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PAPER IN FOREFRONT

Direct molecule-specific glucose detection by Raman spectroscopy based on photonic crystal fiber

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Abstract This paper reports the first step toward the development of a glucose biosensor based on Raman spectroscopy and a photonic crystal fiber (PCF) probe. Historically, it has been very challenging to detect glucose directly by Raman spectroscopy due to its inherently small Raman scattering cross-section. In this work, we report the first quantitative glucose Raman detection in the physiological concentration range (0–25 mM) with a low laser power (2 mW), a short integration time (30 s), and an extremely small sampling volume (~50 nL) using the highly sensitive liquid-filled PCF probe. As a proof of concept, we also demonstrate the molecular specificity of this technique in the presence of a competing sugar, such as fructose. High sensitivity, flexibility, reproducibility, low cost, small sampling volume, and in situ remote sensing capability make PCF a very powerful platform for potential glucose detection based on Raman spectroscopy.

Keywords Glucose detection · Raman spectroscopy · Photonic crystal fiber · Fiber sensor

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Introduction

Diabetes mellitus (types I and II) is an epidemic currently sweeping the world, affecting over 171 million people worldwide (2.8% of the population) and costing the USA over US $132 billion per year. It is projected that the number of patients will increase rapidly to 366 million by 2030 [1]. Although there is no known cure for diabetes, diabetics manage their blood sugar levels in various ways to maintain their health, including daily monitoring, diet, exercise, and use of medications (insulin in the case of type I, oral medications as well as potential insulin usage in type II). It is well known that when the body fails to produce insulin, the metabolic disorder will cause the blood glucose concentration to fall outside the normal range of 4.4–6.6 mM. Therefore, the diagnosis and management of diabetes mellitus requires frequent measurements of blood glucose levels. In fact, glucose biosensors account for 85% of the entire biosensor market due to the huge population of diabetics [2]. The most prevalent technology of glucose monitoring involves "finger pricks" which monitors the hydrogen peroxide evolution from the oxidation of glucose with glucose oxidase; however, it is an indirect electrochemical detection and is inconvenient for sampling [2]. Thus, the development of a glucose detection system that is molecule-specific, sensitive, reliable, flexible, affordable, and convenient has attracted extensive attention in recent years.

Among the biosensing techniques, optical biosensors are attractive because they are usually sensitive, often noninvasive, in some cases molecule-specific, and of relatively low cost. In the past, several optical methods have been developed to detect glucose [3]. One technique, involving mid-infrared absorption, shows sensitivity to temperature and pH and displays interference from water absorption [4–

6]. Laser polarimetry is another method of glucose detection where polarized light is passed through the aqueous humor of the eye where the polarization is rotated by molecules such as glucose [7]. However, this method has drawbacks such as rotation of the polarization by molecules other than glucose. Additionally, detection methods have been developed to monitor fluorescence changes of dye molecules containing arylboronic acids upon binding to glucose [8, 9]. However, molecules with similar structures or properties to glucose, e.g., other sugars such as fructose, can negatively impact the detection of glucose itself.

Compared with other optical techniques, Raman spectroscopy offers the unique advantage of molecular specificity and provides "fingerprint" information about specific molecules since each molecule has its own unique set of vibrational modes [10]. However, Raman signals are usually weak, and it is difficult to detect a low-concentration analyte. Raman detection of glucose at physiological concentrations requires both high laser power and long integration time (typically 250 mW and 5 min, respectively). This level of laser exposure is significantly high for the human body and not practical for clinical measurements [11, 12].

One promising method to overcome these problems is to use a nanostructured noble metal surface to enhance the glucose Raman signal, known as surface-enhanced Raman scattering (SERS) [13, 14]. Van Duyne and coworkers have done extensive work on the SERS detection of glucose. In their study, SERS has been successfully applied to detect glucose at physiological concentrations using thiols to anchor glucose to a silver film-over-nanospheres substrate [15–19]. In another study, Dinish et al. [20] implemented a nanogap SERS substrate with a deep-UV lithography technique for glucose sensing. However, it is challenging to achieve high reproducibility, good uniformity, and long-term stability for the SERS substrates. Moreover, fabrication of SERS substrates is time-consuming and expensive. Additionally, glucose sensing requires a long incubation time (typically 10 min) for the molecules to get close to the metal surface, which inherently adds to the total measurement time.

