

# IN VIVO RAMAN SPECTROSCOPY AND IMAGING OF A SINGLE LIVING FISSION YEAST CELL

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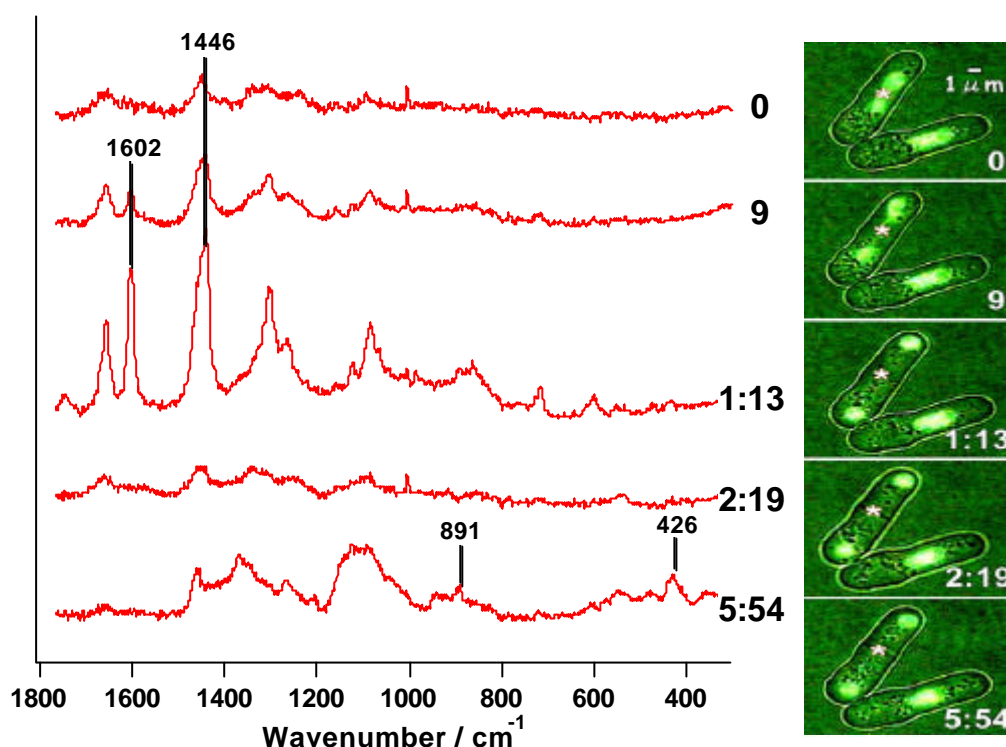
**Keywords:** time- and space-resolved Raman spectroscopy, Raman imaging, single living cell, cell cycle, fission yeast

**Abstract:** Raman microspectroscopy has been used to study the mitosis process of a dividing yeast cell at the molecular level. Several bands arising from molecular species in mitochondria and septum were used to perform Raman imaging experiment to demonstrate its potential for physicochemical studies of a single living cell.

Cell cycle is one of the most fundamental biological processes. A number of detailed molecular mechanisms involved in the cell cycle have been elucidated in recent years with the use of various biochemical approaches. However, biochemical methods are intrinsically lacking in time and space specificities. In the present study, we use Raman spectroscopy to obtain the time- and space-resolved molecular information of living fission yeast *Schizosaccharomyces pombe* (*S. pombe*).

A confocal Raman microspectrometer (*Nanofinder*<sup>®</sup>, Tokyo Instrument Inc.) coupled with a He-Ne laser (632.8nm, 3W on the sample) was used to measure the spectra of a dividing *S. pombe* cell. Spatial resolutions of 250nm in the lateral direction and 1.7 $\mu$ m in the depth direction were achieved by using a 100 $\mu$ m pinhole. They enabled the space-resolved Raman measurements of the organelles, which switch from nuclei to septum, in the centre of the dividing yeast cell. A part of the results of a cell with GFP-labelled nuclei is shown in Fig. 1. The bands observed in each spectrum agree with the known Raman bands of protein, phospholipids and carbohydrates of the irradiated organelles. Although the origin of the strong and sharp Raman band at 1602cm<sup>-1</sup> is not clear, we found that the intensity of this band is closely correlated with the metabolic activity of the cell<sup>1</sup>.

