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Raman imaging from microscopy to macroscopy: Quality and safety control of biological materials

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ABSTRACT

Raman imaging can analyze biological materials by generating detailed chemical images. Over the past decade, significant advancements in Raman imaging and data analysis techniques have overcome problems such as long data acquisition and analysis times and poor sensitivity. In this review article, Raman spectroscopy and imaging are introduced and the corresponding computational methods for image data analysis are discussed. We provide an overview of the applications of this method in areas such as food, pharmaceutical, and biomedical sectors, with emphasis on recent developments that have helped industrialize its applications in various sectors. Finally, the current limitations and trends for future Raman imaging are outlined and discussed with a view toward new research practices for applying this technique more efficiently and adaptably in numerous sectors.

1. Introduction

The Raman effect involves a change in the wavelength of scattered monochromatic visible light and was first described in 1928 by Raman and Krishan [1,2]. The earliest recorded use of Raman spectroscopy occurred in the following year and was confined to studying the vibrational, rotational, and anisotropic (Rayleigh wing) spectra of molecules in gaseous and liquid states, as well as the internal and lattice spectra of mostly clear and transparent crystals. Early applications of Raman spectroscopy aimed to elucidate the structures of molecules and crystals to resolve analytical chemistry problems [3].

In the early stages of Raman spectroscopy, it was exotic and laborious because of the sophisticated sampling method, involving a sample in a long tube illuminated along the length with a beam of filtered monochromatic light generated by a gas discharge lamp or mercury radiation, and the use of relatively large sample volumes. Nearly 40 years after the discovery of the Raman effect, the development of lasers in the 1960s resulted in simplified Raman spectrometers and boosted the sensitivity of the technique. In addition, advancements in photomultipliers, electronic devices, and computer interfaces have revolutionized Raman spectroscopy, enabling instrument commercialization [3]. Long-term continuous advancements in instrumentation and software development have made Raman spectroscopy applicable to many fields, where it can be adopted as a chemically specific, label-free sample investigation technique for detection of micro-to macro-components.

The diverse and uneven nature of chemical distribution in food and pharmaceutical products make these complex heterogeneous samples ideal for analysis. Food and pharmaceutical products have long, complicated manufacturing processes, which require multiple quality checks, including rapid monitoring at critical points. Fast, reliable, and accurate analytical methods are essential to ensure product quality, safety, authenticity, and compliance with labeling. Raman spectroscopy is a powerful tool for the characterization of a wide range of inorganic and biologically relevant analytes and has several potential applications in the food and pharmaceutical

Abbreviations: RHI, Raman hyperspectral imaging; NIR, Near-infrared; IR, Infrared; HIS, Hyperspectral imaging; CCD, Charge-coupled device; AOTF, Acousto-optic tunable filter; LCTF, Liquid-crystal tunable filter; FT, Fourier transform; SNR, Signal-to-noise ratio; SERDS, Shifted excitation Raman difference spectroscopy; WMRS, Wavelength-modulated Raman spectroscopy; PCA, principal component analysis; AirPLS, adaptive iteratively reweighted penalized least squares; SG, Savitzky–Golay; API, Active pharmaceutical ingredient; HCA, Hierarchical cluster analysis; MCR, Multivariate curve resolution; VCA, Vertex component analysis; ICA, Independent component analysis; PLS, Partial least squares; SORS, Spatially offset Raman spectroscopy; SLM, Spatial light modulator; VPH, Volume-phase holography APD; Avalanche photodiode detector PAT, process analytical technology; SARI, Small animal Raman instrument.

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industry for quality and safety control by analysis of the physiochemical properties of a material throughout production. However, a Raman spectrometer only detects a small portion of the sample; therefore, the spectra are sometimes not representative of the whole sample, particularly if the sample has intrinsic chemical variability, which is the case in food and pharmaceutical materials.

As an integrated alternative, Raman spectroscopy with a chemical imaging component (hyperspectral) can obtain both spectral and spatial information from the target. This (Raman) chemical imaging was first incorporated with Raman microspectroscopy to produce images of specimens where the contrast is derived from the chemical heterogeneity [4–6]. The technique has matured at a relatively rapid pace since its introduction approximately two decades ago.

By combining both spectral and spatial information, Raman imaging allows the identification of the chemical species and their localization. The chemical properties and distribution of species in a sample usually influence the quality attributes of both food and pharmaceutical products. Therefore, chemical imaging combined with Raman spectroscopy can be used to generate a chemical map to show distributions of parameters of interest. Early applications of Raman chemical imaging in different disciplines are described in the study by Tripathi and Jabbour [6] and references therein. The term Raman chemical imaging or Raman microscopic imaging is found in some papers [7,8] with the same meaning as Raman hyperspectral imaging (RHI); for this reason, these terms will be used interchangeably in this paper.

