

Advanced Raman Spectroscopy in Nanomedicine

Abbreviations: SERS: Surface-Enhanced Raman Scattering; TERS: Tip-Enhanced Raman Scattering; SPM: Scanning Probe Microscopy; TPL: Two-Photon Luminescence; CARS: Coherent Anti-Stokes Raman Scattering; SRS: Stimulated Raman Scattering

Introduction

Optical microscopy is a key technique to biomedical research. Light microscopy reveals changes in refractive index within cells and tissue, however lacks of biochemical contrast and is only appropriate for monolayers of cells or tissue slices and can produce only the image of the surface or optical contrast in the case of inverted microscopy. Fluorescence imaging is the current major technique in biomedical research. It is based on the labeling of the molecules by fluorescent dyes. The fluorescence can be expressed by modified genes or labelled molecules under investigation. The fluorescence imaging have a number of drawbacks:

- sample modification by labeling;
- label can perturb the behaviour of the tagged molecule of interest;
- photobleaching limits the observation time.

The Raman scattering phenomenon known for more than 80 years, during the last decades is gaining more and more attention in analytical applications and can provide chemical fingerprints of cells, tissues or biofluids. In contrast to established analytical techniques, Raman spectroscopy provides label-free, non-destructive, chemically selective and spatially resolved analysis. The high chemical specificity, simple sample preparation procedure and the ability to use advanced optical technologies in the visible or near-infrared spectral range have recently led to an increase in medical diagnostic applications of Raman spectroscopy. The basic idea of the application of Raman spectroscopy in medicine is the hypothesis that diseases affect the molecular structure of cells and can be detected and quantified by Raman spectroscopy. Based on advanced technical development, Raman spectroscopy can be implemented into diverse setups ranging from confocal microscopes for acquisition of three-dimensional spectral information up to hand-held fiber devices for direct use in clinical diagnostics. Furthermore, recent progress in the field of multivariate data analysis allows for processing such complex spectral data into scientific information for fundamental research as well as for patients to obtain objective data for diagnosis [1]. The new strategies have been developed to overcome the weak signal problems of Raman spectroscopy by application of non-linear optics and localized nanoplasmonic effects to enhance the Raman signals to increase the acquisition speed and accuracy of diagnosis. Application of nanotechnology solutions in Raman spectroscopy and microscopy, like SERS and TERS, open a new directions in Nanomedicine, based on a single molecule science [2].

Editorial

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Valentinas Snitka**Kaunas University of Technology, Lithuania*

***Corresponding author:** Valentinas Snitka, Research Center for Microsystems and Nanotechnology, Kaunas University of Technology, Student str. 65, Kaunas, Lithuania, Tel: +37037451588; Email: vsnitka@ktu.lt

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Physiological investigations of tissues and cells

Raman micro-spectroscopy provides an easy to use, non-destructive, and spectra based imaging tool of probing cell and tissue physiology with diffraction-limited resolution, however in combination with Scanning Probe Microscopy (SPM) the spatial resolution up to 10 nanometers can be achieved [3]. By probing the vibrational signature of molecules and molecular groups, the distribution and metabolic products of molecular activity that cannot be imaged using fluorescent dyes can be investigated. The non destructive imaging and characterization of single cells by Raman spectroscopy was demonstrated by several authors [4]. Authors managed to obtain the Raman spectra from the different organelles of the cells and from isolated chromosomes by micro-Raman spectroscopy with diffraction limited resolution. Since then, Raman spectroscopy has been applied to study the physiology of a wide range of cells, bacteria and viruses, as well as plant cells, and the interaction of drug molecules, nanoparticles and other molecules with cells [5-6].

Drug-cell interactions

The focus for the development of novel therapeutics is moving from classical solid dosage systems such as tablets and capsules to modern drug carriersystems in micro/ nanometer size range. In this tendency, an appropriate analytical analysis and evaluation of interactions of drug delivery systems, their cellular uptake of drugs and nanoparticles and the release mechanism, the kinetics for an incorporated drug in order to improve therapeutics and specific disease treatment protocols are of high importance. The Raman spectroscopy as non-invasive, chemically sensitive analytical technique is opening a new ways in the field [7-9].

