A novel non-imaging optics based Raman spectroscopy device for transdermal blood analyte measurement

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Due to its high chemical specificity, Raman spectroscopy has been considered to be a promising technique for non-invasive disease diagnosis. However, during Raman excitation, less than one out of a million photons undergo spontaneous Raman scattering and such weakness in Raman scattered light often require highly efficient collection of Raman scattered light for the analysis of biological tissues. We present a novel non-imaging optics based portable Raman spectroscopy instrument designed for enhanced light collection. While the instrument was demonstrated on transdermal blood glucose measurement, it can also be used for detection of other clinically relevant blood analytes such as creatinine, urea and cholesterol, as well as other tissue diagnosis applications. For enhanced light collection, a non-imaging optical element called compound hyperbolic concentrator (CHC) converts the wide angular range of scattered photons (numerical aperture (NA) of 1.0) from the tissue into a limited range of angles accommodated by the acceptance angles of the collection system (e.g., an optical fiber with NA of 0.22). A CHC enables collimation of scattered light directions to within extremely narrow range of angles while also maintaining practical physical dimensions. Such a design allows for the development of a very efficient and compact spectroscopy system for analyzing highly scattering biological tissues. Using the CHC-based portable Raman instrument in a clinical research setting, we demonstrate successful transdermal blood glucose predictions in human subjects undergoing oral glucose tolerance tests. Copyright 2011 Author(s). This article is distributed under a Creative Commons Attribution 3.0 Unported License. [doi:10.1063/1.3646524]

I. INTRODUCTION

Most bulk biological tissues are highly scattering even in the near infrared (NIR) regime (with the exception of a few transparent tissues such as the ocular lens). On the other hand, many optical systems can receive light only at a limited range of angles specified by their effective numerical apertures. This is particularly problematic for designing a suitable Raman spectroscopy-based diagnostic device, as efficient light collection is crucial due to the inherently weak nature of Raman scattered light. For this reason, Raman spectroscopy instruments used for the analysis of bulk biological tissues often require a light collection device that redirects the light scattered at steep angles to be collimated within a limited range of conic half angles with respect to the receiving optical system. This had been previously achieved by the use of a half paraboloidal mirror, plano-convex lens, and sapphire ball lens. However, many of these strategies have certain shortcomings. For example, while Raman scattered photons can back-scatter in all directions when the excitation light impinges on the tissue surface (due to the sequence of multiple scattering events in the tissue),
the half paraboloidal mirror surrounds only a portion of the illuminated tissue surface and fails to collimate the light being scattered away from the mirror. The plano-convex collimation lens has a limited acceptance angle defined by its numerical aperture. Finally, the sapphire ball lens itself could create significant Raman signals and confound the intrinsic tissue Raman spectra.

All of these issues can be resolved by using a hollow gold-coated compound parabolic concentrator (CPC). A CPC is a non-imaging optical element that was originally developed for efficiently concentrating collimated light into a smaller area. Conversely, when used as a collimator, the CPC completely surrounds the illuminated tissue site (360°) to efficiently collect Raman scattered light at all conic half angles (−90° to 90°, i.e., 2π solid angle). In addition, the gold-coated surface efficiently reflects the NIR Raman scattered light without generating Raman signatures of its own. However, the utility of CPC, such as the one constructed previously, is limited when a very high degree of light collimation is desired, as its physical dimensions become impractically large for manufacturing or use (Fig. 1). In order to circumvent this problem, we have designed a portable clinical Raman spectroscopy instrument for transdermal glucose measurement that incorporates a non-imaging optical element called compound hyperbolic concentrator (CHC). The CHC was designed to accommodate a focusing lens so that the Raman scattered light from highly scattering biological tissues could be collimated to be within a very narrow range of half angles, while also maintaining practical physical dimensions (Fig. 2).

II. THE COMPOUND HYPERBOLIC CONCENTRATOR (CHC)

A. Motivation for CHC

While the CPC is often used as a concentrator for harvesting solar energy by using its major aperture as input, it can also be used as a collimator by using its minor aperture as input in
applications such as light emitting diode illuminators. Tanaka et al. proposed the use of a hollow gold-coated CPC as a collimator to facilitate Raman scattered light collection from biological tissues by an optical fiber probe with a limited numerical aperture (0.29 NA), and demonstrated seven-fold enhancement in collection efficiency compared with the same optical fiber probe without the CPC. However, many bulk tissue Raman spectroscopy systems call for an even higher degree of collimation than required by the numerical aperture of the collection optical fibers and spectrographs, as certain dielectric and holographic optical filters are effective only at a very limited range of incident angles. For example, both the optical density as well as the band center of the holographic notch filter varies significantly with the angle of the incident beam. While this property is very useful when filtering collimated beams, as it allows fine adjustment of the stopband wavelengths by angle tuning, it could significantly reduce the filter performance when collecting photons that are multiply-scattered in a wide range of angles. This has important implications in bulk biological tissue Raman spectroscopy as most bulk tissue samples are highly scattering, and Rayleigh scattered light needs to be filtered out efficiently in order to obtain a Raman spectrum of acceptable quality.