One potential solution to the aforementioned issues is to utilize a photonic crystal fiber (PCF) for glucose detection based on normal Raman spectroscopy. Since it was first reported in the 1990s [21, 22], PCF has emerged as a powerful and promising platform for various optical sensing techniques, including absorption [23, 24], fluorescence [25], Raman [26, 27], and SERS [28–35]. The unique microstructures of axially aligned air channels in PCF not only create a photonic bandgap for confining light inside the fiber but also lead to a strong interaction between the light and the solution analyte. A recent study showed that PCF probes can enhance the Raman signal by a factor of 50–100 in both Raman and SERS detection of dye molecules [33]. In this regard, we extend the PCF idea for glucose detection, which has been very challenging in Raman spectroscopy.

Here, we report, for the first time, direct and quantitative Raman detection of glucose at the physiological concentration range using a novel liquid-filled PCF probe. The measurements were carried out under a much lower laser power (2 mW), shorter integration time (30 s), and smaller sampling volume (50 nL) than previously reported Raman measurements [11, 12]. In addition, we demonstrate the molecular specificity of this technique in fingerprinting glucose and differentiating it from a glucose–fructose mixture.

Experimental section

Chemicals

D-Glucose was purchased from Fisher Scientific and D-fructose purchased from Sigma Aldrich. Milli-Q water was used to prepare the analyte solutions at different concentrations throughout the present study.

Preparation of fiber probe and Raman measurements

The PCF was purchased from Crystal Fiber A/S (model AIR-6-800), with its cross-section shown in Fig. 1. The central core diameter was around 6 μm, the side length of

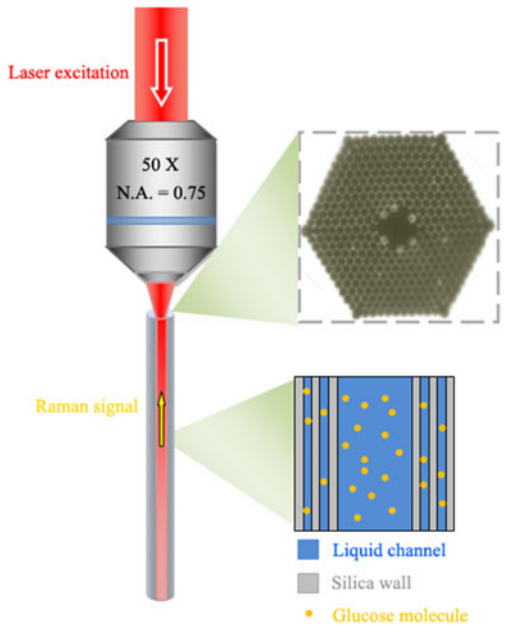

Fig. 1 Schematic of a liquid-filled PCF probe for glucose detection. *Top right* Cross-sectional view of the PCF probe (provided by Crystal Fiber A/S). *Bottom right* Air channels of the PCF filled with glucose solution

the cladding channels was 0.75 μm, and the pitch distance between the cladding channels was 1.6 μm. An 8-cm PCF segment was cut carefully at both ends and one end was dipped into the sample solution for 1 min, allowing the filling of the air channels of the PCF by capillary action. Then, the dipped end was placed under the Raman microscope for measurements. The Raman signals were collected using a Renishaw Raman System (Renishaw Inc., model RM2000) with a ×50 objective lens in backscattering geometry. The excitation laser was operating at 632.8 nm with a laser power of ~2 mW. The integration time for each Raman measurement was 30 s. For the bulk detection, the laser excitation light was directly focused onto the surface of the sample solution with the same laser power and integration time. All the Raman spectra presented in this study were baseline-corrected.

