Hollow core photonic crystal fiber as a robust Raman biosensor

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ABSTRACT

The present work demonstrates the integration of hollow core photonic crystal fibers (HC-PCF), microfluidics, and statistical analysis for monitoring biomolecules using Raman spectroscopy. HC-PCF as a signal enhancer has been proven by many researchers. However, there have been challenges in using HC-PCF for practical applications due to limitations such as coupling, stability, evaporation, clogging, consistent filling, and reusing the same fiber. This limited the potential of HC-PCF to detect low concentrations of liquid samples, which is why HC-PCF still hasn't transcended the lab barriers. The current device is based on an H-design lay-out which uses the pressure difference between the two ends of the fiber for filling and flushing the liquid samples. This mitigated several issues related to device performance by allowing us to fill the fiber with liquid samples consistently, rapidly and reproducibly. The resulting Raman signals were significantly more stable as various concentrations of ethanol in water were sequentially introduced into the fiber. The scheme also allowed us to overcome the barrier of predicting low concentrations by applying Partial Least Square (PLS) technique which was done for the first time using HC-PCF. Thus, the present scheme paves path for the inclusion of HC-PCF in the main stream point-of-care technology.

Keywords: Hollow core photonic crystal fiber, Raman spectroscopy, biosensor, multivariate analysis

1. INTRODUCTION

Hollow-core photonic crystal fibers (HC-PCF) has periodic array of air holes running along the fiber [1]. In recent years HCPCF has gained importance as a Raman biosensor as it allows extremely efficient interaction between light and sample [2-7]. Some of the research reported have used HC-PCF merely as a micro-litre sample container while emphasizing their importance for screening samples whose availability in terms of volume is somewhat limited; especially for characterizing bodily fluids such as blood, saliva etc. While other researchers, including ourselves, have made a conscious effort to preserve the photonics band gap property in a sample filled HC-PCF for initiating stronger modal field overlap with the species of interest, and thus achieved higher molecular detection sensitivity [8-11].

The role of HC-PCF as a 'Raman signal enhancer' has been well established, the question remains whether it possess the necessary attributes for practical sensing applications. There are a number of difficulties associated with implementing HC-PCFs for on-line monitoring of the sample. First, light coupling and guidance is affected by the formation of air gaps or discontinuous sample distribution within the micro-channels. It results in poor quality spectra that don't correlate well with chemical concentration. Second, each sample solution requires new strand of HC-PCF which is not only time consuming but also influence the light coupling conditions. Variation in the light launching condition from one HC-PCF to another alters the propagation properties that again put a question mark on the spectral reproducibility and correlation with the sample concentration. Finally, conventional methods of sample filling into HC-PCF based on capillary action are slow which poses a significant impediment towards reach near real-time sensing.

With this consideration, we present a novel detection scheme that exploits Raman spectroscopy for monitoring samples filled into HC-PCF which is further integrated with a 'differential pressure system'. The present investigation builds on our earlier work on HC-PCF based Raman sensing and demonstrate the integration of a fluidic control system aimed at ensuring repeatability and reusability of the HC-PCF sensor. The sensor layout exhibits an H-shaped differential pressure system common to microelectromechanical systems (MEMS) microfluidic flow control. [12]. The HC-PCF is perpendicularly connected to these channels that are maintained at different pressure. The HC-PCF embedded into differential pressure system enabled fast filling, evacuation and refilling of sample solutions into its hollow core/cladding. The design configuration, as presented in this paper, offers high degree of control on the sample flow rate/direction which facilitates characterization of different sample sets with one single HC-PCF. Other novel features of this study include the application of multivariate analysis, for the first time, on the spectral data obtained from sample filled into HC-PCF.

2. EXPERIMENTAL

2.1 Theory

The light guiding property of non-selectively filled HC-PCF, changes depending on the refractive index of the filled sample. In this case, the guiding principle is still due to bandgap effect but the transmission band supported by the fiber is shifted. The shift in the transmission wavelength of HC-PCF can be determined from the equation given by Russell et al [13], as follows:

$$\lambda' = \lambda_0 \left[\frac{1 - \left(\frac{n_{liq}}{n_{sil}}\right)^2}{1 - \left(\frac{n_{air}}{n_{sil}}\right)^2} \right]^{1/2} \tag{1}$$

λ_0 is the wavelength of the fiber when empty, and λ' is the shifted wavelength of the fiber when filled with the sample, n_{liq} being the refractive index of liquid sample, n_{air} refractive index of the air and n_{sil} being refractive index of the silica.

