Time Domain Diffuse Raman spectrometer based on novel TCSPC camera for depth analysis of diffusive media

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We present a time domain diffuse Raman spectrometer for depth probing of highly scattering media. The system is based on a novel TCSPC camera that simultaneously acquires both spectra and temporal information of Raman photons. A dedicated non-contact probe was built, and time domain Raman measurements were performed on a tissue mimicking bilayer phantom. The fluorescence contamination of Raman signal was eliminated by early time gating (0-212 ps) the Raman photons. Depth sensitivity is achieved by time gating Raman photons at different delays with a gate width of 106 ps. Importantly, time domain can provide a high contrast between two layers Raman signal. As a result, an enhancement factor of 2170 was found for our bilayer phantom which is much higher than the values obtained by spatial offset Raman spectroscopy (SORS), frequency offset Raman spectroscopy (FORS) or hybrid FORS-SORS on a similar phantom.

OCIS codes: *300.6500 Spectroscopy, time-resolved; 170.5660 Raman spectroscopy; 170.7050 Turbid media; 290.0290 Scattering; 290.5860 Scattering, Raman; 300.6450 Spectroscopy, Raman.*

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In recent times, new advancements in Raman spectroscopy have emerged as a promising tool for subsurface probing of biological media [1]. In particular, the invention of spatial offset Raman spectroscopy (SORS) [2][3], transmission Raman [4], and recent frequency offset Raman spectroscopy (FORS) [5] has led to widespread applications of deep layer Raman spectroscopy, covering pharmaceutical to biomedical imaging. By effectively using various concepts in continuous wave diffuse optics, these techniques enabled depth probing of diffusive media. Still, they require either different collection points (SORS) or different excitation wavelengths (FORS). Moreover, they are sensitive to fluorescence contamination or auto-fluorescence which is a key problem while working with biological tissues.

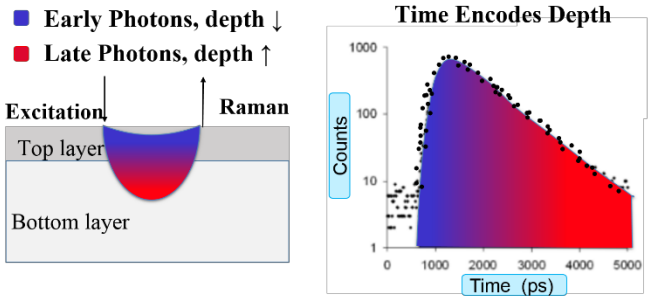

Fig. 1. Principle of time encoded depth in Time domain diffuse Raman spectroscopy, late photons carry depth information.

One possible way to overcome these limitations is by time gating the Raman signal, which can effectively distinguish delayed fluorescence signals from Raman photons [6]. Furthermore, time gating of Raman photons can tag photons arriving from different depths, thus distinguishing the Raman signal from different layers of diffusive media [7][8][9]. Importantly, in time domain we can eliminate the contribution of bottom layer Raman photons by time

gating the early photons which is impossible in continuous wave methods. Fig. 1 shows the concept of time of arrival of Raman photons encoding information from different depths of diffusive media.

Time gating of Raman photons possesses several challenges due to very low Raman cross section, which in turn imposes stringent requirements, like low noise and high performance of detector and gating technology. This gets worse in diffusive media where the signal is further reduced by few orders of magnitude due to high scattering. Previous attempts made in the literature, like Kerr gating of time resolved Raman photons pose complex problems due to bulkiness and efficiency of instrumentation [7][8], also the time gating has to be performed by serial acquisition of Raman signal at different delays. Gating using intensified charge-coupled device (ICCD) cameras has limited SNR due to noise created by intensifiers and also needs serial acquisition at different delays for time tagging photons arriving from different depths. However, work of Ariese *et al.* pioneered the use of ICCD in diffuse Raman spectroscopy, demonstrating interesting applications for two-layered samples [9][10][11]. A single-photon avalanche diode (SPAD) array based time resolved Raman system was demonstrated on a highly Raman active homogeneous sample [6][12], however, the low detector fill factor and high dark counts of the SPAD detector may prove hindrance to detect the Raman signal from diffusive media. Detection technology based on time correlated single photon counting (TCSPC) along with a sensitive time resolved single photon counting detector/camera with high detection efficiency, low noise, cost-effective and compact size could be an optimal solution for time gating of Raman photons. Importantly, in a camera based TCSPC system both Raman spectrum and temporal information are acquired in a single measurement, whereas gating can be performed during post processing of data. A recent work based on a TCSPC system with a single pixel SiPM detector enabled serial spectral acquisition by using a monochromator, but lacked simultaneous acquisition of spectral data and had problems of Raman signal buried in high noise of the SiPM detector [13].