The Raman imaging measurement has been performed on a yeast cell with GFP-labelled mitochondria. In the Raman imaging measurement, the excitation laser spot scanned the yeast cell. The cell was translated by a piezoelectric stage horizontally with 0.5 $\mu$ m step in both x and y directions. Images of this cell were generated using the 2s integrated intensity of the Raman bands at each location of the laser spot. It took 20min to acquire the entire image of the cell. All images were obtained by one measurement. We show in Fig. 2 the Raman mapping images of the band at 1602cm<sup>-1</sup>(origin unclear), 1446cm<sup>-1</sup>(C-H bending in phospholipids), 895cm<sup>-1</sup> and 429cm<sup>-1</sup> (both are from  $\beta$ -Glucan) and the images observed with fluorescence microscope. The mapping result shows that Raman band 1602cm<sup>-1</sup> (Fig. 2c) gives strong signal only in the brightest regions in Fig. 2b. This result indicates that the origin of the Raman band at 1602cm<sup>-1</sup> exists in mitochondria. The bright areas in Fig. 2d also coincide with the positions of mitochondria; while from the other areas of this cell, some signals, though weaker, are also observed. This finding is consistent with the fact that mitochondria are rich in phospholipids because of their double membrane structure, while most of the other organelles are single membrane enveloped ones and therefore contain less concentrated phospholipids. There must also be contributions from the CH bending intensities of proteins. The Raman mapping results shown in the Fig.2e and f are different. Although both of the Raman band 891cm<sup>-1</sup> and 426cm<sup>-1</sup> are observed in the spectrum taken from septum, they seem to arise from different molecular species. The species that giving rise to the band 891cm<sup>-1</sup> exists in both the



septum and the cell wall. The  $426\text{cm}^{-1}$  band, on the other hand, seems to arise from some species that exist only in the septum.

Fig. 1. Time- and space-resolved Raman spectra of a living *S. Pombe* cell. The GFP images are shown at the right hand side, where the bright blobs seen inside show the GFP-labelled nuclei. The Raman spectra obtained at 0 min, 9min, 1h 13 min, 2h 19min and 5h 54 min are shown. The position of the laser spot is shown by \* in the images.

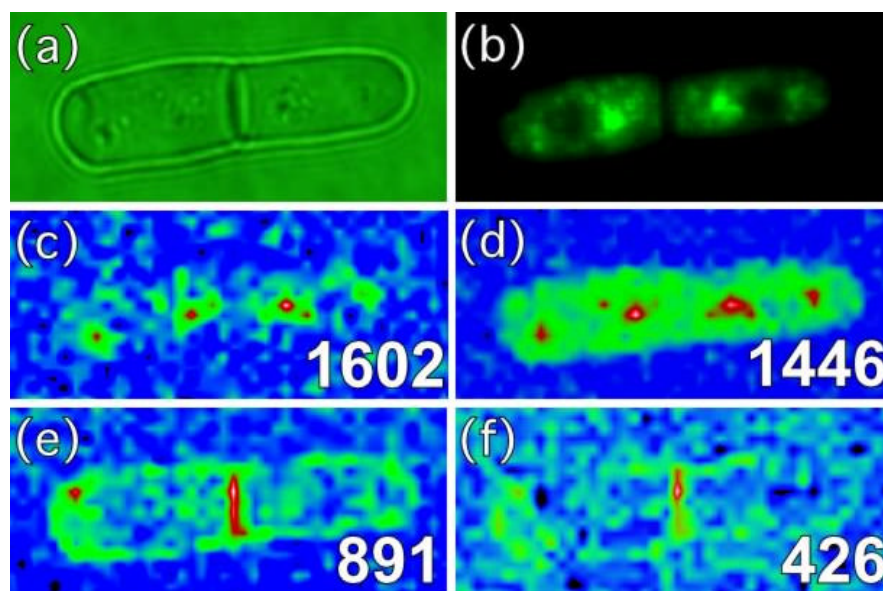


Fig. 2 The Raman mapping patterns of a living *S. Pombe* cell. The microscopic image of the cell (a), the GFP image of mitochondria (b), and the Raman mapping images of Raman bands at  $1602\text{cm}^{-1}$  (c),  $1446\text{cm}^{-1}$  (d),  $891\text{cm}^{-1}$  (e) and  $426\text{cm}^{-1}$  (f).

#### References:

1. Y.-S. Huang, T. Karashima, M. Yamamoto, T. Ogura and H. Hamaguchi, J. Raman Spectrosc., in press.