Microfluidic Raman Spectroscopy for Bio-chemical Sensing and Analysis

Praveen C. Ashok and Kishan Dholakia

Abstract The detection and analysis of bio-chemical analytes are important in the fields of personal healthcare, drug development, and environmental science, among others. The field of microfluidics aims to realize portable devices which can perform fast and sensitive bioanalyte detection with minimal sample preparation. Raman spectroscopy is a powerful tool for analyte detection owing to its high specificity and its ability for multi-component detection in an analyte. Combining microfluidics with Raman spectroscopy would help achieve miniaturized analytical devices that may provide rich information about a given analyte. However, the low cross-section of Raman process demands special geometries to achieve such a convergence. The majority of the previous embodiments were restricted to free-space geometry, limiting portability. However, in recent studies, fiber-based Raman detection system incorporated in microfluidics offers the opportunity to develop portable optofluidic bioanalyte detection devices. Here, we review various approaches used for using Raman spectroscopy in microfluidics for analyte detection, and various analytical approaches that could be used to enhance the detection sensitivity of Raman spectroscopy-based detection. This is followed by a detailed discussion about the fiber-based optofluidic Raman detection systems.

Keywords Analyte detection • Fiber Raman probe • Microfluidics • Raman spectroscopy • Soft lithography

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W. Fritzsche and J. Popp (eds.), *Optical Nano- and Microsystems for Bioanalytics*, Springer Series on Chemical Sensors and Biosensors (2012) 10: 247–268
DOI 10.1007/978-3-642-25498-7_9,

Contents

1 Introduction

The bio-chemical analysis communities are pushing toward the development of cheaper, miniaturized, and portable devices while simultaneously achieving lower detection limits, improved sensitivity, faster analysis, and high throughput with a reduced requirement upon sample volume [1]. This has resulted in the unprecedented advancement of the field that is variously addressed using terms such as "microfluidics and nanofluidics," "Lab on a Chip (LoC)," or "micro total analysis system (μTAS)" [2]. To achieve functionality, LoC technology requires integration of various tools for on-chip sample preparation, control, and sensing. To achieve sensing and detection within microfluidic devices, techniques such as chemical, thermal, or optical sensing are being widely used [1, 2].

Among these, optical detection techniques have played a crucial role in the development of LoC technology. Various optical imaging and spectroscopic techniques have been widely used for bio-chemical sensing and analysis as they have proven track records in achieving the lowest limits of detection while obtaining a high amount of information from the samples [3]. Among these, Raman spectroscopy is a potential optical detection technique which has found several applications within microfluidic platforms [1].

Over the past 3 decades, Raman spectroscopy has emerged as a powerful analytical tool for bio-chemical analysis. Raman spectroscopy is the result of inelastic scattering of photons from samples [4], yielding information about vibrational and rotational modes in a system. As it is a scattering process which does not involve any electronic process from the sample such as absorption or fluorescence, Raman spectroscopy can be performed irrespective of the electronic energy levels of the sample of interest. A vibronic energy level diagram for the Stokes Raman scattering process is shown in Fig. 1. Raman spectra are presented as a Raman shift with respect to the excitation wavelength, and this shift is expressed in wave numbers (units of cm^{-}). The particular advantage of using Raman scattering is

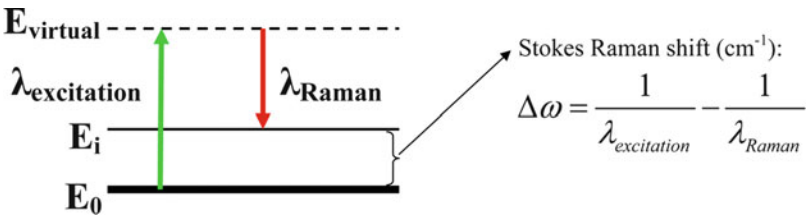

Fig. 1 Energy level diagram of Raman stokes scattering process. "$\lambda_{excitation}$" is the wavelength of the excitation photon, "λ_{Raman}" is the wavelength of Raman photon, "$\Delta\omega$" is the Stokes Raman shift, and "E" is the energy level of the atom or molecule. Raman shift is normally expressed in units of "cm^{-1}"

that it can be performed at a range of excitation wavelengths. Thus, the excitation pulse can be tuned to consider the absorption of the analyte and its surroundings, unlike infrared absorption spectroscopy which is incompatible with the water-rich environment of biological systems due to the strong absorption of water in the infrared region. In contrast to fluorescence spectroscopy, Raman spectra provide sharp, distinctive Raman peaks corresponding to the vibrational characteristics of the sample. Through deconvolution of the resulting spectra, it is possible to achieve simultaneous multi-component detection, effectively giving a "chemical fingerprint" of the composition of the sample of interest [1].

Even though this is a powerful spectroscopic technique, the cross- section of Raman scattering process is orders of magnitude lower than that of fluorescence. The availability of highly sensitive CCDs has given a boost for the field of Raman spectroscopy. One of the solutions to overcome the issue of low Raman cross-section is surface-enhanced Raman spectroscopy (SERS) [5]. However, for the implementation of SERS, one needs to either mix the samples with colloidal nanoparticles or rely on the adsorption of the sample on specialized SERS substrates. Consequently, even though SERS offers enhancement in orders of magnitude, the increased complexity and poor reproducibility of the process make it less favorable when compared to standard Raman spectroscopy.

Implementation of Raman spectroscopy as a viable analytical detection method in microfluidics is still in its infancy when compared to other spectroscopic techniques [6]. This chapter reviews the history of microfluidic Raman spectroscopy (MRS) and discusses some of the recent advancements in this field in terms of implementation. Such developments could lead to the development of microfluidic Raman analytical devices that are portable and alignment free, suitable for field applications.

