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Research Article

Raman Microimaging Using a Novel Multifiber-Based Device: A Feasibility Study on Pharmaceutical Tablets

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Raman microimaging is a potential analytical technique in health field and presents many possible pharmaceutical applications. In this study, we tested a micrometer spatial resolution probe coupled to a portable Raman imager via an indexed multifiber bundle. At the level of the probe, the fibers were arranged in a circular geometry in order to fit to the pupil of an objective. The imaging potential of this Raman system was assessed on pharmaceutical-like pellets. We showed that this setup permits to record, nearly in real time, Raman images with a micrometer resolution. The collected images revealed a marked heterogeneity in chemicals distribution. Further investigations will be led on cells and biological tissues to evaluate the potential of this Raman imaging device for biomedical applications.

1. Introduction

Raman microimaging is a promising analytical technique permitting to combine molecular and spatial information. Several studies have demonstrated that Raman analysis can bring valuable in situ information applicable as well in biological research [1, 2] as in pharmaceutical control [3]. Raman analyses require no staining, no solvent addition nor particular sample preparation, contrary to standard analytical methods. Experiments proved that Raman spectroscopy is an aspiring technique comparable to reference methods in quality control. It was proven that Raman approach is an accurate and precise method for active ingredient quantification when combined to data processing, even when the substance comprises less than 1% of the total mass of the tablet [4–6]. In addition, Raman measure is nondestructive and preserves the distribution of actives over the sample.

Considering Raman spectral imaging, the most widely cited studies consist of point by point mapping of a defined surface by using a motorized plate. Recent works reported the use of line-scanning system exploiting the bi-dimensionality of charge coupled device (CCD) matrix detector [7]. In this technique, the exciting laser beam is used to illuminate a line on the sample along which several Raman spectra can be recorded simultaneously. The sample is subsequently displaced in small increments perpendicular to the direction of the laser line. Although these laboratory equipments present a good efficiency allowing rapid spectral image acquisition, they do not fully exploit the capability of multiplexing, that is, simultaneous collection of several spectra by one-shot reading of the CCD. However, Schulmerich et al. took advantage of this capability to collect transcutaneous *in vivo* Raman spectra of mice tibia. This system, comprising a designed multifiber probe, permits to access in depth information but is not dedicated to spatially resolved surface imaging [8].

The present paper presents an innovative setup based on the coupling between a multifiber microprobe and a portable Raman imaging spectrometer. The basic principle of this imaging instrumentation is the use of an indexed multifiber bundle fitted to the objective exit pupil. After collection, the signal resulting from the different fibers is directed to different lines of the CCD matrix. In this way, each line of the CCD gives a spatially resolved Raman spectrum.