Results and discussion

Figure 1 shows a schematic of the liquid-filled PCF probe operating in the aqueous glucose detection. Specifically, the laser light was coupled into the PCF by the objective lens and subsequently guided inside the PCF due to both the photonic bandgap effect and the liquid inside the fiber channels [33]. More detailed analysis of the light propagation inside a liquid-filled PCF can be found in [33]. By confining both the laser light and the sample solution along the length of the fiber, a much greater laser–analyte interaction volume can be achieved compared with the conventional bulk detection in which the effective volume is limited by the spot size of the laser light in the lateral direction and the depth of focus in the longitudinal direction. Similar to the excitation laser light, the Raman scattered signal will also be confined and (partially) propagated back in the liquid-filled PCF to the Raman spectrometer, which leads to a higher collection efficiency than the bulk detection. Therefore, typically, the liquid-filled PCF probe can provide a sensitivity enhancement of 50–100 times over the bulk detection [33].

It should be noted that in this experiment, sealing the cladding channels of the PCF to make a liquid core PCF, which is widely applied in previous reports of PCF SERS detections [29, 31–35], is not necessary. This is because there is no concern about the large background introduced by the interaction between the metal nanoparticles and silica walls, which exists in SERS experiments [33]. As such, the preparation of the PCF probe is much simplified. Also, the typical fiber length is chosen to be 8 cm, which is not only for easy handling but also is large enough to get the saturated Raman signal.

Figure 2 shows the Raman spectra of glucose solutions obtained in bulk detection and using the liquid-filled PCF at different glucose concentrations. The spectra show characteristic Raman peaks of glucose, which are consistent with the standard spectrum [18]. Taking the major Raman peak at 1,127 cm^{-1} as a reference, the liquid-filled PCF achieves a signal intensity of 3,647 (counts) at a concentration of 50 mM, while the bulk detection scheme only produces an intensity of 525 (counts) at a concentration of 1 M. The units used in these two measurements are the same. The sensitivity enhancement for the liquid-filled PCF in glucose detection is calculated to be 139, consistent with our previous results [32, 33].

Considering the low signal intensity in the bulk detection, which is similar to previous studies and caused by the inherently small Raman scattering cross-section of glucose, pushing the detection limit of glucose to the physiological concentration range (0−25 mM) is therefore extremely difficult and generally requires high laser power and long integration time, which is not desirable for practical measurements [11, 12]. Importantly, the liquid-filled PCF probe shows two orders of magnitude enhanced sensitivity compared with the conventional Raman detection method, and the detection limit is within the physiological concentration range of glucose.

As shown in Fig. 3, a series of Raman measurements of glucose solution were performed at various physiological concentrations, including 1, 5, 10, 15, and 25 mM. It is evident that the major glucose Raman peaks including

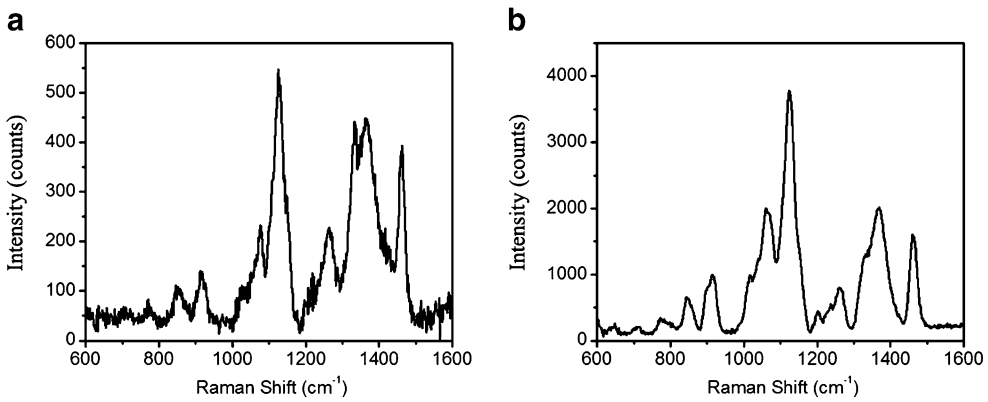

Fig. 2 Raman spectra of glucose solutions collected by conventional bulk Raman spectroscopy at a glucose concentration of 1 M (**a**) and a liquid-filled PCF probe at a glucose concentration of 50 mM (**b**)