2 History of Microfluidic Raman Spectroscopy

It has been over a decade since the birth of MRS. In the first reported work, microfluidics and Raman spectroscopy were combined. Since then, several groups demonstrated the power of Raman spectroscopy as a potential optical detection tool

to interrogate samples in microfluidic chips. Later years saw a wide variety of applications result from the marriage of these two powerful technologies. The reviews by Viskari and Landers (2006) [1] and Hunt and Wilkinson (2008) [3] discuss the implementation of Raman spectroscopy as a potential optical detection technique in microfluidics. MRS has seen variants of its implementation in terms of different microfluidic architectures and different Raman spectroscopic techniques such as normal Raman spectroscopy, confocal Raman spectroscopy, resonance Raman spectroscopy, and SERS [1]. Here, we review the evolution of MRS over last decade, mentioning the major works that helped the advancement of this technology. We present various relevant studies in the correct context and chronologically in order to illustrate the relevance of these works in the advancement of the field of MRS. A special emphasis is given to each study on the various microfluidic architectures and Raman detection schemes adopted for the implementation of this technology for bio-chemical analysis.

2.1 Initial Works

Since the late 1980s, there have been several papers where microcapillary-based electrophoretic systems were interrogated with Raman spectroscopy [7, 8]. A similar approach was used to analyze isotachophoretically concentrated ribonucleotides using Raman spectroscopy [9]. Capillaries, however, had limited surface area, reducing the sensitivity of detection, and the alignment requirement was higher for mounting a capillary on a conventional Raman microscope. These issues led to the implementation of a microchip-based version of the same separation technique, in which on-chip Raman spectroscopy of isotachophoretically separated herbicides—paraquat and diquat—was achieved [10]. In this work, a glass microfluidic chip was coupled to a free-space Raman microprobe; more efficient collection of Raman signal could be achieved with reduced background from the glass by carefully choosing the thickness of the coverslip under the microchip. This study envisages the opportunities for further enhancement of sensitivity by implementing SERS or resonant Raman spectroscopy.

In a later study, time-resolved resonance Raman spectroscopy (TR^3) was employed within a glass microfluidic chip in order to study chromophore structure [11, 12]. This study was achieved through miniaturization of the standard rapid-flow technique used to study photochemical kinetics and combining it with Raman spectroscopic detection. The standard rapid-flow technique was performed using a macro flow device, requiring high amounts of pigments at high flow rates. The implementation of a microfluidic version of the device reduced the required sample volume by shrinking the fluidic channel to match the confocal volume of the Raman detection system.

The pioneering work on the implementation of SERS in microfluidics was the detection of a derivative of trinitrotoluene (TNT) in a glass microfluidic chip using surface-enhanced resonance Raman spectroscopy (SERRS) [13]. In this device,

on-chip preparation of silver colloid was achieved which was further mixed with the sample to be detected followed by detection of SERRS. Microfluidic chip allowed reduction of the required reagent volume, and the sensitivity was improved by two orders of magnitude by using SERRS compared to macro flow cells. In addition, the long-term stability of the flow stream, offered by the microfluidic channel, allowed accumulation of signal for a longer duration.

2.2 Raman Spectroscopy to Probe Reactions

Microfluidics enables real-time study of chemical reactions through micro-reactors using reduced volume of reagents. This enables optimization of various parameters, which is essential for efficient large-scale production of chemicals commercially. Various detection methods—thermal, electrical, and optical—have been combined with micro-reactor-based microfluidic chips in order to probe the temporal and spatial evolution of a chemical reaction. The ability for Raman spectroscopy to perform simultaneous multi-component detection makes it a desirable method to monitor the progress of a reaction within a micro-reactor. There have been several architectures to integrate Raman detection in micro-reactors for temporal and spatial mapping of the chemical composition of the sample in micro-reactors. The formation of diazonium salts in anhydrous conditions and their subsequent chlorination in situ were studied using online, on-chip Raman spectroscopy by probing a glass-based microfluidic reactor using a Raman microscope [14]. Another reported study looked at spatially mapping the evolution of the reaction to form ethyl acetate from ethanol and acetic acid in a Pyrex glass-based microfluidic chip using a confocal Raman microscope [15]. It was observed in this work that SERS or resonance Raman spectroscopy should be employed to enhance the sensitivity and to reduce the acquisition time to achieve real-time monitoring of the reaction. The applicability of MRS on micro-reactors was further extended to studies of biologically relevant processes such as enzyme-catalyzed reactions for the synthesis of peptides from amino acids [16].

The first use of PDMS-based microfluidic chips in conjunction with Raman spectroscopic probing was to study the mixing of ethanol and isopropanol using confocal Raman microscopy [17]. Since PDMS is a polymer, it is highly Raman active and there are specific Raman lines due to PDMS in the fingerprint region. This work discusses the issues associated with the background signals from PDMS unless a confocal configuration is used for Raman detection. MRS for reaction optimization was further taken forward for studying various reaction dynamics with a variety of microfluidic architectures. This included designing specialized micromixers to enhance the efficiency of mixing dynamics [18, 19].

Apart from being used as a technique for studying chemical reactions, MRS was employed to study fluid dynamics. The diffusion of two miscible, non-reacting liquids was studied in a silicon glass-based microfluidic chip [20]. Confocal Raman spectroscopy was used to probe the interdiffusion of chloroform,

dimethylsulfoxide, acetonitrile, and dimethylformamide. The obtained data were used to estimate the diffusion coefficients of the liquids. Through their approach, the authors envisage exploiting the multi-component detection ability of Raman spectroscopy for probing the interdiffusion of more than two liquids, or situations where multiple chemical reactions occur simultaneously.

2.3 *Bio-chemical Detection Using SERS in Microfluidics*

As mentioned in Sect. 1, SERS-based detection is widely used to overcome the low cross-section of the Raman process and has additionally found a variety of implementations in MRS. The feasibility of combining microfluidic separation devices with SERS was demonstrated by integrating metal–polymer nanocomposite in a PDMS microfluidic chip and collecting SERS spectra using a confocal Raman microscope, as shown in Fig. 2a [21]. A mixture of riboflavin and resorufin was detected as a proof of principle to demonstrate the combination of a powerful separation technique (capillary electrophoresis) with structurally information-rich analytical technique (Raman spectroscopy).

