Methods 128 (2017) 119–128

Contents lists available at ScienceDirect

Methods

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

Imaging chemistry inside living cells by stimulated Raman scattering microscopy

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ARTICLE INFO

Article history:
Received 25 March 2017
Received in revised form 6 June 2017
Accepted 20 July 2017
Available online 23 July 2017

Keywords:
Chemical imaging
Stimulated Raman scattering microscopy
Vibrational spectroscopy
In vivo imaging
Raman tag

ABSTRACT

Stimulated Raman scattering (SRS) microscopy is a vibrational imaging platform developed to visualize chemical content of a biological sample based on molecular vibrational fingerprints. With high-speed, high-sensitivity, and three-dimensional sectioning capability, SRS microscopy has been used to study chemical distribution, molecular transport, and metabolic conversion in living cells in a label-free manner. Moreover, aided with bio-orthogonal small-volume Raman probes, SRS microscopy allows direct imaging of metabolic activities of small molecules in living cells.

Contents

Abbreviations: CRS, coherent Raman scattering; SRG, stimulated Raman gain; SRL, 3D, three-dimensional; SRS, stimulated Raman scattering; TPEF, two-photon excitation fluorescence; OPO, optical parametric oscillator; AOM, acousto-optic modulator; EOM, electro-optic modulator; PCA, principle component analysis; MCR, multivariate curve resolution; H&E, hematoxylin and eosin; *C. elegans*, *Caenorhabditis elegans*; DMSO, dimethyl sulfoxide; C–D, carbon-deuterium.

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http://dx.doi.org/10.1016/j.ymeth.2017.07.020
1046-2023/

1. Introduction

A central theme of chemical science is deciphering how molecules function in a complex system, such as a living cell. Such study contributes to the society by providing fundamental knowledge in medical science to improve our lives. Yet, our understanding of chemistry in living system (e.g., how intensive biosynthetic chemical activity drives cell development, function, and inter-cellular communications) is still limited, partly because conventional biochemical assays treat the cell, a highly dynamic structure, as a static bag of molecules. In current paradigms, molecules are extracted from a tissue and analyzed by various analytical techniques such as immunoblotting and liquid chromatography/mass spectrometry. These *in vitro* assays provide very little information about the spatial distribution or temporal dynamics of molecules in real life, thus they are unable to tell the exact roles of molecular activities on cellular functions [1]. Furthermore, the molecular profile of a cell may alter during the extensive sample processing procedures. For *in situ* imaging, fluorescent microscopy is widely used. By measuring fluorescent signals from endogenous species, such as coenzymes nicotinamide adenine dinucleotide (NADH) and Flavin adenine dinucleotide (FAD), cell metabolism can be measured in real time [2–4]. Unfortunately, not all endogenous molecules possess the ideal optical properties. Fluorescent labels allow imaging of proteins and some key metabolites in living cells, but they often disturb the function of small biomolecules, such as glucose and cholesterol, limiting the ability to monitor their activities. These limitations stress the critical need of establishing new platforms for learning chemistry *in situ* in living systems.