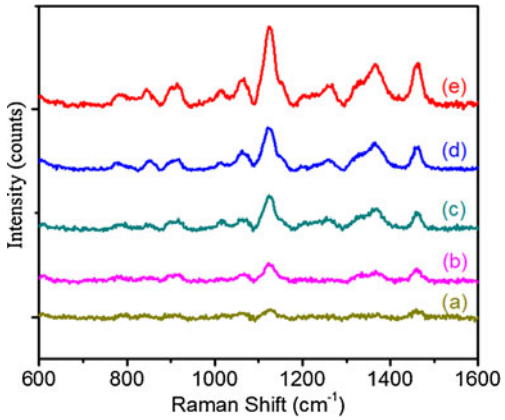

Fig. 3 Raman spectra of glucose solutions collected by the liquid-filled PCF probe at various concentrations: 1 mM (**a**), 5 mM (**b**), 10 mM (**c**), 15 mM (**d**), 25 mM (**e**)

1,065, 1,127, and 1,459 cm^{-1} are observable at concentrations as low as 1 mM. Concentration-dependent Raman intensities of glucose are plotted in Fig. 4 using the strong Raman peak at 1,127 cm^{-1}. A clear linear relationship between the Raman intensity and the concentration is evident, which is critical for the quantitative measurement of the glucose levels. Also, five measurements were taken for each PCF sample at different concentrations. It is shown that the variation of the Raman signal is only 6.0% at a concentration of 15 mM, which is mainly due to the fluctuation of the laser power, the system noise, and the slight change in PCF position under the microscope.

The above results demonstrate glucose detection at the physiological concentrations by Raman spectroscopy, with the lowest laser power (2 mW) and the shortest integration time (30 s). This level of performance is comparable to and even lower than that in the reported SERS protocols [15–20]. Additionally, the PCF detection method has the following advantages: (1) PCF detection is highly reproducible without uniformity and substrate instability issues (common problems in SERS detection), making the quantitative characterization of glucose concentration feasible. (2) PCF probe preparation and measurement is simpler than that in SERS detection, which involves complicated and time-consuming steps such as SERS substrate fabrication and analyte adsorption. (3) The required sample volume is very low. The maximum sampling volume is estimated to be 50 nL, considering the total volume of all air channels in the PCF. The effective sampling volume is expected to be less than the maximum value [33]. (4) Similar to other fiber sensors, the PCF probe possesses the unique advantages of flexibility and potentially in situ remote sensing capability.

To demonstrate the molecular specificity of Raman spectroscopy with the liquid-filled PCF probe, we chose fructose as the competing sugar in the solution. Figure 5 shows the Raman spectra of glucose (10 mM), fructose (10 mM), respectively, and that of a mixture of glucose (5 mM) and fructose (5 mM). The Raman spectra of glucose and fructose can be easily distinguished and are consistent with previous reports [11, 36]. The Raman signal of the mixture is approximately half of the sum of the individual Raman signals of these two molecules at 10 mM as the concentrations are reduced by a factor of 2. This work demonstrates that the PCF probe can quantitatively detect glucose at physiological concentrations as well as fingerprint glucose and differentiate it from fructose. The implementation of this PCF technique in serum or blood sample is clearly of interest, but is much more complex and will be pursued in the next step; a recent study by Khetani et al. [37] has shown some promising results about the performance of the PCF scheme in serum samples. Moreover, handling the high-dimensional Raman spectroscopy datasets within such biological fluids is very compli-