To date, RHI has been applied as a tool for quality control and is a method of choice in scientific disciplines spanning agriculture, biology, and material sciences, e.g., in the analysis of agro-food [9–16], pharmaceutical [17,18, and references therein], biochemical and biomedical [19], and forensic [20] products. RHI has been gaining popularity over the last decade and is accessible to a broad circle of users from diverse fields. Moreover, owing to its instrumental simplicity (rapidity and reproducibility), RHI has been used in a wide range of applications for quality and safety maintenance of food and drug delivery materials in pharmaceutical and biomedical production. Therefore, it is not possible to cover all the applications in this review. Our emphasis here is on recent impressive advancements in RHI, along with the development of state-of-the-art data-processing tools, and interesting applications in different aspects of food and pharmaceutical quality control.

Section 2 will introduce the basic principles of Raman spectroscopy and hyperspectral imaging. Section 3 describes hardware for the construction of a RHI system for application in micro- and macro-scale imaging. Selection of appropriate data analysis methods has great importance in eliminating noise from hyperspectral images and enhancing illustrative features. Therefore, the applications of the most common and effective data analysis methods, including preprocessing, univariate, and multivariate methods, are discussed in Section 4. Applications in the agro-food, pharmaceutical, biological, and biochemical sectors, along with some other interesting recent applications, demonstrate the potential of RHI as a label-free and robust technique in Section 5. Finally, the limitations and future direction of this technique are summarized in Sections 6 and 7.

2. Fundamental principles

2.1. Fundamental principles of Raman spectroscopy

Many papers document the basic theory of Raman spectroscopy, some of which are given in Refs. [7,21–23]. Therefore, only essential features of this technique are outlined here. Raman spectroscopy is a form of vibrational spectroscopy, but unlike infrared (IR) spectroscopy, Raman spectroscopy is based on the exchange of light-photon energy with molecules. In Raman spectroscopy, the target is illuminated with mono-wavelength laser light and the scattered light is than collected with an arrangement of optics and a detector in the spectrometer. When a material is irradiated with mono-wavelength light, most of the scattered energy comprises radiation at the incident frequency, called Rayleigh or elastic scattering, which occurs when only electron-cloud distortion is involved in scattering; in practice, this is filtered out and discarded. The fraction of photons scattered from molecular centers with less energy than they had before the interaction is called Stokes scattered photon. For the anti-Stokes line, the energy of scattered photons is more than that of the incident photons. This is because of scattering of photons from molecules that are in high vibrational states. A simplified energy diagram illustrating these concepts is shown in Fig. 1(a). The Stokes and anti-Stokes Raman peaks are symmetrically positioned around the Rayleigh peak, but their intensities are very different except at low vibrational energy [23]. A schematic Raman spectrum is shown in Fig. 1(b). The much lower intensities

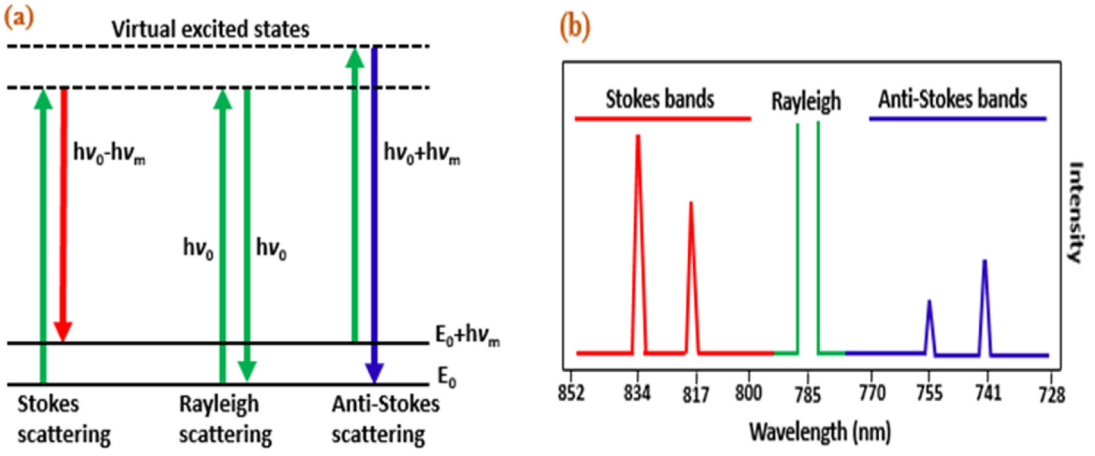

Fig. 1. (a) Schematic representation of Rayleigh and Raman scattering. ν_0 indicates laser frequency (green: no energy difference), Stokes scattering (red: incident photon loss energy), and anti-Stokes scattering (blue: incident photon gain energy). Fig. 1 (b) present illustrative diagram of resulting Raman spectrum (The frequency difference between the incident and scattered radiation is called Raman shift).