In this work, we present time domain diffuse Raman spectroscopy (TDDRS) based on a new time resolved single photon counting camera (LINCam) that enables broadband simultaneous acquisition of spectral and temporal information of Raman photons. The camera was developed at the Leibniz-Institute for Neurobiology (LIN) Magdeburg, Germany under the joint project "TCam4Life" funded by the German Federal Ministry of Education and Research (BMBF). Since 2017, camera systems with sensor diameters of 25 mm or 40 mm are produced by the spin-off Photonscore GmbH (www.photonscore.de) and marketed by PicoQuant GmbH (www.picoquant.de). The LINCam camera provides < 50 ps time resolution and resolves x and y positions of individual photons as precise as a CCD with 1000 × 1000 pixels does. It works on the principle of microchannel plate with very low dark counts, 100% fill factor and a count rate of up to 1 MHz. Further details on the operational principle of the camera are given elsewhere [14].

A compact portable probe was built to perform non-contact time resolved Raman measurements in diffusion regime. The instrument was tested on a phantom mimicking a bilayer tissue. The fluorescence contaminating the signal was effectively removed by time gating the acquired signal, which demonstrates the potential of time resolved Raman spectroscopy in the presence of fluorescence. Furthermore, time gating at different intervals allowed probing at different depths of diffuse media, as late arriving photons carry depth information.

A schematic layout of the portable instrumentation is shown in Fig. 2a. A pulsed laser at 532 nm (PicoQuant: VisUV-532 nm) is used as an excitation source, which is coupled to the compact probe through a 100 µm fiber. The probe works in a non-contact geometry with ring illumination and point collection. An image of the compact probe is shown in Fig. 2c. An axicon lens is used to generate ring illumination of different diameters and a narrow bandpass filter (Thorlabs: FL532-10) at 532 nm provides clean excitation on the sample. The collection optics consists of a long pass filter (Semrock: LP03-532RU) with blocking wavelength at 532 nm, and a series of lenses (L1, L2, L3, L4) which act as Fourier low pass filter to eliminate stray light entering the detection fiber. The detection fiber (1 mm core, 0.39 NA) is projected onto a 200 µm entrance slit of a spectrograph (Acton SpectraPro2150, Princeton Instruments, f/4 system, grating 1200 grooves/mm) and imaged onto a time resolved TCSPC camera (LINCam25, built by LIN/Photonscore).

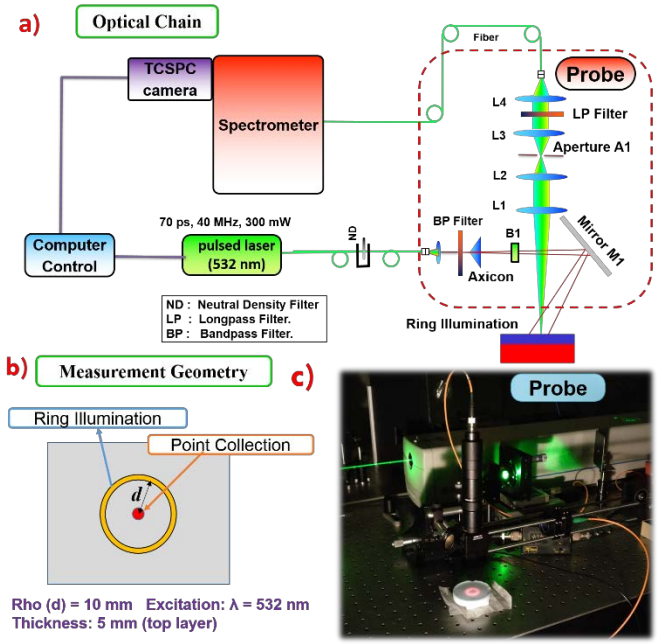

Fig. 2. (a) optical chain of compact TDDRS system, (b) measurement geometry, and (c) image of non-contact probe with ring illumination on bilayer tissue mimicking phantom.

