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Polarized Raman microscopy imaging: Capabilities and challenges for cancer research

Abramczyk H. *, Brozek-Pluska B., Kopec M.

Institute of Applied Radiation Chemistry, Laboratory of Laser Molecular Spectroscopy, Lodz University of Technology, Lodz, Poland

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ABSTRACT

This study explores the possibility of using polarized Raman spectroscopy for discrimination of cancer from normal tissue. In this paper, polarized Raman spectroscopy (PRS) and polarized Raman imaging (PRI) were used to characterize the isotropic and anisotropic vibrational responses in noncancerous and cancerous human breast tissues. This study has been designed to compare whether more accurate information about cancer and margin measurement can be obtained using PRS and PRI imaging compared to conventional Raman imaging which is the type of imaging currently used. We have shown that the approach presented here opens new possibilities in monitoring biochemical composition, structures and symmetry of vibrations in biological tissues.

Additional information gained by using PRS and PRI compared to conventional Raman spectroscopy allow more accurate lesion and margin measurements because the polarized spectroscopy can provide information about molecular orientation and symmetry of the bond vibrations resulting in better visualization of the cancer and normal tissue structures.

This study reveals that PRS has better diagnostic potential than the conventional Raman spectroscopic technique. The Raman images of depolarization coefficients give an insight of the modifications of symmetry, orientation, structure and disorder that accompany the transformation of normal cells into cancer cells. In contrast, the isotropic Raman images remove orientational and conformational disorder and provides information on purely vibrational effects, mainly vibrational dephasing. The Raman isotropic lines becomes narrower and the contrast in the isotropic Raman images increases.

1. Introduction

Label free Raman imaging methods are becoming of increasing value for biomedical applications, especially where there is a requirement for minimal cell perturbation and manipulation. Recently many new Raman approaches have been developed allowing to extract much more information providing unique information on cancer phenotypes in breast [1–2], brain [3], head and neck [4] epigenetic modifications [5]. These novel Raman based strategies have been applied to immunological, neurobiological, cancer and basic cell biology research [6]. Most biomedical applications employ the conventional Raman spectroscopy and Raman imaging where the measurements are performed without any polarization analyzer [1–9]. It indicates that both polarizations – parallel and perpendicular components with respect to the incident linearly polarized monochromatic laser radiation are collected to generate the signal of the Raman scattered light. This approach seems to be a sensible choice [10] in many cases. However, Raman polarization spectroscopy (PRS) can provide additional important information, because it can be useful in assignment of vibrational modes, identification of symmetry of the bond vibrations, determination of molecular orientations and conformation of the molecules in isotropic and ordered phases, the characterization of molecular or solid-state structure, differentiation single crystals vs. polycrystalline materials, detection and characterization of disorder both in the crystalline and amorphous phases [11–14]. PRS is based on the fact that the orientation of the molecular symmetry axes with respect to the polarization of the incident laser beam can greatly influence the efficiency of Raman scattering. All molecules are more or less symmetric and the excitation of the molecule on one or another symmetry axes can lead to a different intensity of Raman scattering. As a general rule, the symmetric stretching modes tend to yield stronger Raman bands [15–18].

In this paper, polarized Raman spectroscopy (PRS) and polarized Raman imaging (PRI) will be used to characterize the isotropic and anisotropic vibrational responses in noncancerous and cancerous human breast tissues.

1.1. Polarized Raman spectroscopy (PRS) and imaging (PRI)

PRS is observed as a result of interference of polarized light with vibrating molecules and measures the fluctuations in the vibrationally-modulated polarizabilities.

* Corresponding author.
E-mail address: abramczy@mitr.p.lodz.pl (H. Abramczyk).

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The Raman intensity $I(\omega)$ of the scattered light is related to the Fourier transform of the polarizability tensor correlation function $C_\nu(t)$, which is induced during the normal vibration [12,19]

$$I(\omega) \propto \int_{-\infty}^{\infty} \exp(-i\omega t)\, C_V(t) \tag{1}$$

where

$$C_\nu(t) = \sum_{i=1}^{N}\sum_{j=1}^{N}\left\langle A'_{i\nu}(0)Q_{i\nu}(0)A'_{j\nu}(t)Q_{j\nu}(t)\right\rangle \tag{2}$$

$A'_{i\nu}$, $A'_{j\nu}$ are cartesian components of the polarizability tensors of molecule for a given vibrational mode ν, $Q_{i\nu}$ is the vibrational coordinate for this mode in the ith molecule $A'_{i\nu} = [\partial A/\partial Q_{i\nu}]_{Qi\nu=0}$.

It is usually reasonable to assume that there are no phase correlations between vibrational coordinates of different molecules

$$C_\nu(t) = \sum_{i=1}^{N}\left\langle A'_{i\nu}(0)Q_{i\nu}(0)A'_{i\nu}(t)Q_{i\nu}(t)\right\rangle \tag{3}$$

Furthermore, it is assumed also that $A'_{i\nu}$, which in general depends on molecular rotation coordinates and intermolecular distances, relaxes independently from $Q_{i\nu}$. This leads to

$$C_\nu(t) = \sum_{i=1}^{N}\left\langle A'_{i\nu}(0)A'_{i\nu}(t)\right\rangle\langle Q_{i\nu}(0)Q_{i\nu}(t)\rangle = C_{rt}(t)C_{\nu ib}(t) \tag{4}$$

where $C_{\nu ib}(t) = \langle Q_{i\nu}(0)\, Q_{i\nu}(t)\rangle$ is the vibrational time correlation function and $C_{rt}(t)$ is the time correlation function of $A'_{i\nu}$. $C_{rt}(t)$ contains information on molecular rotational relaxation and relaxation processes associated with interaction induced dipoles (IR) or polarizabilities (Raman) [20].