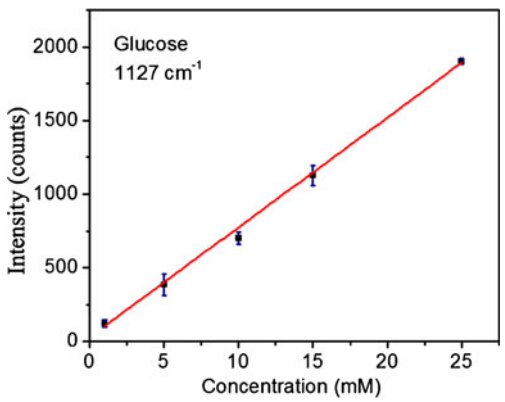

Fig. 4 Concentration-dependent Raman intensities of glucose (1,127 cm^{-1}) measured by the liquid-filled PCF probe. Each datum indicates five measurements for each PCF sample. Each *error bar* indicates the standard deviation

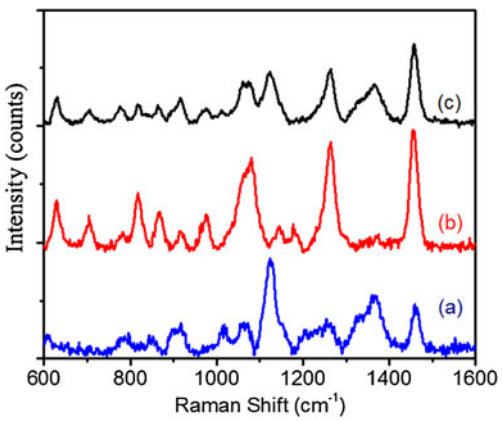

Fig. 5 Raman spectra of glucose (10 mM) (**a**), fructose (10 mM) (**b**), and a mixture of glucose (5 mM) and fructose (5 mM) (**c**) measured by the liquid-filled PCF probe

cated, and an appropriate classification approach, such as principal component analysis, would be applied in future studies [38, 39].

Conclusion

In summary, we have demonstrated that PCF is a powerful tool for quantitative glucose detection at a physiologically relevant concentration range using Raman spectroscopy. Due to the high sensitivity and compactness of the liquid-filled PCF probe, measurements can be conducted under low laser power (2 mW), short integration time (30 s), and small sampling volume (50 nL). The molecular specificity of this technique enables glucose detection in a mixture solution of glucose–fructose. Further improvement in the detection limit can be achieved by optimizing the design of the PCF probe to minimize the absorption and the propagation loss. Furthermore, the PCF probe can be integrated with SERS techniques to further improve the sensitivity, which would be important for potential glucose detection in urine, tear, or sweat. In addition, the potential implementation of a portable PCF Raman system for clinical glucose monitoring will be important [40].

