optical engineering. They feature low cost, immediate availability, compact size, and high quality optical components. There already are a number of reports on the metrological applications of optical pickups [5]. The fact that the components of optical pickups are optimized for ~800 nm light motivates their adoption in biological applications in which near-infrared is usually preferred over visible or UV for probing. In biology, however, only the utilization of optical pickup's automatic tracking capability for flow cytometry was reported recently [6]. The most relevant to our current work is the confocal scanning microscope of Benschop *et al* in which an optical pickup, supplemented by a radial motor and single-mode fiber-optics, is used for 2-dimensional scanning reflective microscopy [7]. We demonstrate, for the first time to our knowledge, the suitability of a commercial optical pickup as a scanning and focusing device for scanning Raman microspectroscopy by building a miniaturized Raman scanning system that incorporates all essential components and subsystems around the optical pickup.

2. System description

The overall structure of the optical pickup-based scanning Raman microspectroscopy systems is shown in Fig. 1(a). Since our main goal is to confirm the suitability of the optical pickup in scanning Raman microspectroscopy, we use a commercially available system for performing the spectroscopic tasks. The confocal configuration is not explicitly pursued in this work for the same reason.

We use the Raman probe with a permanently aligned bundle of two fibers, one for excitation and the other for collected scattering, with appropriate micro-optic components for focusing, collection, and excitation filtering (InPhotonics RPS785). It is cylindrical in shape with a diameter of 12.7 mm and a length of 76 mm. The external control unit (Raman Systems R2001) which includes a diode laser source at 785 nm, a grating-based spectrometer, and a data acquisition unit, is not compact but can readily be miniaturized using custom components. The optical power of the excitation beam is approximately 25 mW and the spectrometer can measure Raman shift up to 2000 cm^{-1}.

The optical pickup is the central part of this scanning Raman microspectroscopy system. Since the structure and operation of a compact disc optical pickup is illustrated in detail in literature, only the portion relevant to this present work is shown in Fig. 1(b). Among the compact disc pickups, we choose the KRS-200A from Sony for its unique structure that suits our purpose. As indicated in Figure 1(b) which is based on the KRS-200A structure, a simple

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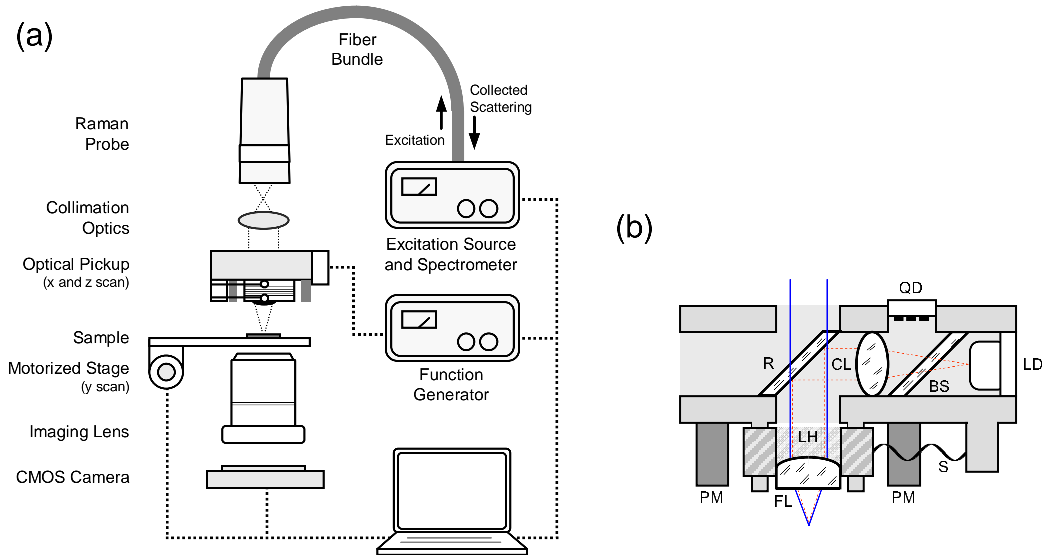

Fig. 1. Schematic views of (a) the lens-scanning Raman microspectroscopy system (drawn not to scale) (b) the optical pickup utilized for this scanning system (BS: beam splitter, CL: collimation lens, FL: focusing lens, LD: laser diode, LH: lens holder, PM: permanent magnet, QD: quadrant diode, R: reflector, S: wire spring; Dashed: beam path for normal pickup operation, Solid: beam path for scanning operation)

removal of the reflector reveals an opening on the other side of the housing that allows a direct access of the focusing lens. In other pickups we survey, either the excessively thick housing or the unfavorable arrangement of other optical components block the beam path. Our survey is by no means exhaustive and leaves potential for a better pickup design. The dashed line in Fig. 1(b) indicates the beam path for the normal pickup operation. In our setup, we remove the reflector and use the beam path delineated in solid lines. The lens holder is situated between a pair of permanent magnets and is coiled in orthogonal directions for the original purposes of automatic focusing and tracking. We isolate the coil from the servo circuitry and control the movement of the lens holder with a dual-channel function generator for scanning in one lateral direction (x) and one axial direction (z).

