High-throughput cell imaging and classification by narrowband and low-spectral-resolution Raman microscopy

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KEY WORDS: Raman microscopy, Low spectral resolution, Narrowband, Cell classification, Cell imaging

ABSTRACT

Multiplex spectral measurement techniques have accelerated Raman microscopic imaging for live-cell analysis without using molecular labeling [1]. The imaging speed of the technique is, however, still far slower than that of fluorescence microscopic imaging. For further improvement of the speed, we developed narrowband Raman microscopy [2]. A typical Raman measurement records 1000 cm^{-1} in range or even wider, whereas the narrowband one records only 100 cm^{-1} or less and hence it allows reduction of readout time of the detector by reducing the number of detector pixels on recording a spectrum. Because the readout time and the effective signal accumulation time for recording a spectrum become comparable to each other for the multiplex measurement, reducing the readout time can improve the imaging speed.

In application of the narrowband measurement, selection of the wavenumber range is important. We selected 1397-1501 cm^{-1} for discriminating cancer and non-cancer human cell lines. In this range, lipid, protein, and nucleotide contribute to the cell spectra while noncellular components (i.e., a buffer solution and a culturing substrate) do not. Furthermore, the fluorescent background is so simple to be approximated by a linear function and is easy to be subtracted. Thanks to these benefits, with baseline-corrected cellwise spectra in this range, we discriminated two cell lines at the accuracy higher than 90%. On acquisition of a Raman image of the cells, the signal readout time and the imaging time were reduced by 24 and 5 folds respectively in comparison to those for a correspondent, 536-3132 cm^{-1} wideband measurement.

To further improve the imaging speed, we employed detector binning. This approach, namely low-spectral-resolution approach, allowed reduction not just of the readout time but also of the signal accumulation time with maintaining signal-to-noise ratio. Hence, improvement of the imaging speed by the narrowband, low-spectral-resolution approach reached at 21 folds, in comparison to a correspondent wideband, high-spectral-resolution one. With the spectra obtained, two cell lines were discriminated at the accuracy higher than 90%.

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