Although a CPC can be designed to achieve a very high degree of collimation to facilitate good filter performance, it would require very large physical dimensions relative to a given input aperture size. For a given input aperture $R_i$ and the maximum collimation half angle $\theta_{\text{max}}$, the length of the CPC, $L$, is defined as follows:

$$L = \frac{R_i (1 + \sin \theta_{\text{max}})}{\tan \theta_{\text{max}} \sin \theta_{\text{max}}}$$

As shown in Fig. 1, the length of the CPC rapidly becomes very long as the desired maximum collimation half angle gets smaller. However, the required length of the nonimaging optical element can be significantly reduced by introducing the CHC (Figs. 1 and 2).

The CHC is defined by the surface of revolution whose cross sectional profile is determined by two hyperbolae that are mirror images about the horizontal axis (Fig. 3(A)). In addition to the reflector itself, the CHC also requires a matching focusing lens with the focal length defined by the
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FIG. 3. (A) CHC formed by two hyperbolae and its associated design parameters. $R_i$: input aperture radius, $R_o$: output aperture radius, $L$: CHC length, $\theta_{\text{max}}$: maximum collimation conic half angle, $\phi$: angle between the two hyperbolae, $f_{1A}$, $f_{2A}$, $f_{1B}$, $f_{2B}$: foci of hyperbolae. The diameter and focal length of the matching plano-convex focusing lens are clearly defined by $R_o$ and the distance between the output aperture and the hyperbolic foci ($f_{2A}$ and $f_{2B}$). (B) CHC design optimized for the clinical Raman instrument.

distance between the output aperture and the focus on the opposite side of the symmetric axis of the hyperbola (Fig. 3(A)). In Fig. 2(B), due to the hyperbolic shape of the CHC reflector, the light is redirected in a manner such that light emanating from point I at the input aperture of the CHC acts as if it were coming from point II, which lies on the lens’ focal plane. Therefore, simply attaching a focusing lens to a CPC does not provide the same performance as the CHC.
In Fig. 2(A), both the CPC and the CHC have an input aperture diameter of 4 mm, and are capable of converting the input light directions from half angles as wide as ±90° to ±3.9° or less with equal collection efficiency. However, the CPC length is 460 mm while the CHC length is only 115 mm.

B. Designing the CHC

The input aperture radius \( R_i \) of the CHC was defined by the distance between the horizontal axis and the focus \( f_{1B} \) (or \( f_{1A} \)) of the hyperbola (Fig. 3). Typically, for bulk tissue Raman spectroscopy measurements, it is desirable to have a large input aperture radius in order to accept as many Raman scattered photons as possible. The major (output) aperture radius \( R_o \) was defined by the distance between the horizontal axis and the intersection of the hyperbola and the line connecting the foci \( f_{1A} \) and \( f_{2B} \) (or, \( f_{1B} \) and \( f_{2A} \)). This dimension was constrained by the largest available lens with a focal length equal to the distance between the output aperture and the line connecting the foci \( f_{2A} \) and \( f_{2B} \). Finally, the maximum half angle \( \theta_{\text{max}} \) of collimated light was defined by the angle between the horizontal axis and the line connecting the center of the output aperture to the focus \( f_{2A} \) (or \( f_{2B} \)). Since the CHC was to be placed in front of a holographic notch filter designed to accept a collimated beam, \( \theta_{\text{max}} \) was minimized for optimization.

With these constraints, the adjustable parameters included the angle \( \phi \) between the transverse axes of the two hyperbolae (Fig. 3), and the shape of the hyperbolae as defined by \( A \) and \( B \) of the hyperbolic equation:

\[
\frac{x^2}{A^2} - \frac{y^2}{B^2} = 1
\]  

The final result of the optimization was a CHC with 2 mm input radius, 26 mm output radius, length of 115 mm, and \( \theta_{\text{max}} \) of 3.9° (Fig. 3(B)).

