18p-C4-10

Large Area Raman Spectroscopic Imaging of Unstained Mouse Brain Slice with Sub-micron Spatial Resolution

Almar Palonpon[1,2], Atsushi Kasai[3,4], Satoshi Kawata[1,2], Hitoshi Hashimoto[3,5] and Katsumasa Fujita[2]

[1] Photonics Advanced Research Center, Osaka University, [2] Department of Applied Physics, Osaka University,
[3] Graduate School of Pharmaceutical Sciences, Osaka University, [4] Institute for Academic Initiatives, Osaka University,
[5] United Graduate School of Child Development, Osaka University
E-mail: almar@ap.eng.osaka-u.ac.jp

1. Introduction

Recently, large area Raman imaging of unstained mouse brain tissues is reported using coherent Raman microscopies featuring high image speed acquisitions resulting from the enhanced Raman signals [1-3]. However, this increase in imaging speed comes at the expense of losing full spectral information. Coherent Raman microscopy typically acquires an image at a single band or a few 100 cm^{-1} in the case of multiplex versions. Here, we report large area Raman spectroscopic imaging of mouse brain slice using a slit-scanning Raman microscope capable of obtaining large images with full spectral information while maintaining sub-micron spatial resolution

2. Materials and Methods

An adult mouse was deeply anesthetized with 50 mg/kg pentobarbital, and perfused transcardially with saline followed by 4% paraformaldehyde in phosphate buffered saline (PBS). The whole brain was dissected and postfixed in the same fixative overnight at 4ºC. The brain was cut into 100-µm-thick slices using a vibratome. Prior to Raman imaging, the slices were sandwiched between two cover slips with PBS.

Raman spectroscopic imaging at the coronal section of the mouse brain was performed at 532 nm excitation using a custom-made slit-scanning Raman microscope modified especially for large area Raman imaging [4]. We used an objective lens with high numerical aperture to achieve sub-micron spatial resolution. To increase the field of view while maintaining the high spatial resolution, we employed a CCD camera with a large imaging area of 27.6 x 27.6 mm and pixel resolution of 13.5 µm to capture simultaneously the Raman scattering signals from the illuminated line. The exposure time for each line was 5 sec. One dataset with 679 scanned lines took about 2.8 hours to complete. The Raman hyperspectral dataset was then post-processed using standard algorithms to remove cosmic rays, reduce noise and take out the fluorescent background.

3. Results

The composite Raman image shown in Fig. 1a was constructed by using the three known Raman signatures of cytochrome *c* at 749 cm^{-1}, lipid at 2850 cm^{-1} and amide I at 1686 cm^{-1}, and assigning them to different colors. The image shows clearly the different spatial distributions of these three chemical species. The lipid contrast mainly shows the location of the lipid-rich fiber structures. The amide I contrast, which shows the distribution of proteins, seems to be high at regions resembling neuronal cell bodies. The strong cytochrome *c* contrast indicates a high concentration of mitochondria in the cortex region. The full Raman spectra (Fig. 1b) covering 600-3000 cm^{-1} show many other Raman signatures that could contain more useful chemical information about the mouse brain, which cannot be obtained by coherent Raman techniques.

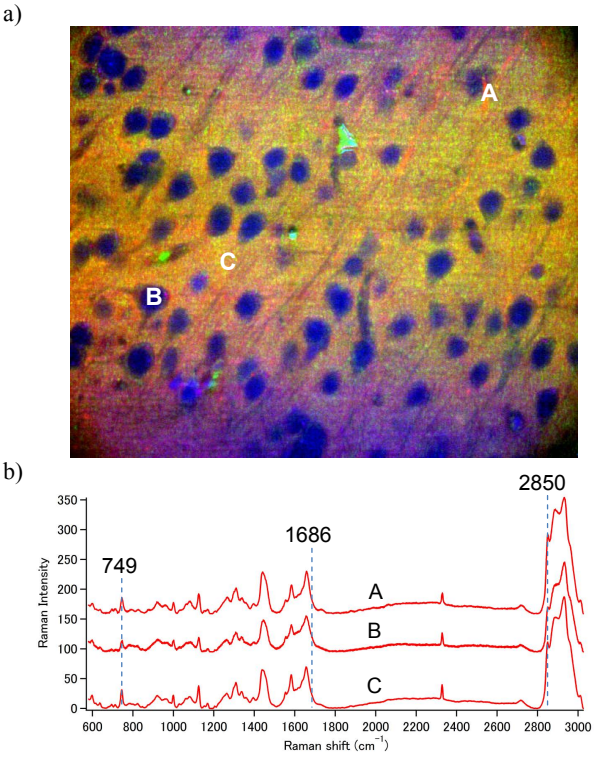

Figure 1. Large area Raman spectroscopic imaging of unstained fixed mouse brain slice. a) Composite Raman images showing distribution of cytochrome *c* (green), protein (blue) and lipids (red). The image size is 260 µm x 220 µm with sub-micron spatial resolution. b) Typical Raman spectra of the mouse brain. Each spectrum was obtained at regions labeled accordingly in Fig. 1a.

References

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