Raman imaging of cells and drug delivery systems

Raman spectral imaging is a method for producing chemical images based on Raman spectra collected during the sample scanning procedure. A complete spectrum is acquired at each and every pixel of the image, and then used to generate a colour

images based on biomaterial composition and structure. A typical experiment uses X-Y sample scanning line by line and spectrum acquisition, repeated many times (up to thousands) to collect data from the area of interest. Raman spectral images can be collected in 2D and 3D dimensions, to produce XY images, XZ and YZ slices, and XYZ data hyperspectra files. The technical progress during the last years led to an increased accessibility and a variety of available instruments on the market [10-13]. Although components and exhibited surface structures varied broadly in pharmaceuticals, images with high spatial resolution and good chemicals selectivity were demonstrated in the work [14]. The resolution of the cellular Raman image is an important factor to select a different molecular response and to capture the subcellular structures up to nanometer size range as well as the nano carriers. The lateral resolution depends on the numerical aperture of the objective in combination with the wave length of the excitation laser light, and the axial resolution depends on the pinhole diameter and the refractive index of the environment [15]. The currently available Raman instruments, produce a lateral resolution down to 200 nm and an axial resolution of 500 nm can be achieved. It makes the Raman microscopy technique feasible for visualization of subcellular structures and drug carriers [16-17].

SERS and TERS

SERS opens a new possibility to dramatically increase the sensitivity of Raman spectroscopy in a wide range of systems and to move to one molecule science and applications. Combining the microscopic technology with optical measurements, single-molecule Raman has been established as a significant spectroscopic method to probe single molecules, exploring the high sensitivity and spectral resolution of SERS [18] and TERS [19]. The SERS nanosensors can be used in various applications like pH sensing, detection of drugs and proteins, toxins, and diagnostic of genes, finger printing of cells. For DNA detection the SERS based nanoprobe can be used as a bioassay in solution or on a chip platform. Gold nanoparticles provide a complex theranostic platform, combining Raman and SERS with luminescence imaging as well as photodynamic therapy and photothermal therapy. Plasmonics-stimulated and optically modulated delivery of nanoparticles into brain tumor in live animals was demonstrated and gold nanoparticle based photothermal treatment of tumor vasculature may stimulate inflammasomes activity, thus increasing the permeability of the blood brain barrier. The imaging method using TPL of gold nanoparticles provides an unprecedented spatial selectivity for targeted nanoparticles delivery to tumor tissue [20].

Beyond the classics

The low Raman scattering cross section of molecules is the major drawback of spontaneous Raman microscopy limiting the application in biomedicine. Therefore, Raman microscopy is a slow technique, with long acquisition time, especially in biological fields. The progress towards faster imaging recently have been made, however the long imaging time still is a limiting factor for wider applications. The auto fluorescence, which is present in many biological samples, is another limiting factor of ten prevents the detection of a Raman signal. In order to solve the problems caused by these limitations, the Raman techniques based on non-

linear optical microscopy, namely CARS and SRS microscopy have been developed during the last years. After an initial period of the development of methodological issues, both schemes have found a lot of applications during the recent years. Recently published review articles give an excellent overview over the current state-of-the-art of biomedical applications of CARS and SRS microscopy [21].

Is Raman Spectroscopy Ready for Clinical Use?

Recent reports and experts opinions [22] demonstrate a fast growing increase in the development and application of Raman spectroscopy based methodologies to address biomedical questions. The label-free ability to obtain specific chemical information about the biological molecules and tissues, makes Raman spectroscopy an attractive analytical tool in clinical diagnostics of cells, tissue biopsies and pathogens. The significant progress has been made towards the application of micro-Raman spectroscopy as a standard test for the fast identification of pathogens and the determination of the pathogens resistance to antibiotics. The Raman spectromicroscopy, as a label-free and video-rate imaging technique may have a tremendous impact on identifying a cancer tumor tissue or other abnormalities. It seems a few years from now we will see the first Raman applications as standard diagnostic or therapeutic tool in a daily clinical practice.

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