C. Manufacturing the CHC

The optimized design was verified for performance by using ray-tracing simulations (Fig. 4, ZEMAX Development Corporation) and transferred to a computer-aided manufacturing program (Mastercam, CNC Software, Inc.). Using a computer numerical control (CNC) lathe machine (HAAS Automation, Inc.), an aluminum mandrel of the CHC was manufactured to high accuracy (error less than 5 \( \mu \)m) and polished to optical grade fineness (0.2 \( \mu \)m). The mandrel was then deposited with a layer of nickel by using a nickel sulfamate electroforming process (Caswell, Inc.). For structural robustness, the CHC nickel shell was electroformed to 3 mm thickness. The mandrel was then released by dissolving the aluminum in a warm (40°C) sodium hydroxide solution while leaving the nickel intact to create the hollow nickel CHC shell. For enhanced reflectivity, the nickel CHC shell was further treated with an electroless bright nickel plating process, which is a well-established and widely used industrial procedure for depositing bright nickel layers on metal workpieces. Afterwards, the CHC was polished for a second time to optical grade fineness (0.2 \( \mu \)m), and electroplated with a uniform layer of pure gold (99.9%) in order to improve NIR reflectivity (>98% at 830 nm) (Fig. 6(B)).

III. DESCRIPTION OF THE CHC-BASED CLINICAL RAMAN SPECTROSCOPY SYSTEM

Prior tissue Raman spectroscopy studies had been mostly performed in back-scattered mode, where the collected Raman spectra were contributed primarily by the outermost tissue surface. On the other hand, recent studies have shown that Raman scattered photons collected in transmission mode configuration have their contribution distributed more uniformly across the entire depth of the tissue, which is more appropriate for probing the bulk volume rather than the surface of the tissue. For example, Raman spectra collected in transmission mode from
FIG. 4. ZEMAX ray-tracing simulation for design verification. The optimized CHC design of Fig. 3(B) was imported into ZEMAX, and coupled with a matching plano-convex focusing lens of 150 mm focal length. Another focusing lens of f/2 was placed in front of the CHC output aperture, and a detector was placed near its focus. In order to simulate the limited numerical aperture (f/2) of the collection fiber bundle (Fig. 5), the detector only accepted light coming in at conic half angles of ±14° or less. With an isotropic light source (conic half angle ±90° or less) at the CHC input aperture, the detector received 78% of the original light intensity.

The skin fold could be more useful for transdermal glucose level measurement than those collected in back-scattered mode, due to the fact that the target glucose-rich dermal and capillary tissues are located underneath the outermost stratum corneum and epidermis layers. For this reason, the clinical Raman instrument was designed to collect Raman and diffuse reflectance (DRS) spectra in transmission mode from skin folds (Fig. 5). The instrument used an 830 nm diode laser (Process Instruments) for Raman excitation, and a broadband source (AvaLight-HAL-S, Avantes) for DRS measurements (DRS provides useful information for tissue turbidity correction in tissue Raman spectroscopy). These sources were delivered to the tissue via separate optical fibers (400 μm core, 440 μm cladding, 0.26 NA, Fiberguide Industries). In particular, the Raman excitation fiber had a shortpass fused silica filter rod (0.62 mm diameter, Barr Associates) at the distal tip, in order to suppress the Raman scattered photons generated by the excitation fiber itself. After the Raman excitation light was scattered and transmitted through a thin tissue (e.g., the thenar fold of an adult human hand, ~3 mm thick), the Raman scattered light was collected from the opposite side of the tissue by the CHC (Fig. 5). The CHC, coupled with a 50 mm diameter focusing lens of focal length 150 mm, redirected any scattered photons (half angles within ±90°) transmitted through its input aperture to be within a narrow range of angles (half angles within ±3.9°), collimating the scattered light to 0.068 NA. The collimated light was then filtered through a 50 mm diameter 830 nm holographic notch filter (Kaiser Optical Systems, Inc.), which efficiently rejected the Rayleigh scattered light while transmitting Raman scattered light. Without collimation by the CHC, the light would have reached the filter at a wide range of angles and the filter performance would have been degraded as its stopband wavelength and optical density varied with the angle of the incident light. The filtered light was then focused onto an optical fiber bundle (1.5 mm diameter, Romack, Inc.) composed of 37 closely packed (76% packing fraction) jacket-stripped collection fibers (200 μm silica core, 220 μm silica cladding, 0.26 NA, Fiberguide Industries) (Figs. 5 and 6). The proximal end of the transmission mode collection fiber bundle (facing the spectrograph input) formed a single 8 mm column of optical fibers to match the height of the CCD detector. The fiber bundle length was limited to 50 cm in order to minimize the generation of undesirable background Raman spectra from the collection fibers, arising from any stray Rayleigh scattered photons that might have leaked through the notch filter. The collected Raman scattered light was then dispersed by the spectrograph.
FIG. 5. Transmission mode clinical Raman spectroscopy setup with CHC. (NDF: Neutral density filter, BPF: Bandpass filter, S: Shutter, FL: Focusing Lens, OFB: Optical fiber bundle.) With the Raman excitation light illuminated on one side of the thin tissue sample (such as the thenar skin fold), the Raman scattered light emerges from the other end of the tissue. The CHC collects Raman scattered light at all conic half angles (±90°) and collimates to within ±3.9°. The collimated light is then filtered through a holographic Rayleigh rejection notch filter and focused onto the collection fiber bundle. On the other end, the collection fiber bundle is arranged to form a column of light with its height matched to the size of the CCD detector.