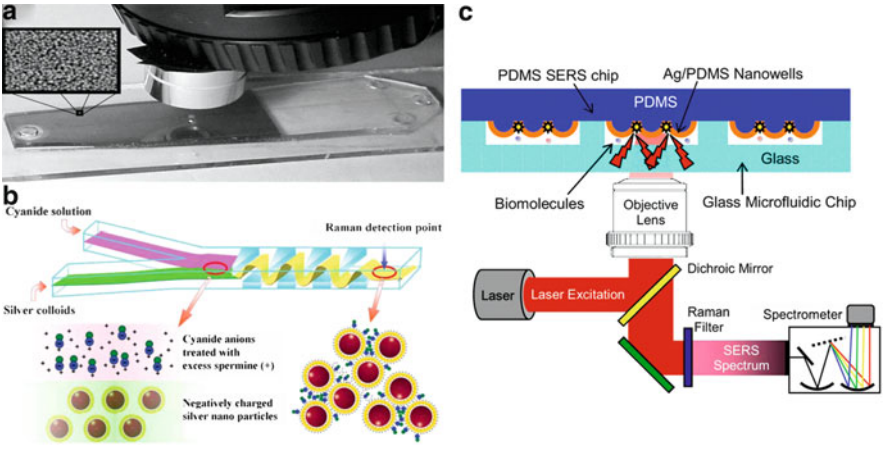

Fig. 2 (**a**) Depiction of integrated microfluidic SERS device under the 106 objective of the LabRam Raman spectrometer. Inset shows an SEM image of the silver-PDMS nanocomposite at approximately 90K magnification ([21], Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission). (**b**) Schematic illustration of alligator teeth-shaped microfluidic channel. The confluent streams of silver colloids and trace analytes are effectively mixed in the channel through the triangular structures, which are located on upper and lower surfaces of the channel in a zigzag way. The flow rate was 5 mL/min ([22], Reproduced with permission of the Royal Society of Chemistry). (**c**) Schematic diagram of the integrated microfluidic chip and the biomolecular Raman imaging system (Reprinted with permission from [23]. Copyright 2005, American Institute of Physics)

Furthermore, the feasibility of using the multi-component detection ability of Raman spectroscopy in microfluidics was demonstrated by several groups through detection of a mixture of dye-labeled oligonucleotides through multiplexed SERRS detection in a PDMS microfluidic chip [24, 25]. These enabled highly sensitive SERS detection on microfluidic platform. The microfluidic architecture has also seen improvements as several groups used PDMS chips with alligator teeth-like structures for the efficient mixing of colloids with samples for SERS detection, as shown in Fig. 2b [22, 25]. Other than colloid-based SERS detection, there have been attempts to develop efficient SERS substrates in PDMS which could be used to obtain reproducible SERS spectra of biomolecules, as shown in Fig. 2c [23].

Even though SERS-based detection offers high sensitivity and proves efficient for qualitative chemical analysis, the quantitative analysis of chemicals using SERS is not easy to achieve as it is difficult to control various experimental parameters such as degree of aggregation, particle sizes of colloidal nanoparticles, and distribution of molecules on a metal surface. The sample manipulation ability offered by microfluidics, however, makes it possible to achieve quantitative studies in SERS-based MRS. With specially fabricated three-dimensional micromixers for efficient sample mixing with colloids, several groups demonstrated quantitative SERS detection of samples [26, 27]. Unlike previous microfluidic SERS embodiments, where Raman measurements were taken in a static condition [24], it was observed that the reproducibility of SERS would be better in a flow mode [26]. The success in the implementation of SERS-based LoC devices urged researchers to use this as a technique to standardize detection achieved through other methods. In one study, confocal SERS detection was used to complement the fluorescence resonance energy transfer (FRET) data of DNA hybridization in a PDMS-based microfluidic system [28]. An exhaustive discussion on the implementation of SERS in microfluidics may be found in the review by Chen and Choo [29].

2.4 Raman Spectroscopic Probing of Microdroplets

Microfluidic devices that can generate sub-nanoliter microdroplets, dispersed within an immiscible continuous phase of oil in a rapid, efficient, and controllable manner, are gaining attention of the analytical community for a wide range of applications [30]. Microdroplet-based microfluidic systems are preferred for bio-chemical reactions and analysis as the compartmentalization provides the advantage of keeping the reactants or the sample isolated. Microdroplets are of interest for biologists as it is possible to mimic a cell-like system within a microdroplet for genomics, proteomics, or system biology studies [30]. There have been a number of reported works where Raman spectroscopy was used to probe the samples in microdroplets generated in a microfluidic system.

In one of the first reported studies of combining a microdroplet-based microfluidic system with Raman spectroscopy, a microdroplet-based micro-reactor was implemented in a PDMS chip and confocal Raman spectroscopy was used to probe

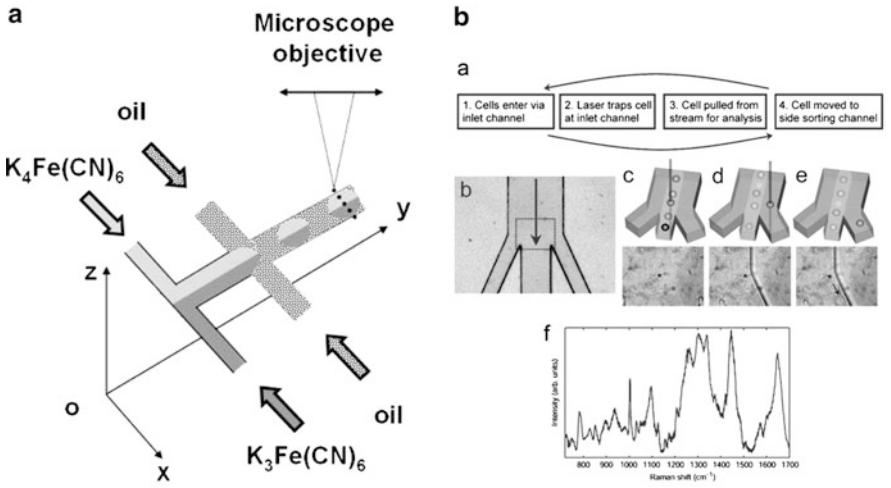

Fig. 3 (**a**) Two fluids co-flow over a short length before they meet two transversal oil streams at the "flow focusing" junction. Droplets presenting two unmixed moieties are dropped off the junction ([31], Reproduced with permission of the Royal Society of Chemistry). (**b**) Illustration of the operating principles of the optofluidic Raman-activated cell sorting (RACS) platform ([32], Reproduced with permission of the Royal Society of Chemistry)

