ACCELERATING RAMAN IMAGING BY MULTI-LINE ILLUMINATION

[1]Kentaro Mochizuki, [1]Shunsuke Maeda, [2]Nicholas Isaac Smith, [1]Katsumasa Fujita
[1]Department of Applied Physics, Osaka University,
2-1 Yamadaoka, Suita, Osaka, Japan
[2]Immunology Frontier Research Center (IFReC), Osaka University,
2-1 Yamadaoka, Suita, Osaka, Japan
E-mail: fujita@ap.eng.osaka-u.ac.jp

KEY WORDS: Raman microscopy, multi-line illumination, label-free imaging, hyperspectral imaging

Improving the image acquisition speed in Raman microscopy is key to fully utilize the powerful analytical capability of Raman spectroscopy in biological applications. Recently, we achieved the Raman image acquisition more than two orders-of-magnitude faster than that in a conventional confocal by using line-illumination [1, 2]. Here, we report a further acceleration of image acquisition rate in Raman microscopy by multiplying illumination lines. We propose the use of multiple illumination lines for parallel detection of Raman spectra from multiple points in a sample. In the developed system, multi-line illumination was generated by using a cylindrical lens array and were focused to a sample via an objective lens. For simultaneous detection of Raman spectra, a multi-slit array was placed at the entrance of an imaging spectrophotometer. To avoid overlaps of Raman spectra in the spectrum axis on a CCD camera for detection, the spectral region was limited by using optical band-pass filters. Each illumination line is scanned simultaneously in order to obtain the distribution of Raman spectra on the field of view. Figure 1 shows a Raman image of 1 μm polystyrene beads measured with 11 illumination lines (excitation wavelength: 532 nm, exposure time: 5 s, detection spectral region: 3000~3100 cm^{-1}). The whole Raman image with 400 x 600 pixels was acquired in about 10 minutes, which is simply 11 times faster than a single-line illumination under the same excitation photon density. This research was partially supported by Nakatani Foundation's grant program for biomedical engineering research and JST-CREST program (JPMJCR1662).

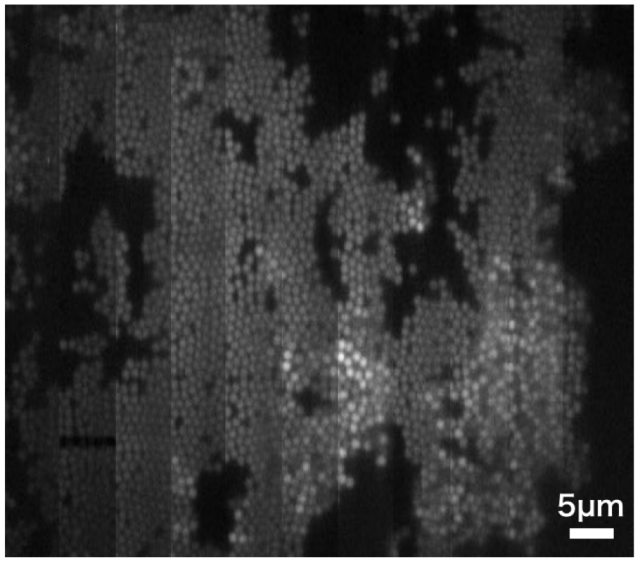

Fig. 1: Raman image of 1μm polystyrene beads, measured with the illumination of 11 lines and constructed with 3055cm^{-1}.

References:

[1] K. Hamada, K. Fujita, N. I. Smith, M. Kobayashi, Y. Inouye, S. Kawata, "Raman microscopy for dynamic molecular imaging of living cells," J. Biomed. Opt., **13**, 044027 (2008).

[2] A. F. Palonpon, J. Ando, H. Yamakoshi, M. K. Dodo, M. Sodeoka, S. Kawata, K. Fujita, "Raman and SERS microscopy for molecular imaging of live cells," Nat. Protoc., **8**, 677-692 (2013).