(f/2, LS 785, Princeton Instruments) and detected by a back-illuminated deep-depletion CCD with 1340×400 array of pixels, thermoelectrically cooled to -70°C (Spec-10:400BR-XTE, Princeton Instruments). The entire setup was fitted inside a wheeled cart (37×18×42 in.) to facilitate portability during its use in the clinical setting.

For convenient access to remote and obscure areas of the human body, the instrument could optionally also use an optical fiber probe (3.5 mm diameter) to collect Raman spectra in backscattered mode (Fig. 7). The optical fiber probe was composed of a nonimaging element (CHC or CPC), a Raman excitation fiber, a DRS excitation fiber, 17 collection fibers (400 μm core, 440 μm cladding, 0.26 NA, Fiberguide Industries), a custom manufactured fused silica notch filter tube (0.97 mm inner diameter, 3.47 mm outer diameter, Barr Associates) and a shortpass filter rod (0.62 mm diameter, Barr Associates)3,22 (Fig. 7). Although a CPC of length 9 mm was sufficient for accommodating the 0.26 NA of the optical fibers, a CHC of a similar size coupled with a matching focusing lens would provide a much higher degree of collimation required for better notch filter performance. In addition, depending on the requirements for the specific tissue targets, other backscattered mode probe configurations such as oblique angle illumination2,23 and dichroic mirrors4 could be employed.

IV. CLINICAL DATA COLLECTION AND PERFORMANCE

Many biomedical Raman spectroscopy applications such as cancer14,24,25 and atherosclerosis detection13,26,27 look for changes in the bulk tissue composition and morphology reflecting the pathology, which are well represented in the tissue Raman spectra. However, in the application of Raman spectroscopy for transdermal glucose level measurement, Raman signatures of aqueous glucose contribute only a very small fraction of the total tissue Raman spectra.28,29 As such, the performance of non-invasive glucose detection depends even more heavily on the quality of the
FIG. 6. CHC-based clinical Raman spectroscopy instrument used for human subject studies. (A) The entire instrument fits inside a wheeled cart to facilitate portability during clinical studies. (B) The manufactured CHC seen from the output aperture. The interior surface was evenly coated with pure gold and polished to optical grade fineness. (C) Side view of the transmission mode Raman spectroscopy setup.

