Optics Communications 402 (2017) 221–225

Contents lists available at ScienceDirect

Optics Communications

journal homepage: www.elsevier.com/locate/optcom

Structured illumination for wide-field Raman imaging of cell membranes

Houkai Chen [a], Siqi Wang [b], Yuquan Zhang [a,*], Yong Yang [c], Hui Fang [a], Siwei Zhu [b], Xiaocong Yuan [a,*]

[a] Nanophotonics Research Centre, Shenzhen University, & Key Laboratory of Optoelectronic Devices and Systems of Ministry of Education and Guangdong Province, College of Optoelectronic Engineering, Shenzhen University, Shenzhen 518060, China
[b] Tianjin Union Medical Center, Tianjin 300121, China
[c] Institute of Modern Optics, Nankai University, Tianjin 300071, China

ARTICLE INFO

Keywords:
Raman microscopy
Super-resolution
Surface enhanced Raman scattering

ABSTRACT

Although the diffraction limit still restricts their lateral resolution, conventional wide-field Raman imaging techniques offer fast imaging speeds compared with scanning schemes. To extend the lateral resolution of wide-field Raman microscopy using filters, standing-wave illumination technique is used, and an improvement of lateral resolution by a factor of more than two is achieved. Specifically, functionalized surface enhanced Raman scattering nanoparticles are employed to strengthen the desired scattering signals to label cell membranes. This wide-field Raman imaging technique affords various significant opportunities in the biological applications.

1. Introduction

Raman spectroscopy has long been an attractive analytical method that is used in many and an ever-growing number of analytical areas to obtain characteristic features of samples. However, single-dimensional spectral information is not sufficient, and a multi-dimensional approach including the spatial and spectral information is imperative. Raman imaging techniques, especially the scanning imaging, have developed greatly over the past decade [1–4], and they are now capable of capturing multi-dimensional information of samples. Different from scanning imaging scheme whose capture time depends on image pixel, the wide-field Raman imaging based on filters can offer higher imaging speeds; however, the diffraction limit constrains the spatial resolution.

To increase the spatial resolution of optical systems, many techniques have been developed such as stimulated emission depletion microscopy (STED) [5], photoactivated localization microscopy (PALM) [6], and stochastic optical reconstruction microscopy (STORM) [7], etc. One technique called standing-wave total-internal-reflective microscopy (SW-TIRM) is the first choice among different super-resolution techniques to extend the spatial resolution of wide-field Raman imaging because of its intrinsic wide-field nature and no special properties requirement [8,9]. SW-TIRM combines advantages of evanescent waves and structured illumination microscopy (SIM) to realize resolution enhancements and the high signal-to-noise signals from surface excitation of the samples.

Herein, the SW-TIRM technique with narrow band-pass filters is used to extend the spatial resolution of field-wide Raman imaging. Sub-100 nm spatial resolution is achieved in one-dimensional evaluation imaging. The isotropy of spatial resolution is better than that reported in previous work and the technique is more suitable for surface Raman imaging because of small penetration depths of evanescent waves [10]. Further, functionalization of surface enhanced Raman scattering (SERS) nanoparticles is implemented for biological imaging. The wide-field Raman imaging of cell membranes with SERS tags is successfully implemented. Multiplexed high-speed Raman imaging is achievable using different SERS nanoparticle labelling with various Raman reporters. The potential of the technique is significant for high resolution and high-speed wide-field Raman imaging, especially in time-resolved imaging.

2. Experiment Methods

SIM uses spatially-patterned light to illuminate specimens, generating a Moiré effect to encode the undetected high-frequency information into a detectable frequency domain of conventional microscopy [11]. A series of intermediate images are captured by varying the structured patterns to separate high- and low-frequency information. They are shifted to the corresponding positions in the frequency domain to extend and eventually enhance the spatial resolution.

Here we employ the SIM technique into Raman imaging and Fig. 1 shows the experimental setup of the wide-field Raman imaging system

* Corresponding authors.
E-mail addresses: yqzhang@szu.edu.cn (Y. Zhang), cxyuan@szu.edu.cn (X. Yuan).

http://dx.doi.org/10.1016/j.optcom.2017.06.021
Received 12 April 2017; Received in revised form 2 June 2017; Accepted 4 June 2017
0030-4018/

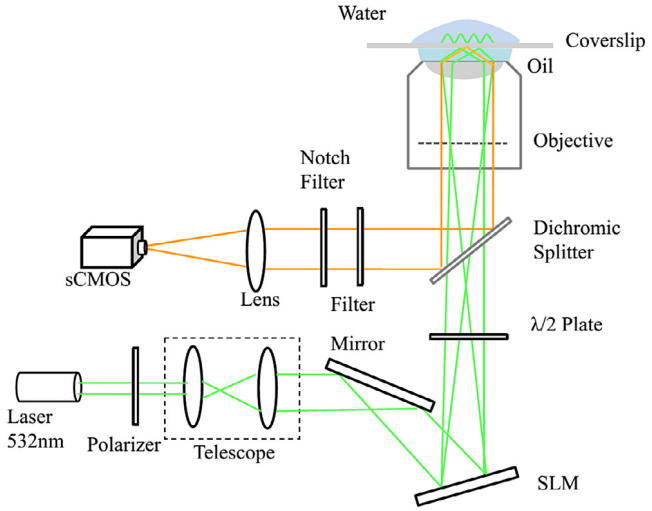

Fig. 1. Schematic diagram of the wide-field Raman imaging system.