the microdroplets [31]. It was observed that the droplet-based micro-reactors are ideal as they can host a rapid exothermal chemical reaction in a controlled condition. In situ probing of the composition of the droplets was achieved using Raman spectroscopy, and the mixing dynamics within the droplets were studied—a schematic of which is shown in Fig. 3a. However, acquisition time had to be kept high to achieve sufficient sensitivity for probing the sample; hence, multiple droplets would pass through the interrogation region during an acquisition, giving only the signal averaged to several droplets. There would also be interference in the spectra from the oil phase which had to be subtracted when post-processing the spectra. Another embodiment of microdroplet-based MRS studied on-chip photopolymerization of benzyl methacrylate in a borosilicate glass-based microfluidic device using a low-resolution Raman system. In this study, a fiber probe was used instead of an objective to excite and collect the Raman signal [33]. However, comparatively long acquisition time (3 min) made this system unsuitable for online monitoring of the process.

In order to avoid issues of sensitivity and background from the oil, SERS detection was combined with a microdroplet-based glass microfluidic system [34]. Samples mixed with gold colloids were sequestered in microdroplets and SERS signals were acquired from the sample with an acquisition time of 1 s. Such a detection scheme has the potential for use in process diagnostics in which online detection of drugs, water pollutants, or food additives is required. The dynamics of microdroplets was studied using confocal Raman spectroscopy through monitoring the isotopic exchange reaction between D_2O and H_2O [35]. Even though the comparatively long acquisition time created issues with obtaining average signals

from multiple droplets, this system could be used to study interdiffusion mixing and droplet mixing within a microfluidic channel through spatially resolved concentration maps of the sample.

2.5 *Microfluidic Raman spectroscopy in Cell Science*

The use of microfluidic systems for cell biology research is a rapidly growing area of research [36]. The main use of microfluidic systems in cell biology is to obtain the chemical information of cells and tissues at a molecular level. The ability of Raman spectroscopy to probe the molecular fingerprint of a sample makes it desirable to be combined with microfluidics to answer fundamental questions in cell biology. The relatively long acquisition time of Raman spectroscopy (a few seconds to several minutes) makes it necessary to have a tool which can noninvasively hold the cell within the microfluidic channel, away from the fluidic channel walls, while Raman spectra are acquired. A solution to this is to use optical tweezers which has its proven track record for its ability in single cell manipulation [37]. Interestingly, the combination of optical tweezers with micro-Raman spectroscopy has developed as a field of its own, finding a variety of applications in the area of biophotonics [38]. The combination of micro-Raman tweezers technology with microfluidics turns out to be a powerful technology where microfluidics adds the advantage of providing more controlled environment for single cell studies.

One of the first attempts toward this was by combining micro-Raman tweezers with microfluidics to study the oxygenation cycle of red blood cells (RBC) [39]. A single RBC was trapped in a PDMS-based microfluidic chip, and the oxygenation cycle of hemoglobin with respect to the surrounding environment was studied through confocal resonance Raman spectroscopy. The same group used this system for further studies on protein denaturization in RBC [40] and for studies of several globin-containing cells, where in vivo conditions were mimicked in a microfluidic channel [41]. In another study, instead of micro-Raman tweezers, combined with microfluidics, a PDMS-based microfluidic system was used along with a dual beam fiber optical trap to trap HL60 human Promyelocytic leukemia cells, and a confocal Raman system was used to record Raman spectra of the cells [42]. In another study, in situ chemical characterization of Chinese Hamster Ovary cells (CHO-K1) with spatial and temporal resolution was achieved by recording SERS of immobilized cells mixed with gold nanoparticle in a microfluidic channel using a confocal Raman microscope [43].

The prospect of obtaining Raman spectra of single cell in a flow-based microfluidic system, in combination with micro-Raman tweezers, offers the opportunity to develop Raman-activated microfluidic cell-sorting devices. Such a system was realized in which sorting of leukemia cells was demonstrated as a proof of principle. In this study, optical tweezers trapped the cells, the Raman signals of which were acquired by using a confocal Raman microscope,

as shown in Fig. 3b [32]. After analyzing the Raman spectra, the optical tweezers moved the cell toward the corresponding sorted channel. This technique, however, had very low throughput due to relatively long Raman acquisition time.

2.6 *Recent Developments*

The ultimate goal of LoC technology is to create analytical devices that are portable so that they can be used for point-of-care testing and field analysis. This makes miniaturization of MRS devices important. In a recently reported study, a microfluidic system for confocal SERS detection of dipicolinic acid and malachite green was realized [44]. Specially fabricated micropillars on the PDMS chip was used to achieve efficient mixing of colloidal nanoparticles with the sample. The notable achievement of this work is the implementation of the detection on a benchtop Raman microscope, as shown in Fig. 4. This is a step forward toward developing MRS devices for real field test applications.

