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Mitochondria Specific Raman Microspectroscopy of Fission Yeast Cells with Simultaneous Raman/GFP Observation

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Abstract

Mitochondria are one of the crucial organelles that maintain cell living status. Many researches have been carried out on mitochondria to gain more knowledge about life. Here, we report the first mitochondria specific Raman spectra of fission yeast, *Schizosaccharomyces pombe*, using a novel bio-spectroscopic technique, “simultaneous Raman/GFP microspectroscopy”.

The GFP-labelled *S. pombe* strain was obtained by tagging Coq3 protein using pSLF272L-GFPS65A vector. Raman excitation laser (632.8 nm He-Ne laser) and GFP excitation light (filtered microscope backlight) irradiated the sample yeast cells simultaneously. GFP signal was monitored in the anti-Stokes region where interference from Raman scattering was negligibly small. A total of 13568 Raman spectra were collected from 19 yeast cells at three different points of the growth curve. Mitochondria specific Raman spectra were obtained by an MCR-ALS analysis in the Stokes region of 1389 spectra that showed strongest (top 10%) GFP intensity.

Fig. 1: Two mitochondria specific Raman spectra of *S. Pombe*.

Two mitochondria specific Raman spectra, Spectrum A and Spectrum B, have been obtained. Spectrum A is dominated by protein Raman bands and Spectrum B by lipid Raman bands, being consistent with the known molecular composition of mitochondria. In addition, Spectrum B shows a strong band of ergosterol at 1602 cm⁻¹, previously reported as “Raman spectroscopic signature of life of yeast”. We have also found that the ratio of protein to lipid changes with GFP intensity that can be regarded as an indicator of mitochondria activity.

Keywords: anti-Stokes Raman spectroscopy, mitochondria, Raman spectroscopic signature of life, *Schizosaccharomyces pombe*, simultaneous Raman/GFP microspectroscopy, yeast

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