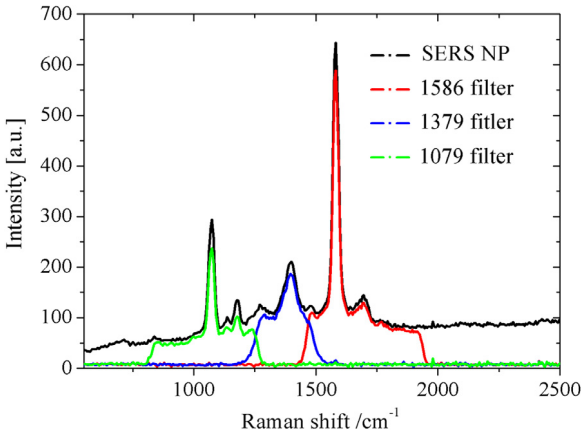

Fig. 2. Raman scattering spectrum of the SERS nanoparticles and filtered spectra using different narrow band-pass filters.

built based on the phase-type spatial light modulator (SLM, Holoeye Pluto, Germany) with a pixel size of 8 μm. A laser beam with a wavelength of 532 nm is expanded to illuminate the SLM. Although it is not the most efficient excitation, this wavelength is the only available one. The polarization direction of the incident beam is along the major axis of the SLM. The laser beam is split into two beams with opposite angles and the beams are focused onto the rear aperture plane of an objective lens with high numerical aperture (UApo N, 100 × /1.49 oil, Olympus, Japan) to form the illumination fringes on the sample plane. The parameters of the beams are regulated by the SLM. The designed holographic pattern of the SLM can be simply described by the phase:

$$phase = \begin{cases} \exp\left(ik\dfrac{x^2+y^2}{2f} + iky\sin\theta + \varphi_{i=1,2,3}\right) & y > 0 \\ \exp\left[ik\dfrac{x^2+y^2}{2f} + i(-k)y\sin\theta\right] & y \le 0 \end{cases} \quad (1)$$

where k is the wave vector of light in vacuum, f the focal length of the focused beams, and φ_i the phase of the illumination fringes. To reconstruct the resolution enhanced images, at least three intermediate images are needed to be taken at various phases of the illumination fringes. Here we choose phase shifts

$$\varphi_{i=1,2,3} = \{0, 2\pi/3, 4\pi/3\}. \quad (2)$$

The parameter θ controls the incidence angle of the interference beams. By precisely controlling θ, the incidence angle of the beams at the interface of glass coverslip and water can be tuned to be larger than the critical angle for the total internal reflection $\theta_c = \sin^{-1}(n_w/n_g) = 61°$, where $n_w = 1.33$ is the refractive index of water, and $n_g = 1.516$ the refractive index of glass coverslip. Therefore, a standing-wave evanescent field is generated to illuminate the specimen at the interface of the coverslip and water. In experiments, the −1 diffraction order is used while other orders are blocked, and details are given in a previous work [12].

Different oriented illumination patterns can also be realized to achieve enhancements of lateral resolution in different directions. The S-polarized beams are usually used to obtain a maximum contrast of the interference patterns in the structured illumination configuration. However, in our imaging system, the polarization of the incidence beams is fixed along the 45° diagonal to eliminate effects of polarization on the efficiency of the dimer enhancement between nanoparticles [13]. Furthermore, the imaging setup is simplified because no complicated rotators or mechanic drivers are needed to modulate the polarization. Circular-polarized beams is a better choice if structured illumination is implemented in more than two directions [14].

In experiments, the periods of the illumination fringes are ~188 nm in the vertical direction (0°) and ~193 nm in horizontal direction (90°). To enhance the weak classic Raman scattering signals in practical applications, SERS nanoparticles are used as specimens to evaluate the lateral resolution of the wide-field Raman imaging system and to label the component of biological specimens. The nanoparticle has a metal core coated with 4-mercaptobenzoic acid (4-MBA) molecules and is encapsulated in a poly(vinylpyrrolidone) (PVP) shell, making a diameter of ~50 nm [15]. These nanoparticles are deposited onto a 170 – μm thick glass coverslip and treated as point sources of Raman scattering. The Raman scattering signals pass through the same objective lens, and wide-field images are captured using a monochromatic sCMOS camera (C11440-22CU, Hamamatsu, Japan). To separate the Raman scattering signal from the original laser signals, two sets of dielectric filters are employed. The first set including two long-pass filters are used to separate the reflected laser beam from the Raman scattering signals. The second set, including various narrow band-pass filters, are used to extract specific peaks of the Raman scattering spectrum. Fig. 2 shows a typical Raman spectrum for the SERS nanoparticles and filtered spectra from the different narrow band-pass filters. Although this result is restricted by experimental conditions at present, a more precise extraction of peak signals can be realized using filters with narrower bands.

3. Experiment results

3.1. Wide-field Raman imaging of SERS nanoparticles

Raman imaging of SERS nanoparticles is firstly performed to demonstrate its superior spatial resolution capability. To obtain the resolution-enhanced image, three intermediate wide-field Raman images at phase shifts $\{0, 2\pi/3, 4\pi/3\}$ of the illumination fringes are captured in the direction of the illumination patterns. The background image without illumination is subtracted from each raw image. One intermediate image at Raman shift of 1586 cm^{-1} is shown in Fig. 3(a). Fig. 3(b) gives out the reconstructed images. The full width at half maximum (FWHM) is improved from 219 nm to 97 nm, as shown in Fig. 3(c)–(d), after applying the reconstruction algorithm and deconvolution method to suppress side lobes generated during reconstruction [16,17]. Compared with the wide-field image from conventional microscopy, the resolution is improved more than two-fold in terms of the FWHM.

By manually changing the narrow band-pass filters, wide-field images at different Raman peaks are captured. Next, the SW-TIRM algorithm is applied to achieve enhancements in spatial resolution at