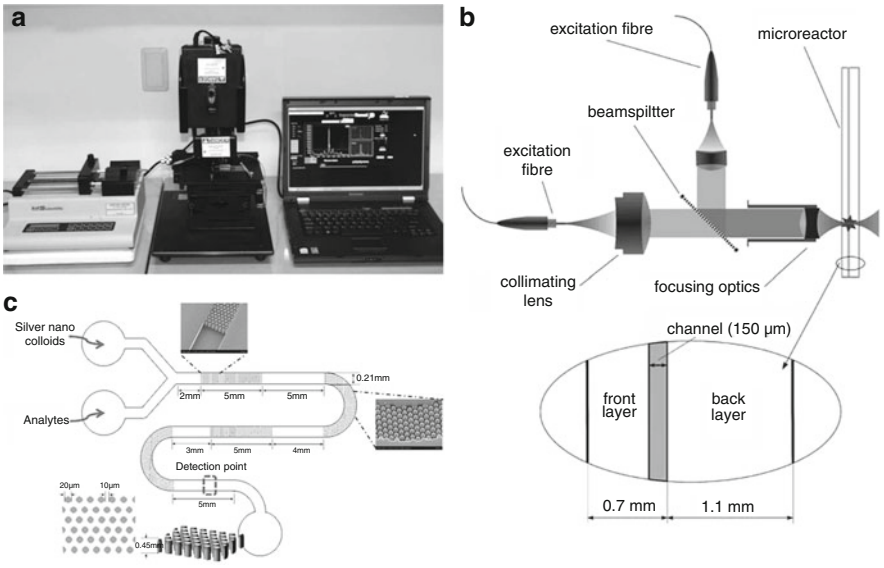

Fig. 4 (**a**) Schematic illustration of a portable Raman system combined with a pillar array PDMS microfluidic channel. (**b**) Schematic illustration of a pillar array PDMS microfluidic channel for the SERS detection of hazardous materials. Dimensions of the PDMS device are 20 mm (width), 24 mm (length), and 8 mm (height). SEM images at the insets show the structure of the micro-pillars ([44], Reproduced with permission of the Royal Society of Chemistry). (**c**) Raman optical setup and micro-reactor cross-section schematic ([45], Reproduced with permission of the Royal Society of Chemistry)

In all previously mentioned studies, conventional Raman microscopes were used to record Raman spectra from the microfluidic channels using high numerical aperture objectives. A recently reported study looks into the aspects of optimizing Raman signal collection from microfluidic channels [45]. In this work, a glass-based micro-reactor was used to probe the acid catalyzed esterification of butanol with acetic anhydride to produce butyl acetate and acetic acid. It was shown that for a non-confocal setup, a miniature aspheric lens would be better than high numerical aperture microscope objective to collect Raman spectra from the sample in the microfluidic channels. This paper contains a detailed discussion regarding the issues that can arise when detecting Raman signals from a microfluidic chip. The relatively high power of excitation (100 s of mW) needed to overcome the low Raman cross-section issue can create localized heating which can affect the reaction dynamics. This issue would be serious when high numerical aperture objectives are used to collect Raman signal in which Raman excitation beam is tightly focused to a very small volume, resulting in relatively high power density at the focal spot. The refractive index change induced by photo-thermal effects can affect the collection efficiency. There may also be the creation of bubbles inside the microfluidic channels due to cavitations, which would disrupt the flow inside the microfluidic channel. Another issue is the localized imperfections on the substrate that might affect the quality of the acquired data. The impurities that might be presented in the substrate would introduce unwanted fluorescence background which is difficult to remove through processing.

3 Microfluidic Raman Spectroscopy: State of the Art

Sect. 2 gives a detailed overview of various works where Raman spectroscopy was used as a potential optical detection method to probe samples in microfluidic chips. These works proved that MRS is a powerful analytical approach that can have various applications in bio-chemical analytics. However, there are various issues associated with this approach that makes it less favorable compared with other spectroscopic detection schemes [1]. This section gives a summary of the state of the art of MRS. Also, we present a discussion on the advantages and disadvantages of the conventional MRS approaches.

The advancement of MRS has been mainly application driven. The technology found applications in sensing and bio-chemical process monitoring. There has been several microfluidic architectures with which MRS was implemented. Microfluidic chips, fabricated in various substrates like glass [15], PDMS [21], and polymer films [43] were used for different applications of MRS. Specialized SERS substrates [21, 23] and micromixers [22, 25] were fabricated in microfluidic chips to obtain efficient SERS detection. Raman spectroscopy was also used to probe droplets in microdroplet-based microfluidic systems [31]. Raman detection schemes were another aspect on which different novel approaches have been attempted so as to enhance the sensitivity. SERS, SERRS, and confocal Raman

spectroscopy were some of the approaches used to improve the detection sensitivity. There have been various approaches on the Raman detection setup such as commercially available confocal Raman setups [21, 46], portable Raman microscopes [44], low-resolution Raman systems using fiber probes [33], and specially designed Raman probes.

Even though the previously mentioned studies differ in applications, microfluidic architecture, or the Raman detection scheme, they all probed the microfluidic chips from outside, using a lens [45], fiber probes [33], or high numerical aperture objectives [3]. On this basis, these works can commonly be categorized as free-space microfluidic Raman detection. One of the advantages of the free-space Raman approach is the fact that a conventional Raman microscope could be used for implementing MRS. The microfluidic chip could be used to analyze a sample on a commercially available Raman microscope [39]. This detection scheme also offers the opportunity to achieve spatial mapping of microfluidic channels to study the reaction or fluidic dynamics within a microfluidic channel [15, 20].

Even though MRS proves to be a powerful analytical tool, some inherent limitations make it less favorable to many applications. One of the major concerns associated with MRS is the inherent low cross-section of Raman scattering process when compared to fluorescence. SERS-based approaches [21] were implemented mostly to overcome this issue and to enhance the detection sensitivity. Another concern is the background signal from the substrate when Raman spectra are collected from a microfluidic chip [1, 45]. In free-space geometries, the Raman excitation beam and the Raman signal must pass through the substrate from which the microfluidic chip is fabricated. In order to obtain a good SNR for the obtained Raman spectra, it is essential to reduce the fluorescence background from both the sample and the substrate. It is, therefore, essential to make careful choice on the material and thickness of the substrate on which the microfluidic chip is fabricated. Even though polymer substrates like PDMS and PMMA are relatively cheap, they are less favorable for MRS as these polymer materials are highly Raman active and their Raman peaks may interfere with those of the analyte in the fingerprint region [29]. A solution for this would be to use confocal Raman systems [15, 17]; however, this results in an increase in the acquisition time. Because of this, many researchers continue to use a glass substrate as it remains relatively transparent in the visible and near-IR region when implementing MRS using free-space systems.