Since the Raman probe is designed for probing samples through container walls, it has a standard working distance of 5mm. To collimate the diverging beam, a pair of lenses are inserted. This can be eliminated in future implementations through the redesign of the Raman probe itself. The diameter of the collimated beam is ~5 mm which is enough to fill the clear aperture of the optical pickup focusing lens completely.

To complement the missing one lateral scan along y axis, we use a motorized translation stage which features 30 nm scanning resolution (New Focus 8301 Picomotor). We mount the sample on a cover glass and control the focal spot position using the monitoring system consisting of a microscope objective imaging lens and a CMOS image sensor. The entire operations of spectroscopic data acquisition, the 3-dimensional scanning, and the image monitoring are controlled by a centralized LabVIEW program. The completed system measures 5×5×16 inches excluding the fiber, the control unit, and the function generator.

3. System characterization

We characterize the scanning resolution of the optical pickup's magnetic actuator by obtaining 1-dimensionally scanned Raman responses from a microstructure of known dimensions. We choose a 10 μm diameter polystyrene (PS) microsphere as our sample for its immediate availability and well-known Raman spectrum. The characterization setup is shown schematically in Fig. 2(a). A PS microsphere is attached to the bottom side of a microscope cover glass and placed near the focal spot of excitation beam which is formed by the optical

Received 26 April 2005; revised 7 June 2005; accepted 7 June 2005
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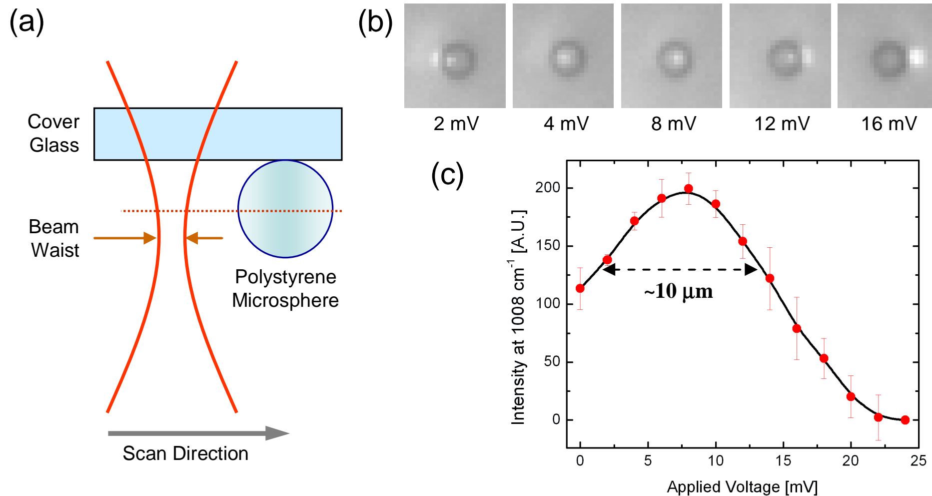

Fig. 2. (a) Schematic illustration of the scanning resolution characterization (b) Visual images of 1-dimensional lateral scanning and (c) Observed change in the intensity of Raman signal at 1008 cm-1 as the actuation voltage is varies from 0 to 20 mV. The error bars indicate the standard deviation obtained from 20 sets of 10 second integration results.

pickup focusing lens positioned above. The actuation voltage (VA) is applied to the coil around the lateral magnetic actuator and is varied from 0 to 20 mV. As indicated in Fig. 2(a), we position the waist of the focused excitation beam at the midpoint between the "south pole" and the "equator" of the PS microsphere to minimize the Raman signal generated inside the cover glass. To obtain more detailed images for determining the scanning resolution, we replace the monitoring system with an inverted microscope (Zeiss-Axiovert200) and a cooled CCD camera during the characterization. The images of lateral scanning and the corresponding Raman response at 1008 cm^{-1} are shown as functions of the applied actuation voltage in Fig. 2(b) and (c), respectively. The lateral scanning resolution can be deduced from the change of intensity in Raman response plotted in Fig. 2(c). The error bars indicate the standard deviation obtained from 20 sets of 10 second integration results. The Raman response rises from VA = 2 mV and returns to the same level when VA~12 mV, which indicates that the scanning resolution of the optical pickup magnetic actuator is approximately 1 µm/mV. As can be noticed from the images of Fig. 2(b), the determination of this scanning resolution is mainly limited by the finite width of the focused beam waist. The long tail in Raman response beyond VA~12 mV is due to the diverging portion of the excitation beam beyond the focus and the 3-dimensional geometry of the microsphere. We also measure the axial scanning resolution of the magnetic actuator by reproducing the monitored focal spot variation using a vertical translation stage with a micrometer drive. The axial scanning resolution is approximately 1 µm/mV as well.