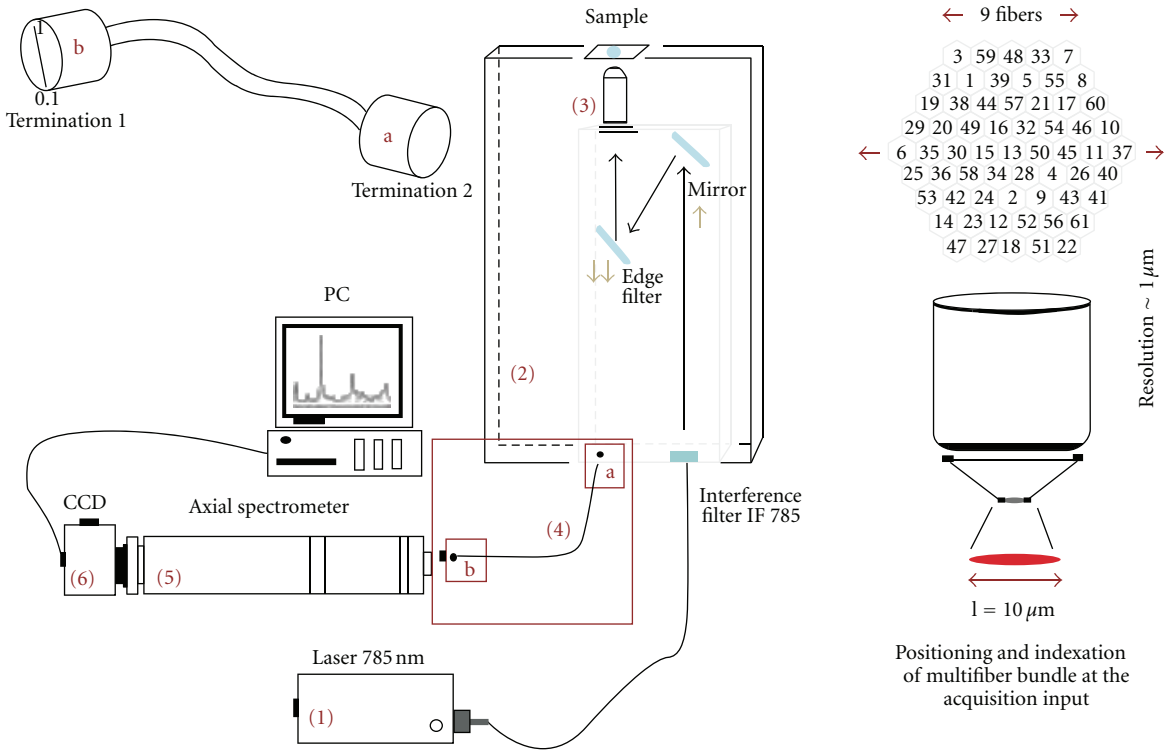


Figure 1: Schematic view of the Raman imaging probe setup.

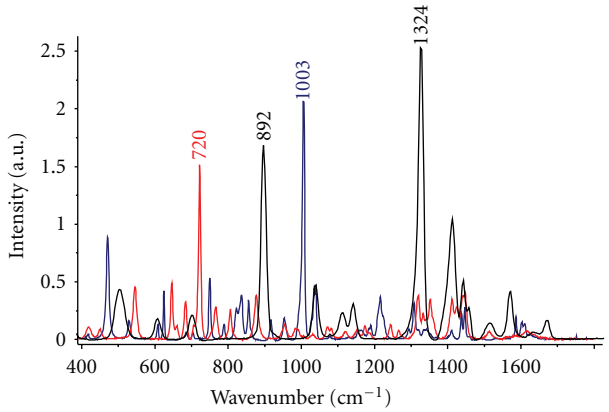


Figure 2: Raman spectra of phenylalanine, methionine, and glycine. Specific vibrations used for the detection of each of the 3 amino-acids are indicated.