A fiber-based on-chip Raman detection scheme provides a possible solution to the issues of background from the substrate and also improves the portability of the system. Recently, our group implemented fiber-based Raman spectroscopic detection schemes in microfluidics (discussed in detail in Sects. 4 and 5).

4 Fiber Probe-Based Microfluidic Raman Spectroscopy

Embedding optical waveguides such as optical fibers is a route to the manufacture of microfluidic chips which incorporate optical detection schemes while retaining portability and eliminating the need for optical alignment. This also helps to

minimize the number of required optical components [6]. This approach has been successfully implemented for various optical detection methods. However, implementation of fiber-based Raman spectroscopic detection schemes in microfluidics was not exploited until recently due to practical difficulties.

In order to explain this issue, it is worthwhile exploring how fiber-based detection schemes were implemented for other spectroscopic methods. In one of the first studies where a fiber-based on-chip fluorescent excitation was used in microfluidics, a fiber insertion channel was etched into the microfluidic chip in such a way that the tip of the fiber, when fiber was inserted in the fiber insertion channel, would remain 190 μm away from the fluidic channel [47]. In a recently reported study, on-chip absorption spectroscopy was implemented using optical fiber-based excitation and collection for high-throughput cell screening [48]. As before, the fiber insertion channel is separated from the fluidic channel by a wall.

Due to the inherent characteristics of Raman spectroscopy, a slightly different approach is necessary when implementing fiber-based Raman systems. Due to the low Raman cross-section, it is essential to enhance the collection efficiency and reduce the fluorescence background in order to achieve satisfactory detection sensitivity. There can be significant fluorescence contribution from the optical fiber itself when Raman excitation beam gets guided through it. To reduce this background, low hydroxyl (OH) optical fibers are used for near-IR applications. Also unlike other spectroscopic probes, specialized filters must be introduced at the probe head so as to filter out the fluorescent background from the excitation fiber and prevent Rayleigh-scattered photons getting into the collection fiber [49].

When embedded fiber probes were used for optical detection in microfluidic chips, a wall separated the fiber tip within its insertion channel from the fluidic channel through which the analyte flows. This approach is not desirable for a fiber-based Raman detection scheme in microfluidics as there will be interfering signal from the substrate. A modified microfluidic design is required to implement fiber-based Raman detection in microfluidics such that the fiber probe is embedded in a chip without walls that physically separate the tip of the probe from the fluidic channel.

Our group demonstrated the first implementation of on-chip fiber-based Raman spectroscopic detection on a PDMS-based microfluidic platform [50]. In our work, a PDMS microfluidic chip with a predefined fiber probe insertion channel was fabricated and a specially designed split fiber probe was embedded into the fluidic chip, thus enabling fiber-based on-chip Raman excitation and collection. This work achieved the desired characteristics for a fiber-based Raman detection system in microfluidics and demonstrated that the key ideas which enabled the implementation of this system were the design of the microfluidic chip, the design of Raman probe, and the Raman detection geometry.

The PDMS chip was fabricated using conventional soft lithography [51]. A specialized protocol was used to define the fiber probe insertion channel by fixing a sleeve with the same dimension as that of the probe head on the mould [50]. As a result, it will be possible to define a pre-aligned fiber insertion channel within the fluidic chip, in which the fiber probe can be inserted and embedded in a leak-

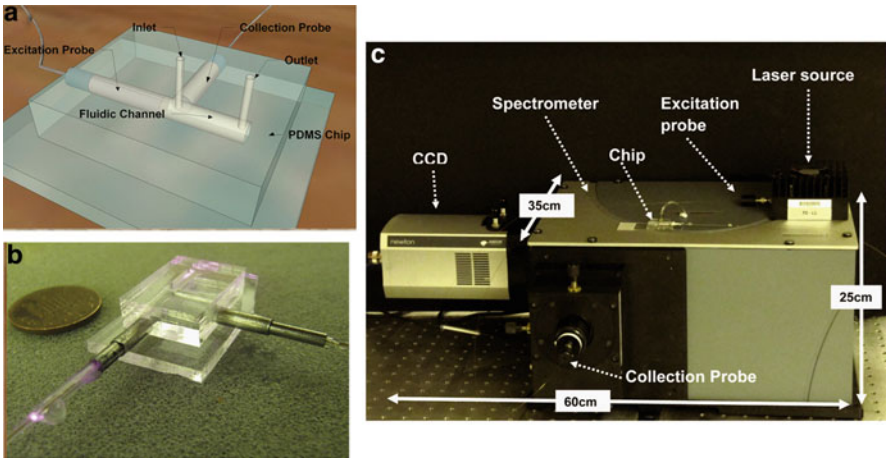

Fig. 5 (**a**) Design of the microfluidic chip. The head of the fiber probe is inserted into the chip, and the analyte to be detected is injected into the chip through inlet and goes out through outlet. (**b**) Photograph of the PDMS-based chip where collection and excitation probes are inserted. (**c**) Photograph of the probe-based microfluidic Raman detection system ([50], Reproduced with permission of the Optical Society of America)

proof manner. The design of the chip is shown in Fig. 5a. As mentioned previously, fiber-based Raman probes require specialized filters at the probe head in order to avoid fluorescent background from the fibers. Conventional Raman probes that are used for tissue imaging [49, 52, 53] are designed with a backscattering geometry, and the excitation and collection part of the probes can be combined in a single housing. This demands complex optics with micro-optic elements at the probe head, and since the beam is not collimated in passing through the filters, the filtering efficiency in some designs will be compromised [53]. In our system, since the microfluidic design determines the collection geometry, we decouple the excitation and collection parts of the probes into two different housings, making it a split Raman probe, simplifying the design of the probe heads. As shown in Fig. 5, the excitation and collection probe head includes a filter sandwiched between a pair of collimating and focusing achromatic doublet lenses of diameter 2.5 mm. The excitation probe head contained a bandpass filter to remove the fluorescent background while passing the excitation wavelength from the 785-nm excitation laser. A long-pass filter with cut-off wavelength at 795.2 nm was used for the collection probe head to prevent the Rayleigh-scattered photonics in the excitation wavelength getting into the collection fiber. Due to the collimating and refocusing geometry, it was possible to make the filters work at their maximum efficiency, while the split configuration avoided the requirement of specialized dichroic mirror or filters at the probe head. Though the diameter of the fiber used for the probe was 250 μm, the diameter of the probe head was 3 mm, mainly due to the difficulty in obtaining smaller size filters.

