Hybrid fluorescence-Raman microscopy for the visualisation of protein-metabolome interaction in living cells

Liang-da Chiu$^{1,2}$, Taro Ichimura$^3$, Hideaki Fujita$^{3,4}$, Takeaki Ozawa$^1$, Katsumasa Fujita$^2$

$^1$ Dept. of Chemistry, the Univ. of Tokyo, $^2$ Dept. of Applied Physics, Osaka Univ., $^3$ QBiC, RIKEN, $^4$ iFReC, Osaka Univ.

E-mail: fujita@ap.eng.osaka-u.ac.jp

1. Introduction

In recent years, metabolomics has risen as a hot topic of study. Deciphering how the metabolome is regulated by the central dogma, i.e. DNA, RNA or protein expression, is of huge interest to the scientific community now [1]. However, although well-established methods are available for the independent analysis of protein expression or metabolome profile, it has been difficult to connect metabolome profiling results to protein expression patterns in living cells.

In this study, we have developed a new method that combines the use of fluorescence microscopy with Raman spectroscopy through the anti-Stokes excitation of fluorophores. Fluorescence microscopy, especially when combined with genetically-encoded fluorescent proteins, is the most widely used technique for the visualisation of protein expression in living cells [2]. On the other hand, Raman spectroscopy is also a widely used method for the metabolome profiling of living cells [3]. Combining the two methods enables the study of protein-metabolome interaction in living cells for the first time.

2. Experiments and Results

The developed hybrid fluorescence-Raman microscope is a very simple system that has an extremely similar optical design to a normal Raman microscope. The only difference is that the dichroic mirrors used to reject the Rayleigh scattering signal are substituted with notch filters so that the anti-Stokes signal can be detected at the same time as well. This makes our hybrid imaging method easy to implement a technique that can be readily adapted to any existing Raman microscope systems without the need of any expensive equipment, such as pulsed lasers.

The result of hybrid fluorescence-Raman imaging of histone-CFP expressing HeLa cell is shown in Fig. 1. Minimal cross-contamination of the fluorescence and Raman signals is observed in the hyperspectral dataset, indicating that the two imaging modes are well separated with each other even though they are simultaneously acquired in the same spectrum. We have further applied the hybrid imaging setup to the study of chemical profiling of embryonic stem cells with and without Oct-4 expression, and the chemical analysis of nucleus and Golgi body directly in living HeLa cells. The details about the later experiments will be revealed in the talk.

3. Conclusions

We have successfully developed the first hybrid fluorescence-Raman imaging setup that can perform chemical profiling of living cells according to fluorescent protein expression guide. Further applications of the chemical analysis of fluorescent protein-expressing cells also demonstrated the enormous potential of the technique to help advance our understanding of the fundamentals of proteome-metabolome regulation at the cellular level.

Fig. 1 A hybrid Raman-fluorescence image of a histone labelled HeLa cell.

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References