Raman-scattering based vibrational spectroscopy has been a powerful tool for non-invasive, label-free analysis of chemicals. Raman scattering is an inelastic scattering process, in which an excitation photon loses energy to a certain molecular vibration mode, resulting a scattered photon with a different wavelength. Such energy losses are directly related to vibrational transitions of a molecule, showing as peaks in Raman spectrum (Fig. 1a). Therefore, analysis of Raman-scattered photons can be used to identify chemical species quantitatively. Raman microscope, which is now commercially available, allows chemical imaging with submicron spatial resolution [1]. However, because Raman scattering is a feeble process, the image acquisition speed of current Raman microscopes (at least tens of minutes per frame) is insufficient to follow chemical dynamics *in vivo*. To improve the imaging speed, line illumination has been adopted for ultra-fast Raman imaging, allowing several minutes per frame imaging speed (Nanophoton, Osaka, Japan). To overcome the speed limitation, coherent Raman scattering (CRS) microscopy [5] has been developed to enhance the Raman signal level. In CRS microscopy, two excitation beams, known as pump (ω_p) and Stokes (ω_s), are used. When the laser-beating frequency ($\omega_p - \omega_s$) is in resonance with a molecular vibration frequency (Ω), four major CRS processes occur simultaneously, known as coherent anti-Stokes Raman scattering (CARS), coherent Stokes Raman scattering, stimulated Raman gain (SRG), and stimulated Raman loss (SRL) (Fig. 1b). These nonlinear optical processes offer a large signal that allows live-cell imaging at a speed three to four orders of magnitude faster than Raman microscope. As nonlinear optical process, CARS and SRS microscopy offers inherent three-dimensional (3D) sectioning capability. Fur-

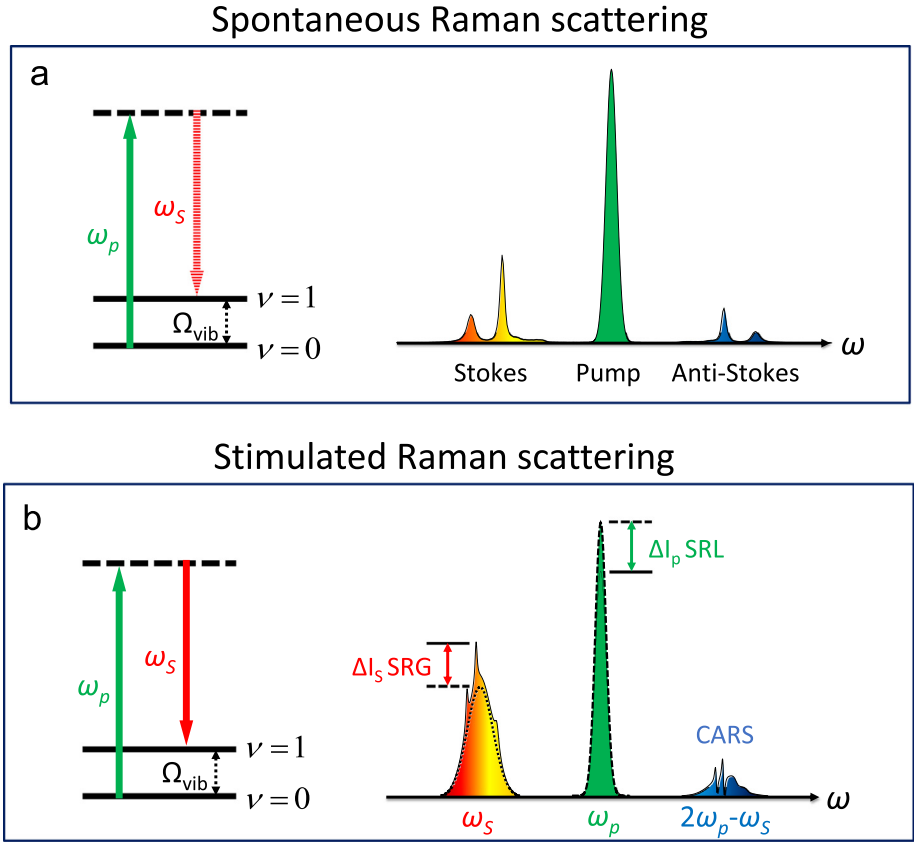

Fig. 1. Spontaneous and coherent Raman scattering processes. (a) Energy diagram of spontaneous Raman scattering and representative spectra. (b) Energy diagram of SRS and CARS processes and representative spectra. Broadband coherent Raman scattering induced by a pump field at ω_p and a Stokes field at ω_S. Solid arrows indicates laser excitation and dashed arrow indicates the spontaneous scattering process Ω_{vib} denotes the vibrational energy.