2.2 Flow rate

In all the experiments, the HC-PCF sensor was filled with fluidic samples and the corresponding Raman spectra were recorded. An important point to note is that this is a special case of sample filling/re-filling in a micro-litre volume cylinder (HC-PCF). It is because the experimental demonstration involved sequential filling of different samples into a single HC-PCF followed by recording their Raman spectrum. Each cycle was preceded (or followed) by purging the HC-PCF with de-ionised water while completely removing the sample solution. Subsequently, new sample solution had been injected that pushed the de-ionised water out of HC-PCF. In other words, ascertaining various parameters of fluid dynamics such as flow rate, optimal pressure difference (across the HC-PCF) took into account that one sample (e.g. water) faced resistance by already existing sample (e.g. ethanol) in HC-PCF. Under this situation, we used a modified form of Hagen-Poiseuille equation to determine sample flow rate (F) [14]:

$$F = \frac{r^4 * \Delta P}{\mu_{avg} * l * 1.625 * 10^{-8}} \tag{2}$$

where r and l denote the radius and length of the HC-PCF, respectively. The pressure difference across the HC-PCF is represented by ΔP. Since the average viscosity of the system continuously changes as the 2nd liquid displaces the 1st, the modified Hagen-Poiseuille equation crudely approximates as the average viscosity (μ_{avg}) of both liquids.

2.2 Experimental Set-up

The layout of HC-PCF sensor is shown in Fig. 1. It consists of two segments-optics and H-shaped differential pressure system. The optical assembly employed a single mode 100-mW, 14-pin butterfly pin laser (Photonic Solutions) with a central wavelength of 785-nm. The laser beam was passed through a bandpass filter (BP) centered at 785-nm (+/- 2nm) to filter out other wavelength components around 785-nm from the laser diode. Then, it was directed through a dichroic filter (R785RDC, Chroma Technologies Corp.) which reflected 785-nm (+/-5nm) at an angle of 45^0 and transmitted 790-1000 nm band. The dichroic filter acted as a reflector for the laser beam which was further focused onto the tip of the HC-PCF by a 40x microscopic objective lens (L1) with numerical aperture (N.A) as ~0.22. Furthermore, the dichroic filter acted as a high pass filter for the light scattered backward from the sample-filled HC-PCF, thus allowing only the Raman wavelength to pass through it. The filtered Raman light was then imaged onto a fiber bundle (Fiberoptic System Inc., 26 multimode fiber, NA= 0.22) by another 6.3x microscopic objective lens (L2) with N.A as ~0.22. The output of the fiber bundle was interfaced into a Kaiser f/18i Spectrograph with a TE-cooled Andor CCD camera. The Andor SOLIS software was used for spectral data acquisition and spectra were monitored on the data acquisition computer.
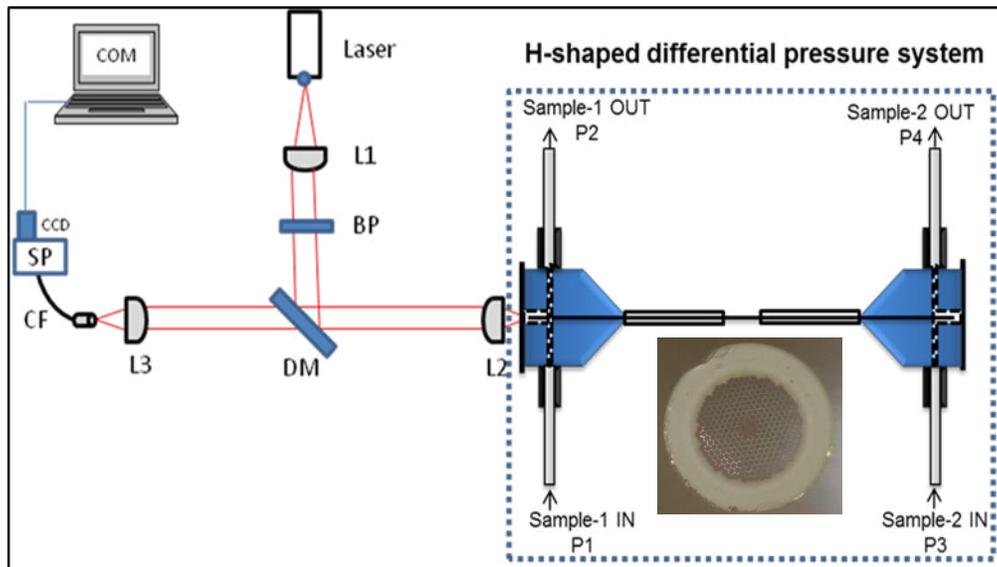