We also characterize the edge response of the completed system by obtaining Raman response through an abrupt 1-dimensional edge. We choose the poly(dimethylsiloxane) (PDMS) as our sample material for the ease of microstructure fabrication via soft-lithography and distinct Raman response. The procedure of the Raman scanning is shown in the inset of Fig. 3(b). The PDMS slab is attached to a glass slide and mounted on a translation stage for rough alignments. The focusing (axial) actuator is utilized first to place the focal spot exactly on the surface of the PDMS slab. Then we actuate the lateral scanner of the optical pickup in a point scanning mode by discretely increasing the current in order to synchronize with the rather slow spectroscopic scanning (approximately 1 minute per scan). The spectral results are plotted in Fig. 3(a). It can be easily noticed that the intensity of collected Raman spectra is being decreased as the focal point passes through the edge. Since the Raman signal is most intense at 492 cm^{-1}, we use the peak as the indication signal. Figure 3(b) shows the change of

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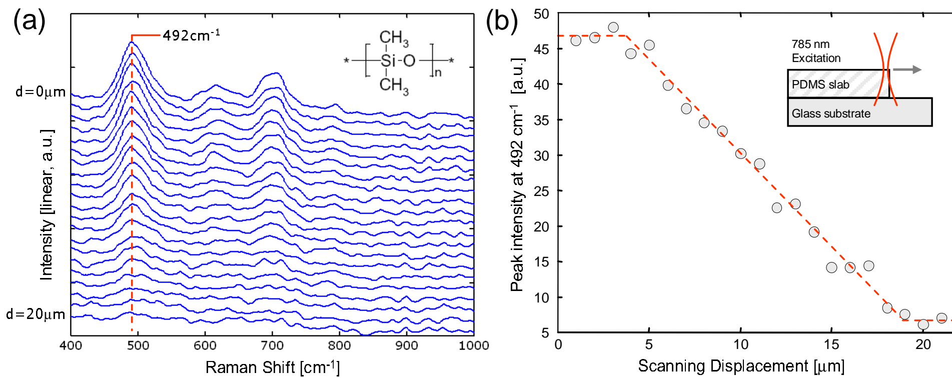

Fig. 3. (a) The obtained Raman spectra from the 1-dimensional scanning of the PDMS (chemical structure shown in the inset) microstructure. The scanning range is 20 µm. A couple of distinct Raman peaks are observed. (b) The peak intensity at 492 cm^{-1} is plotted as a function of the displacement (open circles). The residual Raman signal that causes the gradual edge response is due to the diverging portion of the excitation beam beyond the focal spot and is consistent with the observation in Fig. 2(c).

peak intensity at 492cm^{-1} as a function of the displacement d. Again, the divergence of the excitation beam manifests itself as the gradient edge response.

The full-width half-maximum (FWHM) waist of the optical pickup-focused beam is measured to be approximately 3.5 µm from the images. This FWHM is much larger than the diffraction-limited focal spot size required for the original purpose of compact disc pit reading. We attribute this broadening to the complicated optical path between the Raman probe and the optical pickup including the Raman probe focusing lens, the collimation lens, and the focusing lens. A series of focal spot measurements in which the collimation distance, i.e., the spacing between the Raman probe and the collimation lens, is varied around the perfect collimation point reveal that the optical pickup focusing lens in our setup generates a nearly diffraction-limited focal spot when the incident beam is slightly diverging rather than perfectly collimated. This configuration, however, compromises the collection efficiency of the Raman scattered light and degrades the signal to noise ratio. Given a specific sample to be scanned, we try to balance the spot size and the collection efficiency by adjusting the collimation distance. This drawback can be eliminated by using a custom-designed Raman probe that does not require collimation optics.