Another advantage of the split probe was the ability to choose the most efficient detection geometry, simply by modifying the microfluidic chip design. We compared collinear and orthogonal collection geometry, and it was observed that the orthogonal collection geometry is the desired geometry with minimal fluorescence background; this geometry helps to eliminate most of the forward scattering photons at excitation wavelength [50].

In order to prove the ability of this device to detect bio-analyte, concentration analysis of urea solution was performed. At 200 mW excitation and 5 s acquisition time, the minimum detection limit of this device was estimated to be 140 mM. This value is relevant when human physiological limit of urea in human urine is considered. Consequently, this system has the potential to be used for analyte detection and monitoring with relatively low acquisition times. This system is the first stepping stone toward implementing fiber-based Raman detection scheme in a microfluidic platform. With a simple fluidic system and embedded fiber probes, a completely alignment-free microfluidic system for Raman spectroscopic analyte detection was achieved. This fluidic chip can be incorporated into a chemical reactor for online process monitoring, or when combined with a portable Raman spectrometer, this could be used for field testing of analytes.

Even though the fiber probe-based microfluidic Raman system proved to be rugged and portable, there are certain limitations which make it less favorable for wider applications. Since the diameter of the filter is one order larger than that of the fiber used to collect the signal, there is a throughput (Etendue) mismatch at the probe head which would reduce the collection efficiency of the probe. Another concern is further miniaturization. The size of the fluidic channel depends on the size of the probe head, and the size of the probe head mainly depends on the size of the filters used. Obtaining filters of smaller sizes is difficult and expensive. This makes it difficult to achieve fluidic devices in true microfluidic dimensions with this approach. This motivated the development of the second generation of a fiber-based microfluidic Raman spectroscopic system, explained in the following section.

5 Waveguide Confined Raman Spectroscopy

As mentioned in Sect. 4, the successful implementation of fiber-based Raman detection system in microfluidics should ensure maximum collection efficiency and minimum fluorescent background from the substrate as well as the sample. The significant Etendue mismatch at the probe head and the issue of scalability limit the applicability of probe-based MRS. Our group recently demonstrated an alternative approach called waveguide confined Raman spectroscopy (WCRS) which solves the issues of collection efficiency and scalability [54].

The basic concept of WCRS is the confinement of the Raman signal excitation and collection region through embedded waveguides along with confinement of the analyte through the microfluidic channel. In WCRS, two embedded waveguides, one for excitation and one for collection of the Raman signal, meet at the signal

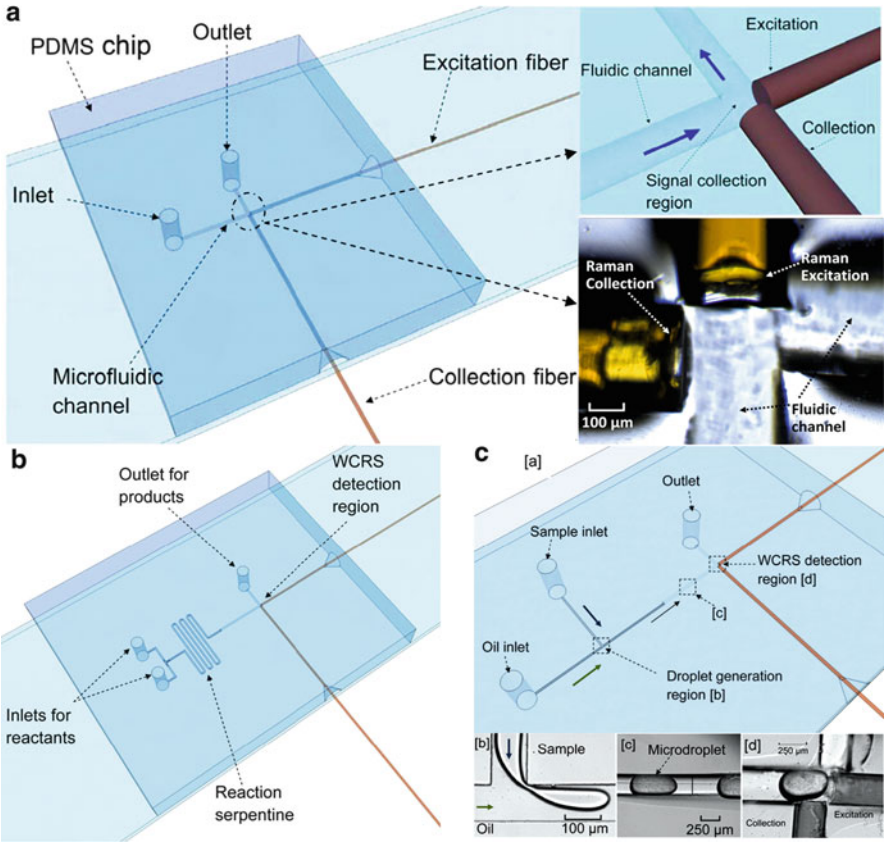

Fig. 6 (**A**) Schematic of the PDMS microfluidic chip where WCRS was implemented. (**B**) Design of the WCRS incorporated micro-reactor chip (only a representative drawing of the serpentine region is shown; in the actual microfluidic chip, the serpentine region is longer). (**C**) (a) Design of the microfluidic chip for microdroplet generation combined with WCRS. (b) Microdroplet generation region. (c) Microdroplet flowing through the microfluidic channel. (d) WCRS detection of microdroplets ([54], Reproduced with permission of the Royal Society of Chemistry)

collection region; neither waveguide has any optical elements at the tip to modify the beam profile. This system has been implemented with a pair of embedded low OH multimode fibers for excitation and collection, as shown in Fig. 6a. The distance between the ends of excitation and collection fibers should be of the order as the size of the fiber core. Since the Raman signal collection is confined to the close vicinity of the ends of the fibers, the diameter of the incident beam is comparable to the size of the core within the collection region despite the divergence of the incidence beam on emergence from the fiber. The same applies to the acceptance angle of the collection fiber, maximizing the collection efficiency of the system. The orthogonal collection geometry eliminated collection of the unscattered incident beam, ensuring that the Raman signal is not masked by the

high-intensity incident radiation or background fluorescence from the probe fiber. When compared to its probe-based counterpart, there is no Etendue mismatch at the collection side of WCRS. This improved collection efficiency results in better sensitivity of detection for WCRS when compared to its probe-based counterpart, even though the fluorescent background might be higher due to the lack of filters at the collection region of the waveguides.