Measurements were performed on phantoms mimicking a bilayer tissue: the top layer was a 5 mm thick silicone elastomer, whereas the bottom layer was a marble slab (calcite CaCO3, thickness = 17 mm) simulating bone tissue. The optical properties (absorption μ_a, reduced scattering μ_s') of both layers were kept similar to that of biological tissues with top layer (μ_a = 0.11 cm^{-1}, μ_s' = 16.5 cm^{-1}, refractive index =1.41 @532 nm) and bottom layer (μ_a = 0.03 cm^{-1}, μ_s' = 16.5 cm^{-1}, refractive index = 1.66 @532 nm) and the absorption of both layers were around the above mentioned values over the measured Raman spectral range. The optical properties of top layer were tailored using black silicone for absorption and calculated fraction TiO2 powder for scattering. The bilayer phantoms were accurately characterized using a time domain diffuse optical spectrometer [15], which was validated in various phantom studies [16][17]. The temporal histogram of Raman photons was acquired for 300 seconds. Laser power of 100

mW was injected over the excitation ring radius (d) of 10 mm, with 1 mm as ring width. The ring radius was so chosen to allow sufficient diffusion process of the Raman photons. Vertical binning was performed.

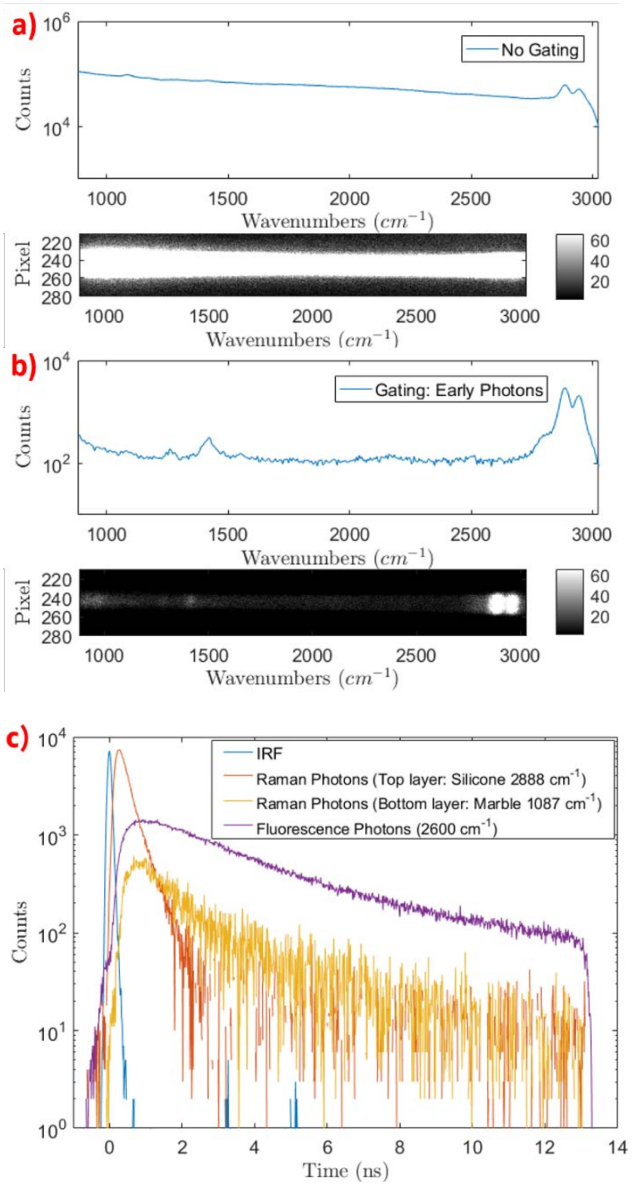

Fig. 3. Raman spectrum of bilayer phantom (a) In the absence of time gating the Raman photons are buried in fluorescence (b) Time gating of early photons enhancing Raman photons against fluorescence background, (c) Temporal profile of instrument response function (IRF), Fluorescence photons, Raman photons of top and bottom layer. IRF was collected using white paper reflection placed at the sample plane.