In linear vibrational spectroscopy on isotropic systems, $C_{\nu ib}(t)$ is equal to of the isotropic Raman spectrum I_{iso}

$$C_{\nu ib}(t) \cong C_{iso}(t) \tag{5}$$

where

$$I_{iso} = I_{II} - \frac{4}{3}I_{\perp} \tag{6}$$

where I_{II} and $I_\perp$ are the Raman scattering intensities when the polarization directions are perpendicular and parallel with respect to the incident beam, respectively [12,21,22].

The approximation in Eq. (5) corresponds to neglecting the contribution to $C_{rt}(t)$ from interaction-induced polarizability relaxation. The validity of approximations (3)–(5) varies with the vibrational mode, intra- and intermolecular interactions and thermodynamic state [23], but most cases can be approximated in this manner.

Since every vibrational mode has a unique differential polarizability ellipsoid and the Raman scattering intensity depends on the polarization of the incident and scattered light, the Raman intensities I_{II}, $I_\perp$ vary from mode to mode. The ratio $\frac{I_\perp}{I_{II}}$ is known as the depolarization coefficient.

$$\rho = \frac{I_\perp}{I_{II}} \tag{7}$$

The depolarization coefficient is useful to identify the symmetry of the vibrational mode and the order in the sample. In a liquid PRS measurements are sampling all orientations of the molecule in space, whereas in a crystal the molecules are all oriented in space in accordance with the crystal class to which the compound or element belongs [10].

The forms of the Raman polarizability tensors are dictated by the symmetry of the vibration. In isotropic media, when averaging is performed over ensembles of all orientations, the depolarization coefficient ρ is expressed as [24]

$$\rho = \frac{3\overline{\gamma}^2}{45\overline{\alpha}^2 + 4\overline{\gamma}^2} \tag{8}$$

where:

$\overline{\gamma}$ - anisotropy of tensor polarizability derivative with respect to normal vibration mode Q.

$\overline{\alpha} = \frac{1}{3}(\alpha_{xx} + \alpha_{yy} + \alpha_{zz})$ polarizability derivative with respect to normal vibration mode Q.

In contrast, the forms of the Raman polarizability tensors for crystals are dictated by the symmetry of the crystal. Thus, in perfectly ordered crystals whose structures differ from that of isotropic liquids as well as in partially ordered systems like liquid crystals and other mesophases, the Eq. (8) is no longer valid. Changing the configuration of the PRS experiment one can expect to see the Raman bands of different symmetry, e.g. configuring the Raman analyzer parallel to the incident polarization selects the A_1 Raman bands [10,19,25–28].

PRS has been employed for various biomedical applications, such as collagen orientations in bones [29–31], orientation of collagen fibers in biological tissues [32] the intercalation of ethidium bromide with DNA [33], discrimination healthy epidermis and basal cell carcinoma [34,35], cervical cancer [36], gastric cancer detection based on blood plasma surface-enhanced Raman spectroscopy [37]. PRS has been used also to study hemoglobin and cytochrome *c* for which the phenomenon of the inverse polarization was noticed [38]. Polarization data for an activated lymphocytes in the inflammation state indicated the inversion polarization effect [39].

We will demonstrate that PRS and PRI are powerful tools to enhance the accuracy of Raman diagnosis about the cancer over conventional Raman spectroscopy.

To the best of our knowledge, only a few literature reports [36] are available on the characterization of cancer human tissues by PRS and the first report by PRI when polarized Raman images of epithelial cells in human tissue are shown. It is also the first implementation of polarized Raman imaging to study alterations in epithelial cells in human breast induced by cancer development.

2. Experimental

2.1. Samples preparation and equipment requirements

We have employed polarized Raman spectroscopy and Raman imaging to measure the human breast noncancerous and cancerous tissues. The Raman technique has been applied to record Raman spectra and images of ex vivo fresh human breast tissue samples without any fixation. The ex vivo samples were obtained during the resection surgery from the tumor mass (cancerous tissue) and the safety margin (noncancerous tissue). All tissue specimens were frozen and stored at −80 °C. Before the Raman measurements the frozen samples were cryosectioned at −20 °C with a microtome (Microm HM 550, Sermed) into a 6 or 16 μm-thick slices. The slices are used for Raman analysis without typical for histology examination paraffin embedding procedure. The parallel slices were used for standard histology examination. The thin sections were placed onto calcium fluoride windows (CaF_2, 25 × 1 mm) and examined by Raman imaging. Standard single Raman spectra and 2-D images were collected on thin sections. After the Raman measurements these sections were stained and histologically examined. The adjacent part of the tissue was paraffin embedded and also cut into 6 μm-thick sections for conventional histological analysis. All tissue procedures were conducted under a protocol approved by