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References

1. Wild S, Roglic G, Green A, Sicree R, King H (2004) Diabetes Care 27:1047–1053
2. Wang J (2008) Chem Rev 108:814–825
3. Steffes PG (1999) Diabetes Technol Ther 1:129–133
4. Klonoff DC, Braig J, Sterling B, Kramer C, Goldberger D, Trebino R (1998) IEEE LEOS Newsletter 12:13–14
5. Lambrecht A, Beyer T, Hebestreit K, Mischler R, Petrich W (2006) Appl Spectrosc 60:729–736
6. Vrančić C, Fomichova A, Gretz N, Herrmann C, Hoecker S, Pucci A, Petrich W (2011) Analyst 136:1192–1198
7. Cameron BD, Gorde HW, Satheesan B, Cote GL (1999) Diabetes Technol Ther 1:125–143
8. Russell RJ, Pishko MV, Gefrides CC, McShane MJ, Cote GL (1999) Anal Chem 71:3126–3132
9. Tong A, Yamauchi A, Hayashita T, Zhang Z, Smith B, Teramae N (2001) Anal Chem 73:1530–1536
10. Schrader B, Bougeard D (1995) Infrared and Raman spectroscopy: methods and applications. Weinheim, VCH, 787 pp
11. Berger AJ, Koo TW, Itzkan I, Horowitz G, Feld MS (1999) Appl Opt 38:2916–2926
12. Lambert J, Storrie-Lombardi M, Borchert M (1998) IEEE LEOS Newsletter 12:19–22
13. Campion A, Kambhampati P (1998) Chem Soc Rev 27:241–250
14. Kneipp K, Kneipp H, Itzkan I, Dasari RR, Feld MS (2002) J Phys: Condens Matter 14:R597–R624
15. Shafer-Peltier KE, Haynes CL, Glucksberg MR, Van Duyne RP (2003) J Am Chem Soc 125:588–593
16. Yonzon CR, Haynes CL, Zhang XY, Walsh JT, Van Duyne RP (2004) Anal Chem 76:78–85
17. Stuart DA, Yonzon CR, Zhang X, Lyandres O, Shah NC, Glucksberg MR, Walsh JT, Van Duyne RP (2005) Anal Chem 77:4013–4019
18. Lyandres O, Yuen JM, Shah NC, Van Duyne RP, Walsh JT, Glucksberg MR (2008) Diabetes Technol Ther 10:257–265
19. Yuen JM, Shah NC, Walsh JT, Glucksberg MR, Duyne RP (2010) Anal Chem 82:8382–8385
20. Dinish US, Yaw FC, Agarwal A, Olivo M (2011) Biosens Bioelectron 26:1987–1992
21. Birks TA, Roberts PJ, Russell PSJ, Atkin DM, Shepherd TJ (1995) Electron Lett 31:1941–1943
22. Cregan RF, Mangan BJ, Knight JC, Birks TA, Russell PSJ, Roberts PJ, Allan DC (1999) Science 285:1537–1539
23. Ritari T, Tuominen J, Ludvigsen H, Petersen JC, Sorensen T, Hansen TP, Simonsen HR (2004) Opt Express 12:4080–4087
24. Jensen JB, Pedersen LH, Hoiby PE, Nielsen LB, Hansen TP, Folkenberg JR, Riishede J, Noordegraaf D, Nielsen K, Carlsen A, Biarkley A (2004) Opt Lett 29:1974–1976
25. Smolka S, Barth M, Benson O (2007) Appl Phys Lett 90:111101
26. Konorov SO, Addison CJ, Schulze HG, Turner RFB, Blades MW (2006) Opt Lett 31:1911–1913
27. Pristinski D, Du H (2006) Opt Lett 31:3246–3248
28. Amezua-Correa A, Yang J, Finlayson CE, Peacock AC, Hayes JR, Sazio PJA, Baumberg JJ, Howdle SM (2007) Adv Funct Mater 14:2024–2030
29. Han Y, Tan S, Oo MKK, Pristinski D, Sukhishvili S, Du H (2010) Adv Mater 22:2647–2651
30. Yan H, Liu J, Yang C, Jin G, Gu C, Hou L (2008) Opt Express 16:8300–8305
31. Zhang Y, Shi C, Gu C, Seballos L, Zhang JZ (2007) Appl Phys Lett 90:193504
32. Shi C, Lu C, Gu C, Tian L, Newhouse R, Chen S, Zhang JZ (2008) Appl Phys Lett 93:153101
33. Yang X, Shi C, Wheeler D, Newhouse R, Chen B, Zhang JZ, Gu C (2010) J Opt Soc Am A 27:977–984
34. Yang X, Shi C, Newhouse R, Zhang JZ, Gu C (2011) Int J Opt 2011:754610
35. Yang X, Gu C, Qian F, Li Y, Zhang JZ (2011) Anal Chem 83:5888–5894
36. Mathlouthi M, Luu DV (1980) Carbohydr Res 78:225–233
37. Khetani A, Tiwari VS, Harb A, Anis H (2011) Opt Express 19:15244–15254
38. Patel II, Martin FL (2010) Analyst 135:3060–3069
39. Patell II, Trevisan J, Singh PB, Nicholson CM, Krishnan RK, Matanhelia SS, Martin FL (2011) Anal Bioanal Chem 401:969–982
40. Yang X, Tanaka Z, Newhouse R, Xu Q, Chen B, Chen S, Zhang JZ, Gu C (2010) Rev Sci Instrum 81:123103