The microfluidic channel also allows confinement of the required sample volume. Since a diverging beam is used for sampling, the whole cross-section of the microfluidic channel is probed unlike the fiber probe-based MRS system or confocal MRS systems.

The sensitivity of the WCRS system was compared to that of its probe-based counterpart by detecting urea under similar experimental conditions. The minimum detectable limit was found to be 80 mM, an improvement over the probe-based system, while the required sample volume was five orders lower in the WCRS-based system. Since bare fiber or embedded waveguide can be used for WCRS, without any other optical elements, this technique is scalable solely depending on the size of the waveguide used for excitation and collection. Low OH multimode fibers are commercially available in a range of sizes ranging from 100 μm – 300 μm, enabling implementation of WCRS in true microfluidic dimensions (channel size in the order of 100 μm).

In practice, these features make WCRS an ideal candidate for developing microfluidic devices where rapid and alignment-free Raman spectroscopic detection is possible with minimal background from the substrate. Due to the highly sensitive detectors used for Raman detection, it is necessary to darken the whole microscope when bulk microscope-based MRS systems are used to take Raman measurements in broad daylight. As waveguides are used for exciting and collecting Raman signal, it is only necessary to darken the fluidic chip in the case of WCRS, making MRS more amenable for field applications.

Fundamentally, WCRS is an architecture that allows Raman spectroscopic sensing in microfluidics. The applicability of this technology is not limited to analyte sensing. An attractive feature of this architecture is its compatibility to be combined into other microfluidic platforms with multiple functionalities, where WCRS would act as the detection scheme of the device. We recently reported implementation of two proof-of-principle experiments to demonstrate the extended functionality of WCRS to be used as a generic sensing technique that can be implemented in a wide variety of applications [54]. The first of these was the use of WCRS for reaction monitoring in a micro-reactor, the design of which is shown in Fig. 6b. The progress of a binary reaction could be monitored in real time (with relatively low acquisition times) for different flow rates of the reactants. Another implementation of WCRS demonstrated its use for probing microdroplets in a microdroplet-based microfluidic chip, as shown in Fig. 6c. It was shown that it is possible to probe single microdroplet using WCRS-based detection, which could lead to Raman-activated microdroplet sorting devices in the future.

Our recently reported work discusses the implementation of WCRS for acquiring standard Raman spectra [54]. However, techniques like surface enhancement or

fluorescent suppression could help to improve the sensitivity of the acquired Raman signal [29, 55–57]. Such techniques can be easily incorporated in WCRS to further enhance the sensitivity of the device. To demonstrate the feasibility of such modifications [58], we implemented a recently developed fluorescent suppression technique using continuous modulation of excitation wavelength [55, 59] in WCRS. In this form, the same WCRS architecture can be used in which the only modification was the use of frequency-modulated Raman excitation source. With the modulated Raman spectra, a sevenfold enhancement in the sensitivity could be achieved for urea detection [58].

WCRS has proved to be a generic technology, providing a solution to the fundamental limitations of MRS detection systems. The relevant ease with which this technology can be combined with other techniques increases the relevance of this approach. Portable, alignment-free MRS devices realized using WCRS have the potential to affect several fields such as point-of-care diagnosis, environmental sensing, process monitoring, drug development, cell biology, and proteomics.

5.1 Future Outlook

Moving to embedded or integrated waveguide-based architecture from a bulk optics approach will help MRS to be realized in a form which is both portable and free of alignment. This will instigate the future translation of techniques currently only possible with bulk optics to the fiber-based platform with the result that the technology is more amenable for field applications. The fiber-based detection scheme would not, however, completely replace the benchtop implementation of MRS; despite the capability for WCRS to achieve temporal mapping of an evolving process within a microfluidic channel, it is unable to achieve spatial mapping of a process as its detection region is fixed.

Further investigations are necessary on WCRS to extend its applicability to all types of samples. For example, detection of turbid analyte is yet to be demonstrated using WCRS where necessary normalization algorithms need to be incorporated to take into account the scattering and absorption properties of the sample [60]. Another aspect is the demonstration of the ability of WCRS to be used for cell biology applications. There is also opportunity to improve the architecture of WCRS by designing an integrated waveguide-based WCRS chip, where the microfluidic channel size can be further reduced and the collection geometry at the Raman collection region can be optimized.

6 Conclusion

The field of Raman spectroscopy has seen significant advancement both in technology and in application over the last two decades, which has made it a powerful tool for bio-chemical analysis. Combining Raman spectroscopy with microfluidics has

resulted in the development of devices that can achieve rapid bio-chemical analysis of samples using Raman spectroscopy with a significantly reduced sample volume. However, there are several limitations such as weak Raman signal, background from the substrate, requirement for alignment, and lack of portability, which limited the wider applicability of MRS. Moving from a bulk optics-based architecture toward a fiber-based architecture has helped to overcome majority of the aforementioned issues [50, 54]. The fiber-based implementation of MRS will help the field of microfluidics to exploit the advantages of Raman spectroscopy, resulting in the development of efficient, alignment-free, portable Raman spectroscopy-based microfluidic devices for bio-chemical sensing and analysis.

Acknowledgments We thank the UK Engineering and Physical Sciences Research Council for funding. KD is a Royal Society-Wolfson Merit Award holder. The authors are grateful to Dr Andrew McKinley for critical reading of the manuscript and useful discussions.

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