Fig. 1. Schematic of the setup. Laser: Laser source; L1: Collimating lens; BP: Band pass filter; DM: Dichroic Mirror; L2: Microscope objective lens for light coupling; H-shaped differential pressure system with hollow core photonic crystal fiber; L3: Microscope objective lens for backward light collection; CF: Collection fiber; SP: spectrograph; CCD: CCD camera; COM: Computer.

The other segment of sensor configuration comprised of two parallel channels (tubing)-one for sample (ethanol) input/output and other for purging fluid (water) input/output. One end of each tubing was dipped into sample (ethanol)/purging liquid (water) contained in a glass vial (source reservoir). The other ends were connected to empty vials for collecting (ethanol)/purging liquid (water) which acted as collection reservoir. The terminal ends of each of these channels were maintained at different pressures P1, P2, P3 and P4 as shown in Fig. 1 which could be varied to control the flow rate and direction of fluids inside the channels. The HC-PCF was connected perpendicularly to each of these two channels via 4-way micro fluidic cross and mounted on a Thorlab flexure stage. The integration of HC-PCF perpendicularly with two parallel fluidic channels looked like an H-shaped structure as shown in the Fig.1.

In order to flow sample 1(sample mixture) inside the fiber channels, average pressure P1+P2 was kept higher than the P3+P4 and for sample 2 (water) the pressure were reversed. This ensured the liquid can be filled as well as rinsed just by switching the pressure. The P1, P2, P3, P4 value are determined depending on the required flow rate needed for the HC-PCF to be filled in a given time.

For filling HC-PCF with sample 1 (ethanol and water mixture):

$$\frac{P1+P2}{2} > \frac{P3+P4}{2}$$

(3)

For purging HC-PCF with sample 2 (water):

$$\frac{P1+P2}{2} < \frac{P3+P4}{2}$$

(4)

3. RESULTS & DISCUSSION

In the first phase of the research, we investigated the effect of varying the pressure difference across HC-PCF on the time it takes to completely fill the HC-PCF with the sample. We determined the sample filling time by measuring the time it takes for the Raman signal intensity of ethanol to reach maximum from zero. Fig. 2 shows that the experimentally determined time of sample filling in HC-PCF decreased almost exponentially with the rise of pressure difference in the range of 15 to 60 psi. It indicates that pressure difference of 60 psi reduced the sample filling time as low as ~4 min. Further time reduction down to 1min. is possible by increasing the pressure further or by decreasing the length of the fiber. Our experimental values are validated by theoretical values of sample filling time as shown in Fig. 2
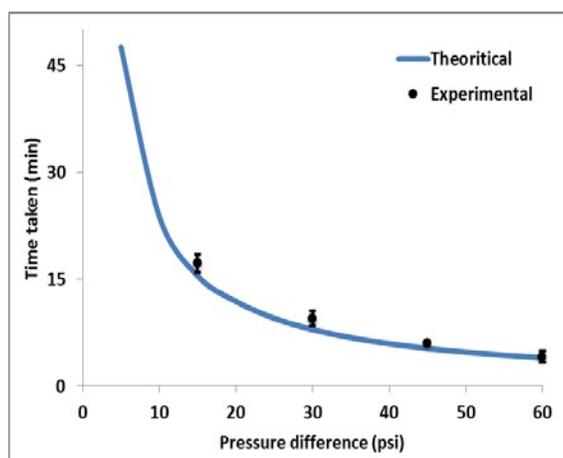

Fig. 2 Theoretical and experimental time for filling sample into HC-PCF at different pressure