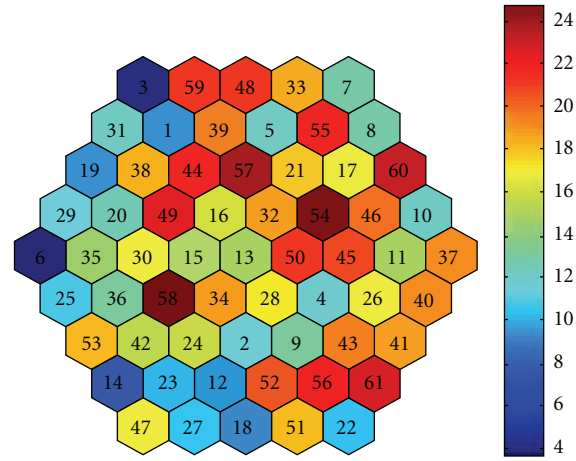
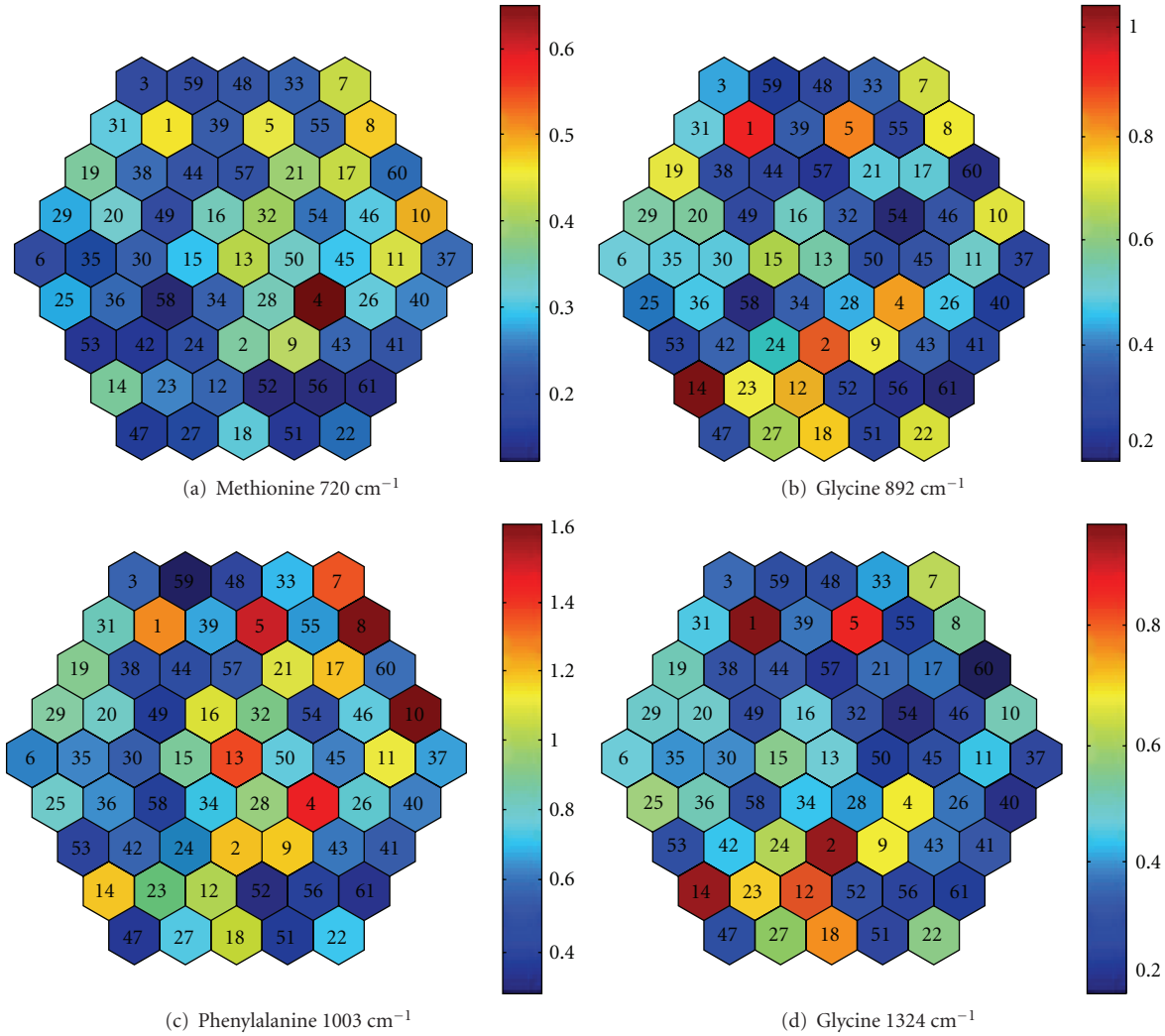


Figure 3: Fibre response image constructed from silicon Raman vibration at 520 cm^{-1}. The color code bar represents intensity variation (a.u.) of the vibration.

