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In-vivo Time- And Space-resolved Raman Spectroscopy Of A Living Yeast Cell

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Ultrafast time-resolved Raman spectroscopy is well established as a useful tool for studying the structure and dynamics of short-lived intermediate species existing in homogeneous molecular systems like liquids and solutions [1]. Time-resolved Raman spectroscopy should also be very powerful in tracing much slower dynamics of larger inhomogeneous molecular systems like living cells, if the measurement is appropriately space-resolved (time- and space-resolved Raman spectroscopy).

In the present paper, we report our recent results of time- and space-resolved Raman spectroscopy of a single living cell of fission yeast, *Schizosaccharomyces pombe* (*S. pombe*) [2]. The experiment was carried out with the use of a confocal Raman microspectrometer (Tokyo Instruments, *Nanofinder*) with a time resolution of 100 s and a spatial resolution of 250 nm. The 632.8 nm line of a He-Ne laser was used for Raman excitation with a power of ~1 mW at the sample point. Figure 1 shows the time- and space-resolved Raman spectra of the central part of a single *S. pombe* cell. The fluorescence images obtained simultaneously under the same microscope are also shown in Figure 1. The nuclei of the yeast are labeled by green fluorescent protein (GFP) and they are seen as bright blobs in the fluorescence images. The Raman measurement starts from the early M phase (0 min). After 11min, the nuclei are separating and moving symmetrically toward the perimeter of the cell. At the G1/S transition (31min), the nuclei are completely separated into two. In the S phase (41 min and 62 min), the septum starts to be formed from the plasma membrane. When the septum formation is completed, the cell separates into two daughter cells (72min). As the cell division progresses, the Raman spectrum changes drastically reflecting the changes of molecular compositions of the organelles existing at the central part of the cell. The Raman bands observed at 0 min are ascribed to the proteins in the nucleus. It is interesting that no strong Raman bands assignable to nucleic acids are observed. The spectra at 11 and 31 min are dominated by the Raman bands of phospholipids, which are most likely to exist in the double membrane of mitochondria. The spectra at 41 and 62 min resemble that of glucan, indicating that glucan is the primary molecular component of the septum. The Raman spectrum at 72 min is quite different from those of the septum and is ascribed to the proteins in the cell wall.

In this way, the changes of the molecular composition of the central part of the yeast cell have been successfully traced by Raman spectroscopy, as the organelles existing at the central part alter from a nucleus to mitochondria and then to the septum.

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[2] Y. –S. Huang et al., J. Raman Spectrosc. 34 (2003) 1.

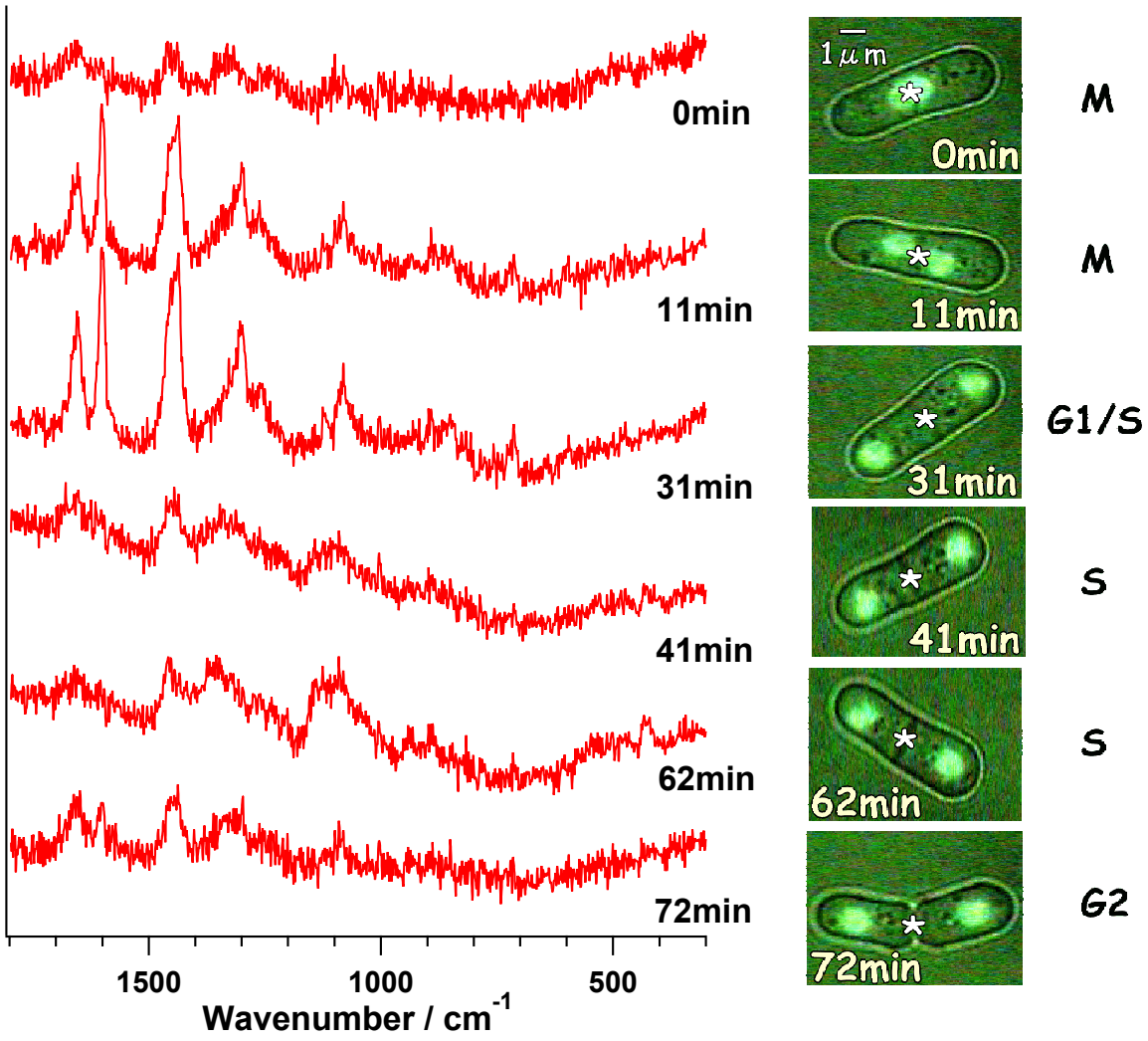

FIG. 1. Time- and space-resolved Raman spectra of a dividing *S. pombe* cell.

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