FIG. 7. Back-scattered mode Raman spectroscopy system with an optical fiber probe. (NDF: Neutral density filter, BPF: Bandpass filter, S: Shutter, FL: Focusing Lens, OF: Optical fiber, OFB: Optical fiber bundle.)
Our CHC-based clinical Raman instrument was used successfully in a study that aimed to determine the clinical feasibility of using Raman spectroscopy for non-invasive glucose measurement, and this study is currently underway in our laboratory. In that study, Raman spectra were collected from the thenar skin fold of the hands of 18 human volunteers in transmission mode during oral glucose tolerance tests (OGTT), where the subjects ingested a glucose-rich beverage to induce changes in blood glucose levels (Figs. 8 and 9). At the same time, reference blood glucose levels were measured by using conventional finger-stick glucometers (SureStep Flexx, Johnson and Johnson). The human volunteers were non-diabetic and were not pregnant, with the following demographics: 56% male (44% female), 78% Caucasian, 22% Asian, and a mean age of 33.2 years (ranging from 18 to 64 years). Prior to the beginning of each study, the human subject was required to fast for at least 8 to 12 hours, to ensure that the study was started at the fasting glucose level. If the fasting glucose level was above 125 mg/dl, the study was canceled and the subject was discharged (as this indicates abnormality in the subject’s glucose regulation ability and it may also prevent the collection of spectral and blood glucose data over a wide enough range of glucose levels). Once the subject passed this fasting glucose level test, the subject was given a standard glucose solution drink which is widely used in the clinics for OGTT (75 g glucose, Trutol, Thermo Scientific). Over the course of 2 to 3 hours, Raman spectra were collected every 5 minutes from the thenar skin fold in transmission mode (830 nm laser at 100 mW, 1 minute acquisition). Concurrently, blood glucose measurements were taken by finger-sticks every 10 minutes. This protocol produced a set of skin Raman spectra (each composed of 1340 CCD pixel intensity values) with corresponding reference blood glucose values. Figure 8(A) shows the raw thenar skin fold Raman spectra collected at various blood glucose concentrations obtained from one human subject during an OGTT (26 data points). In addition, Fig. 8(B) shows the time course of glucose concentrations measured during an OGTT, as measured by conventional finger-prick glucometer and Raman spectroscopy via leave-one-out cross validation.

A calibration algorithm relating the Raman spectra to the corresponding glucose concentrations can be developed by using various chemometric techniques, such as partial least squares (PLS), principal component regression and support vector regression. We have used PLS on the data obtained with the clinical Raman instrument to create a calibration model for non-invasive glucose detection. Leave-one-out cross-validation of this model was performed on the dataset and plotted on a Clarke error grid (Fig. 9), which is an analysis tool widely used in the diabetic research community to characterize the performance of a glucose sensing device. In brief, regions A and B represent clinically acceptable glucose prediction performance while C, D and E regions represent poor performance that would have adverse clinical implications. As shown in Fig. 9, our results showed good correlation between the concentrations predicted by Raman spectroscopy and the conventional finger-stick glucometer readings (Fig. 9, R²=0.81, 100% in A or B region, root-mean-squared-error-of-prediction=16.8 mg/dl), which was obtained from 28 OGTTs performed in 18 individual human subjects. This was achieved while using an 8 mm-height CCD detector with 1340×400 array of pixels (Spec-10:400BR-XTE, Princeton Instruments), 100 mW Raman excitation laser power and 1 minute spectral acquisition time for each data point. Previous OGTT studies using a laboratory-based back-scattered mode instrument including a half paraboloidal mirror and a 25.4 mm-height CCD detector with 1340×1300 array of pixels (Roper Scientific) required 200∼300 mW Raman excitation laser power and 3 minute acquisition time for each data point, in order to achieve similar level of performance. All studies involving human subjects were approved by the Massachusetts Institute of Technology Committee On the Use of Humans as Experimental Subjects (COUHES).

V. CONCLUSION

We have developed a portable clinical Raman instrument for transdermal glucose measurement using a CHC and a matching focusing lens. The CHC and lens combination achieved a very high degree of collimation for enhanced tissue Raman scattered light collection and efficient notch filter performance, while maintaining physical dimensions practical for manufacturing and clinical application. The instrument has been used successfully for collecting skin Raman spectra from human
FIG. 8. Clinical data obtained from one human subject (26 data points) using the Raman spectroscopy instrument during an oral glucose tolerance test. (A) Raw thenar skin fold tissue Raman spectra obtained at various blood glucose concentrations. (B) Time course of glucose concentrations measured during an oral glucose tolerance test, as measured by conventional finger-prick glucometer (○) and Raman spectroscopy via leave-one-out cross validation (×).
FIG. 9. Leave-one-out cross validation results showing glucose concentrations measured by Raman spectroscopy and PLS with respect to reference glucose concentrations measured by a conventional finger-stick glucometer. ($R^2=0.81$, Root-mean-squared-error-of-prediction=16.8 mg/dl, 18 human subjects, 28 OGTTs, 730 data points.)

volunteers during OGTT, while using lower Raman excitation laser power and shorter acquisition times compared with previous bulkier laboratory-based systems with similar glucose measurement performance. The proposed CHC design is also general enough to have many other uses in compact spectroscopy systems requiring efficient collimation and collection of nearly isotropically scattered light for disease diagnosis, as well as for chemical process monitoring and pharmaceutical analysis in a variety of industrial spectroscopic instruments.

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