This microimaging multiplexed setup enables to obtain directly, by one single acquisition, a spectral image with an adjustable spatial resolution depending of the employed objective. Herein, the potential of the setup is assessed on pharmaceutical-like samples.

2. Description of the Setup

A schematic view of the instrumentation is illustrated in Figure 1. The setup consists of a 785 nm laser diode as excitation source (1), a probehead including interferential and edge band pass filters for filtering incident laser line and Rayleigh scattering respectively (2), an objective for focusing the excitation beam and capturing the scattered light (3), an indexed multifiber bundle (4), an axial-model-spectrometer (Horiba Scientific, Villeneuve d'Ascq, France) (5), equipped with a 100 μm wide entrance slit and a 256 × 1024 pixels



(a) Methionine 720 cm^{-1} (b) Glycine 892 cm^{-1}

(c) Phenylalanine 1003 cm^{-1} (d) Glycine 1324 cm^{-1}

FIGURE 4: Constructed images showing the distribution of methionine (a), glycine ((b) and (d)), and phenylalanine (c) within the tri-amino-acid pellet. The color code bars represent intensity variations (a.u.) of the respective vibrations.

CCD detector (Andor) (6). A grism associating a prism and a 600 lines/mm grating density permits to achieve a spectral resolution of 4 cm^{-1}, on 170 cm^{-1} to 2200 cm^{-1} as spectral range. As shown in Figure 1, the multifiber bundle is composed of 61 indexed fibers of 50 μm diameter each. At the level of the objective exit pupil, the fibers are arranged in a circular manner as shown in the inset (a) of Figure 1. Here, the experiments were performed by using a 50x objective (Olympus, NA = 0.75 Mplan). The objective can be changed according to the required spatial resolution. The coupling with the 50x objective provides a spatial resolution between 2 fibers of about 1 micrometer. At the level of the spectrometer entrance slit, the fibers are arranged along a line following the indexation indicated in the inset (b) of Figure 1. In order to visualize the probed surface of samples, the system is also equipped with a camera. The laser power at the sample was measured to 40 mW. The acquisition time corresponds to the time necessary to read the 256 × 1024 pixels of the Andor CCD. It was measured to 10 seconds.

3. Samples and Calibration Products

Neon light was used for wavenumber calibration of the system. The position in wavenumber of several neon lines was verified, and the error between theoretical and experimental values was calculated and set to be lower than one detector pixel if needed. In order to take into account, the wavenumber response of the CCD, calibration measurements were performed using a NIST calibration standard (US National Institute of Standards & Technology Gaithersburg, MD, USA). This system mimics a black body on the considered range of near infrared spectrum. For the experiments, Raman spectra of a pellet composed of a mixture of 3 amino acids (1/3 glycine, 1/3 methionine and 1/3 phenylalanine; 1/3 : 1/3 : 1/3 W) and of a pharmaceutical pill of Efferalgan (paracetamol : vitamin C, 500 mg : 200 mg; detected by specific vibrations at 1347 and 1062 cm^{-1} resp.) were recorded. For the pellet, the three amino acids were selected for presenting specific nonoverlapping vibrations

