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学 位 論 文 要 旨

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題目: Raman spectroscopic studies coupled with MCR-ALS applied on some biomedical systems

（MCR-ALS法を組み合わせたラマン分光法による医・生物学的応用研究）

Raman effect is one of light scattering effects which was reported first by C. V. Raman in 1928. When a light with a particular wavelength such as laser light is irradiated on to a substance, several processes can happen (absorption, scattering etc.). Raman scattering is an inelastic scattering process in which the irradiated light either loses or gains energy as a result of interaction with the molecule. Since Raman spectrum conserve unique molecular information called 'molecular finger print', Raman spectroscopy (RS) has been gaining attention as a valuable molecular characterization tool especially in the context of biological and medical research. Label-free molecular imaging and low or non-invasiveness are some of the major beneficial points. However, there are various vibrational modes even in a single molecule and each mode has a unique Raman signature. To further complicate the matter, there are numerous molecules in any given biomolecular system (cells, tissues etc.). So each Raman spectrum contains signatures from a variety of biomolecules. Mainly, contributions from unexpected molecules pose a big challenge for spectral interpretation. Therefore, application of classical univariate analytical methods to Raman spectral data obtained biological and medical systems are insufficient. Researchers often employ multivariate analysis such as principal components analysis (PCA). Though useful in some cases, obtained spectral information does not contain pure molecular information and lacks physical meaning. In order to overcome this problem, in addition to PCA, I employ multivariate curve resolution – alternating least square (MCR-ALS) which is also a multivariate analytical technique. In MCR-ALS analysis, a matrix approximation is sought by a linear combination of desired number of spectral components and its corresponding abundances. The extracted spectral components represent pure molecules while the abundances can be thought of their relative concentrations. To study complex systems such as biological and medical samples, the usefulness of MCR-ALS is reported in several model studies whereas the number of practical applications are still very few. It is probably the reason why deep consideration in the context of both biology and physics is still needed to understand results of MCR-ALS. Hence, this thesis consists of two practical applications of MCR-ALS to describe its usefulness and how to process entire analysis in biological and medical field.

1) Visualizing wax ester fermentation in single *Euglena gracilis* cells by Raman microspectroscopy and multivariate curve resolution analysis

Global demand for energy is on the rise at a time when limited natural resources are fast depleting. To address this issue, microalgal biofuels are being recommended as a renewable and eco-friendly substitute for fossil fuels. *Euglena gracilis* is one such candidate that has received special interest due to their ability to synthesize wax esters that serve as precursors for production of drop-in jet fuel. However, to realize economic viability and achieve industrial-scale production, development of novel methods to characterize algal cells, evaluate its culture conditions, and construct appropriate genetically modified strains is necessary. Here, we report a Raman microspectroscopy-based visualization method to visualize important metabolites such as paramylon

and ester during wax ester fermentation in single *Euglena gracilis* cells in a label-free manner.

We measured Raman spectra to obtain intracellular biomolecular information in *Euglena* under anaerobic condition during which wax esters are synthesized and stored viz wax ester fermentation from paramylon. First, by univariate approach, we identified Raman markers corresponding to paramylon/esters and constructed their time-lapse chemical images. However, univariate analysis is severely limited in its ability to obtain detailed information as several molecules can contribute to a Raman band. Therefore, we further employed MCR-ALS to obtain chain length-specific information and their abundance images of the produced esters. Accumulated esters in *Euglena* were particularly identified to be myristyl myristate (C28), a wax ester candidate suitable to prepare drop-in jet fuel. Interestingly, we found accumulation of two different forms of myristyl myristate, one of which does not show univariate Raman marker. As a method of studying wax ester fermentation, our exploratory MCR-ALS is powerful to make the most of Raman hyperspectral data.

2) Identification of Molecular Basis for Objective Discrimination of Breast Cancer Cells (MCF-7) from Normal Human Mammary Epithelial Cells by Raman Microspectroscopy and Multivariate Curve Resolution Analysis

RS which is a non-invasive and label-free method has been suggested to improve accuracy of cytological and even histopathological diagnosis. To our knowledge, this novel technique tends to be employed without concrete knowledge of molecular changes in cells. Therefore, identification of Raman spectral markers for objective diagnosis is necessary for universal adoption of RS. As a model study, we investigated human mammary epithelial cells (HMEpC) and breast cancer cells (MCF-7) by RS and employed classical univariate analysis, various multivariate analyses (MA) including principal components analysis (PCA), linear discriminant analysis (LDA), and support vector machine (SVM) to estimate diagnostic accuracy. Furthermore, to elucidate the underlying molecular changes in cancer cells, we utilized MCR-ALS with non-negative constraints to extract physically meaningful spectra from complex cellular data. Unsupervised PCA and supervised MA, such as LDA and SVM, classified HMEpC and MCF-7 fairly well with high accuracy but without revealing molecular basis. Employing MCR-ALS analysis we identified five pure biomolecular spectra comprising DNA, proteins and three independent unsaturated lipid components. Relative abundance of one lipid component seems to be strictly regulated between the two groups of cells and could be the basis for excellent discrimination by chemometrics-assisted RS. The lipid component was unambiguously assigned to linoleate rich glyceride with comparison of pure standard lipids. Although both cell lines used in this study are of epithelial source, it is important to understand that most tumors are like organs and have more than one type of cell. Therefore, while the model holds true to this breast cancer cell line with a specific excitation at 633 nm in our measurement system, it is imperative that we further test on large numbers of other cell lines and with different excitations wavelengths as well to have general consensus. This study successfully identified Raman spectral markers and demonstrated the potential of RS to become an excellent cytodiagnostic tool that can both accurately and objectively discriminates breast cancer from normal cells.

These results from two practical studies successfully show that Raman spectroscopy coupled with MCR-ALS is strong analytical tool to extract biomolecular information for both exploratory and diagnostic purposes. Since results of this technique depends on initial parameters and is not a unique solution, it presently needs deep consideration for exploratory analysis, this problem can be addressed by combining a large standard Raman spectral library and user-friendly interface to extract pure spectra with various initial parameters and check whether extracted result is physically meaningful. In the future, I believe Raman spectroscopy coupled with MCR-ALS technique probably endows an opportunity to researchers in biological and medical fields for easy reading of "molecular finger print" as it is without needing expertise in spectroscopy.