The final phase of investigation focused on applying partial least square (PLS)-an important quantitative analysis tool of multivariate analysis- on the Raman spectrum of various samples that were injected into the HC-PCF. The PLS calibration model is constructed where the response Y-variable (analytical data) depends on more than one explanatory X-variable (Raman shift wave number) analytical data. The PLS has been widely used with Raman spectroscopy for trace level detection of bulk samples (cuvette) [15]. However, its application to Raman spectral data collected from sample filled HC-PCF has not been reported to-date. This is due to a number of reasons. Conventionally, different HC-PCFs are employed for different sample sets. However, replicating the exact light coupling condition for every single HC-PCF is difficult as different modes are excited depending on the alignment of HC-PCF tip with respect to the focusing optics. Moreover, sample filling into HC-PCF by capillary action, as commonly done, may not ensure complete coverage of core and cladding channels, or the sample distribution may vary from one HC-PCF to another. As a result, light guidance within HC-PCF gets severely impacted and both the Raman signal and the spectral background fluctuates from one sample to another. In such a situation, any kind of multivariate analysis tool, including PLS, fails to establish a proper correlation between the Raman signal and the species concentration which ultimately leads to false prediction of sample constituent.

With this consideration, we injected different sample solutions into a single HC-PCF followed by recording their respective Raman spectrum, without altering the light coupling condition. Between the consecutive injections of any two sample solution, we rinsed the HC-PCF with water to completely remove the traces of the preceding sample solution.

This has resulted in obtaining high quality Raman spectrum as the 'differential pressure system' provided a better control on the flow direction/rate within the HC-PCF. Our spectral data set exhibited least spectral background fluctuation and was therefore suitable for direct multivariate analysis, without requiring spectral pre-processing. Fig. 3 shows the Raman spectrum of different samples where the ethanol concentration varied with respect to water. Partial least square was applied to the spectral data to obtain a calibration curve as shown in the inset of Fig. 3. The calibration model predicts the concentration of ethanol in the range of 5-60% with a low root mean square of 0.9%. The coefficient of determination (R2) was found to be 0.997 which indicated the Raman spectral data of different ethanol-water samples correlated extremely well with the concentration of ethanol.
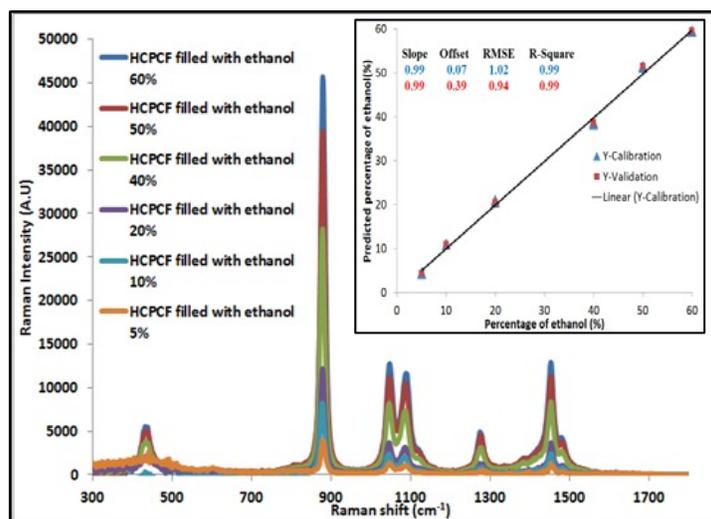

Fig. 3 Raman spectra of different concentration of ethanol filled in HC-PCF and PLS prediction

CONCLUSION

We have demonstrated HC-PCF as a robust biosensor by integrating pressure-driven flow. The present scheme improved the stability, the speed of filling and the reusability of HC-PCF and thus paves the path for a HC-PCF based Raman sensing platform to be utilized in real-time monitoring and diagnostic applications. Our experimental configuration allowed complete filling of samples into HC-PCF which was consistent for all sets of sample mixture. This enabled stable operation of HC-PCF based Raman sensor for the first time and resulted in high quality spectral data. With the achievement of stability and reproducibility, we have demonstrated the ability of using of partial least square (PLS) analysis on the spectral data to accurately predict the concentration of ethanol in sample mixture.

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