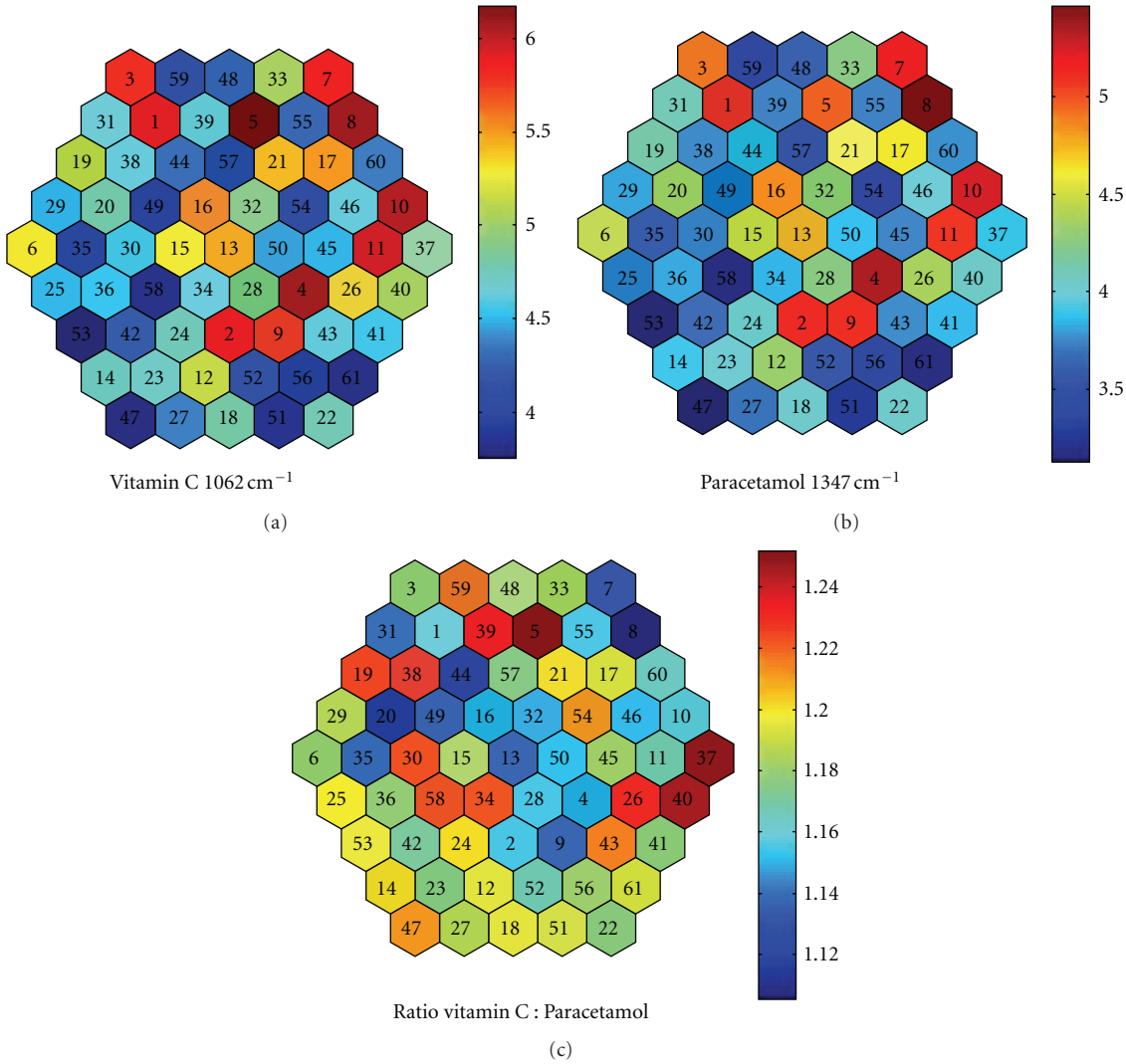


Figure 5: Constructed images showing the distribution of vitamin C (a) and paracetamol (b) within the Efferalgan tablet. Distribution ratio is displayed in (c). The color code bars represent intensity variations (a.u.) of the respective vibrations.

(Figure 2) offering easy processing of spectral data for images reconstruction.

4. Correction of the Difference of Signal Transmission between the Bundle Fibers

When working on a plane homogeneous surface such as a pure silicon wafer, one expects that the Raman signal presents a similar intensity for all the fibers. In practical manner, due to the laser focalization and fibers position with respect to the optical axis of the objective, the transmission of the Raman light varies from one fiber to another. Figure 3 displays a color-coded image revealing the intensity variations of the 520.7 cm^{-1} silicon Raman vibration for the set of fibers. This silicon signal was used to correct the differences of signal transmission between the fibers. Indeed, for each fiber, the silicon Raman intensity permits to determine a coefficient of transmission efficiency. In the experiments, the signal of each fiber was thus divided by the corresponding coefficient.