Fig. 3a shows the Raman spectrum of the bilayer phantom integrated in time dimension, the spectrum was extracted by vertically binning (210-280 pixels) the Raman signal. In the absence of time gating it is evident that the Raman signature of marble (1087 cm−1) and silicone (1260, 1411, 2888, 2841 cm-1) are buried in the overwhelming fluorescence. However, the fluorescence background can be suppressed by time gating the Raman photons, since Raman is an instantaneous process as compared to Fluorescence photons which have long decay time. Fig. 3b shows the spectrum of early Raman photons (0-212 ps), demonstrating clear fluorescence elimination. Time-gating to suppress fluorescence in Raman signals has been explored using different techniques (for a review and implications in case of diffusive media see [18]). The key advantages of our approach are: i) fully parallel acquisition both in spectral and temporal domain with no gaps, ii) maximum freedom in post-processing to select time windows; iii) compactness of the setup.

The high temporal resolution of the LINCam camera can be used to understand the temporal distribution of fluorescence photons and Raman photons arriving from top and bottom layers of the bilayer phantom. Fig. 3c shows the temporal profile of Raman photons of the top layer (photons integrated under 2888 cm^{-1}), bottom layer (1087 cm^{-1}), and fluorescence (2600 cm^{-1}). The absorber in top layer contributed to fluorescence whereas the bottom layer fluorescence arises from unknown organic contaminants in the marble slab, both layers have comparable fluorescence intensities. As expected, fluorescence had a long decay time whereas the top layer signal (2888 cm^{-1}) arrived at early time as compared to the bottom layer signal (1087 cm^{-1}). Though Raman is an instantaneous process, due to diffusion of Raman photons the broadening of the temporal profile is observed for the top and bottom layer. The Raman photons of the bottom layer arrived with a delay as compared to top layer photons which is attributed to the long diffusion path taken by Raman photons from bottom layer.

To understand further the temporal relation between Raman photons of top and bottom layer, we plotted the Raman spectra of the bilayer phantom at different temporal delays (gate width in step size of 106.2 ps) (Fig. 4), as anticipated early gates are dominated by top layer signal (2888 cm^{-1}), which vanishes with increasing bottom layer signal (1087 cm^{-1}) at late gates. This change in Raman signal contribution of different layers with changing delay could be used for depth resolving of diffusive media [7]. Importantly, scattering and thickness of the top layer play a crucial role in the arrival time of Raman photons from the bottom layer. A delay in arrival time and attenuation is expected with increasing scattering or thickness of the top layer, this could be attributed to the increased photon migration pathlength due to increased scattering.

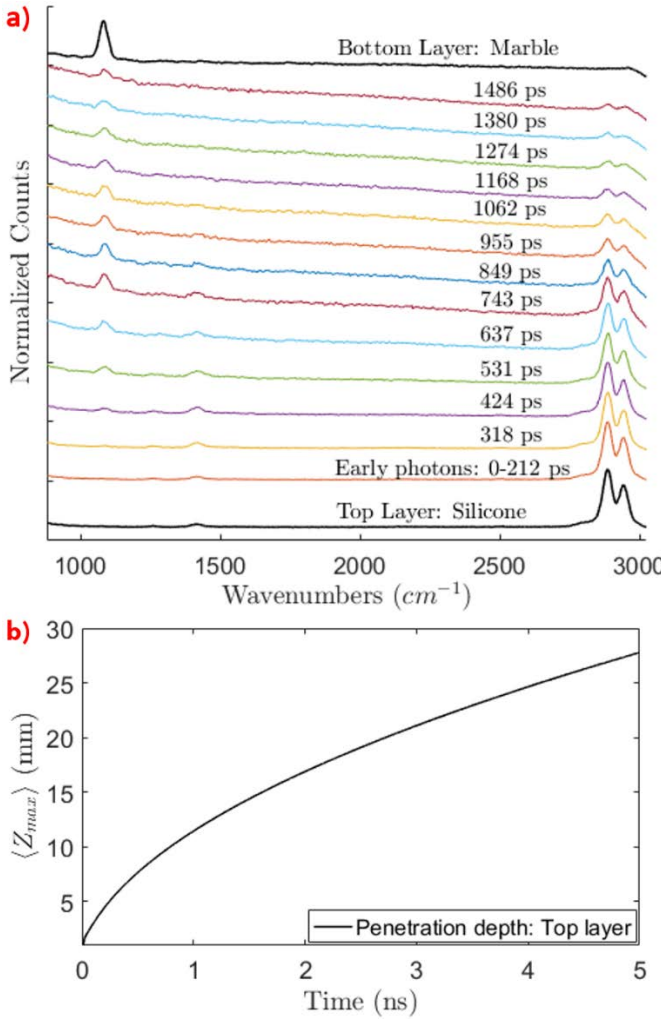

Fig. 4. (a) Raman spectra of bilayer medium with time gating (106 ps gate width) at different temporal delays, (b) theoretical estimation of maximum penetration depth of top layer Raman photons with time.