4. Two-dimensional Raman imaging

Using the completed Raman microspectroscopy system, we perform the Raman imaging of nanoscale structures of Adenine. The sample is prepared by drying Adenine (Sigma Aldrich A2786-25G) on a microscope cover glass. As the Adenine solution solidifies, a number of spots with dimensions ranging from a few to a few tens of microns appear as shown in Fig. 4(a). To fully demonstrate the Raman imaging capability of our miniaturized setup, we use the original CMOS camera and miniature spectrometer for microscopic and spectroscopic tasks, respectively. While the resolution of the CMOS camera is satisfactory for given applications, its limited dynamic range makes the focal spot larger than its original size confirmed by the cooled CCD camera. Again, the axial actuator is used to set the focal spot located on the Adenine spot before starting the lateral scanning. Considering the size of the Adenine spot to be scanned, we set the scanning interval at 10 µm for both x- and y-directions and the number of pixels at 9×8. Among the Raman peaks of Adenine within the measurement range of our spectrometer, we choose 726 cm^{-1} since it exhibits the strongest contrast above the baseline. The integration time is set at 20 seconds. Figure 4(b) shows the unprocessed mosaic image. The original shape of the Adenine spot is clearly reconstructed as shown in Fig. 4(c) when the raw image is interpolated into a 50×50 pixel image. The resolution of the Raman imaging can be improved by adopting a spectrometer with a better sensitivity for shorter integration time.

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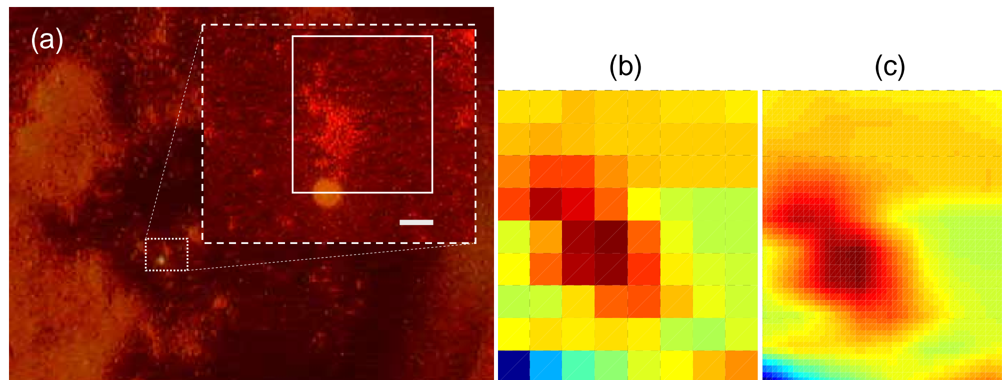

Fig. 4. (a) CMOS camera image of the sample area. Brighter areas are solidified Adenine. The dotted box contains the magnified image (Scalebar: 20 µm). The orange circle is the excitation beam focused by the optical pickup. The inner, solid-lined box indicates the area of Adenine spot to be scanned. (b) The 9×8 mosaic Raman image constructed by collecting Raman response at 726 cm^{-1}. Each pixel is a 10 µm×10 µm square. (c) A 50×50 interpolated version of the Raman image. The shape of the Adenine spot is clearly reconstructed.

5. Conclusion

We have implemented and characterized a miniature, lens-scanning system for Raman microspectroscopy using the compact disc optical pickup technology. Based on the 1-dimensional Raman scanning of a polystyrene microsphere, we demonstrate the feasibility of using the optical pickup for Raman microspectroscopy with little structural modification, for the first time to our knowledge. The magnetic actuators of the optical pickup exhibit 1 µm/mV scanning resolution in both axial and lateral directions, which is adequate for the Raman imaging for cellular or microfluidic structures. The successful 2-dimensional scanning of micron-scale biochemical substances further corroborates this feasibility of optical pickups. The whole system, excluding the supporting electronics and spectrometer, fits within 5×5×16 inches rendering itself adequate for future table-top and portable analysis systems. Currently the major limiting factor of this system is the low sensitivity that allows operations for solidified samples only. In fact, the Raman spectroscopy of diluted liquid samples has been considered to be difficult even with high sensitivity equipments due to the continuous movement of the molecules. In near future, the technique of surface-enhanced Raman scattering (SERS) associated with the surface plasmon resonance around metallic nanoparticles will play an important role in improving the sensitivity of the whole detection process. SERS-induced enhancement of Raman sensitivity up to 10^{10} has already been reported [8]. In conjunction with the development in plasmonic techniques, the present system will serve as a application platform for the SERS-based molecular detection.

Acknowledgments

This work is supported by Intel Corporation. The authors appreciate the help and discussion of Dr. Selena Chan and Dr. Andrew A. Berlin with Intel Corporation.

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