5. Data Processing

For collection of spectral images, the acquisitions were performed with Labspec 5 (Horiba Scientific, Lille, France) in CCD image mode. Data preprocessing and processing were performed with home-made software with a friendly user graphical interface operating under Matlab environment (The Matworks, Inc., Natick, MA, USA). This software enables to import spectral images directly from Labspec and to correct the data from dark current, CCD response, and signal transmission variations from one fiber to another. The following step of the data processing consists of a baseline correction. Indeed, the baseline is due to a fluorescence background and to the parasitic Raman signal from the optical fibers. This baseline is different from one spectrum

to another; it can be modeled by a polynomial mathematical function. The assumed order of the polynomial function was specified by the operator. The coefficients of this function were estimated by an iterative method based on least squares minimization [9]. For each recorded spectrum; the polynomial function was computed and removed from the spectrum. In our experiments, a 3-order polynomial proved to be appropriate for baseline correction.

6. Results

6.1. Raman Imaging of the Tri-Amino-Acid Pellet (1/3 Methionine; 1/3 Glycine; 1/3 Phenylalanine). Figure 4 reports the spectral color-coded images collected on the tri-amino-acid pellet and representing the intensity variation of Raman bands of methionine (a), glycine ((b) and (d)), and phenylalanine (c) (720 cm^{-1}; 892 and 1324 cm^{-1}; 1003 cm^{-1} resp. (Figure 2)). These images are the result of one single acquisition. They characterize the relative repartition of these 3 amino-acids within the pellet. Despite the intensity difference, the images constructed from glycine bands at 892 cm^{-1} and 1324 cm^{-1} (Figures 4(b) and 4(d)) show large similarities. Comparison between the images relative to methionine, glycine, and phenylalanine (Figures 4(a), 4(b), 4(d), and 4(c)) reveals a heterogeneous distribution of these 3 amino-acids within the pellet; for example, each of fibers 8, 10, and 13 presents high variations of the signal.

After the experiments on the three amino-acids pellet, a pharmaceutical sample was measured. An Efferalgan tablet, composed of a mixture of paracetamol and vitamin C, was chosen.

6.2. Raman Imaging of the Efferalgan Pill. To assess the relative distribution of vitamin C and paracetamol within the pellet, the Raman bands at 1062 cm^{-1} and 1347 cm^{-1} were used, respectively. Figure 5 displays the corresponding bundle images. In first inspection, vitamin C and paracetamol repartitions seem to be quite similar within the tablet (Figures 5(a) and 5(b)). To highlight differences of repartition between the 2 components, the pseudo-colored ratio image was calculated on the basis of the 1062/1347 intensity ratio. Indeed Figure 5(c) illustrates much better the relative distribution of these two compounds and shows clearly the heterogeneity existing within a microscopic area of the sample (diameter ~10 μm).

7. Conclusion

This innovative instrumentation permits to construct Raman images, in a multiplexed manner, with a microscopic spatial resolution depending on the objective magnification. The setup demonstrated the ability to collect an image of 61 spectra in one single acquisition. It gives the possibility to collect, nearly in real time, spectral images for various applications, particularly for quality control in pharmaceutics. The acquisition time corresponds to the duration of CCD pixels reading; this time can be reduced by using new high speed and sensitive camera. In addition, the setup presents no mobile optic set and is easily transportable. Besides, the home-made developed software gives the possibility to access to each spectrum from the spectral image, offering the way to analyze in more details information contained in the Raman vibrations (i.e., molecular interaction, structural information, etc.). This innovative imaging setup could also be of interest for biomedical approaches such as cell and tissue analysis. Further analyses are in progress on *ex vivo* tissue samples to assess the potential of the setup in this field of application.

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