Fig. 4b shows a simulation of Raman photon mean maximum penetration depth <Z_{max}> with time, it takes minimum 250 ps (approx.) for photons to reach the bottom layer for 5 mm thickness of the top layer. As predicted, Fig. 4a shows no sign of bottom layer Raman signal till 212 ps, which is in agreement with the simulation. Simulations were based on a simplified heuristic analytical Raman forward solver (see Eq. (12) in [19], originally proposed in [20][18]) and on the theoretical approach described in [21]. The selection of optical properties similar to biological tissues in this study enabled the simulation of Raman temporal profiles expected for biological media.

One way to quantify the enhancement of the Raman signal from the bottom layer against top layer is by calculating the enhancement factor, which is defined as

$$\eta = \frac{I(t)_{Bottom}}{I(t)_{Top}} * \left[\frac{I(t_0)_{Bottom}}{I(t_0)_{Top}}\right]^{-1} \quad (1)$$

where t and t_0 are the Raman photon arrival times denoting extreme contrast points of bottom to top layer in Raman signal. Fig. 5 shows the temporal dependence of η for marble (1087 cm^{-1}) to silicone (2888 cm^{-1}) Raman signal. The enhancement factor was estimated to be 2170 which is much higher as compared to SORS (2.8), FORS (2.8) or hybrid FORS-SORS (6) as shown for a similar phantom in our previous work [5]. In theory, due to delay in arrival time of bottom layer Raman photons, the enhancement factor for the time resolved measurement on a bilayer phantom or tissue is infinite. However, in practice this parameter is limited by measurement conditions (optical properties, source detector separation, layer thickness) and signal to noise ratio (SNR) of the overall system. The observed high enhancement factor in our measurement is a result of negligible dark counts created by our detection system thus significantly improving the SNR of the system.

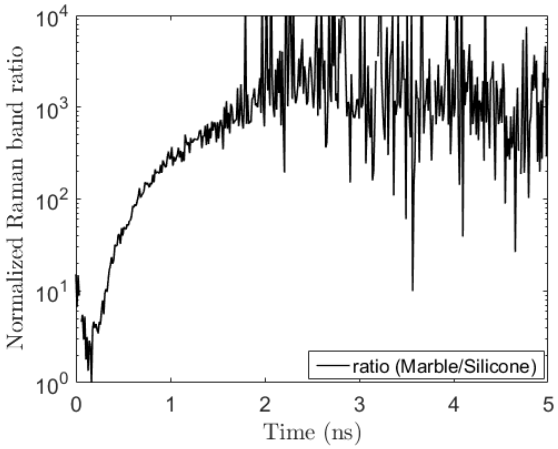

Fig. 5. Normalized Raman band ratio of marble (1087 cm^{-1}) to silicone (2888 cm^{-1}) Raman peak as a function of arrival time of Raman photons.

The signal collection efficiency of the system can be greatly improved by using a high throughput spectrometer, exploiting a greater area of the camera using fiber bundle, performing measurement at different geometry (short *d*) thus reducing acquisition time to few seconds with same SNR as presented here. However, attention should be paid to the maximum count rate of the camera (1 MHz) which might saturate in the presence overwhelming fluorescence. Also, complex phantoms with common bilayer spectral features can be explored to understand the complex un-mixing process.

In conclusion, we have demonstrated the TDDRS with a TCSPC camera based compact instrumentation on a tissue mimicking bilayer phantom. We have developed a dedicated non-contact probe for performing TDDRS measurements. An enhancement factor of 2170 was observed for the bilayer phantom which is 3 orders higher as compared to SORS or FORS on similar phantom [5]. Our work is a precursor that demonstrates the future of time domain diffuse Raman spectroscopy for depth probing of biological media. With advent of novel photonic devices [22] and diffuse Raman methods [2][5][19] the proposed technique shows strong potential for biological applications.

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