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Fast reconstruction of Raman spectra from narrow-band measurements based on Wiener estimation

Shuo Chena, Yi Hong Ongb, Quan Liu*a
a Division of Bioengineering, School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore 637457
*Corresponding author: quanliu@ntu.edu.sg

ABSTRACT

Raman spectroscopy has demonstrated great potential in the study of biological molecules in a variety of biomedical applications. But slow data acquisition due to weak Raman signals from these molecules has prevented its wide use especially in an imaging setup. We propose a novel method to reconstruct the entire Raman spectrum from a few narrow-band measurements based on Wiener estimation. This method has been tested on Raman spectra from individual cells and shown fast speed and excellent accuracy. This method represents a new direction to speed up Raman data acquisition in an imaging setup to investigate fast changing phenomena.

Keywords: Raman spectroscopy, spectral reconstruction, compressive sensing, Wiener estimation, narrow-band imaging

1. INTRODUCTION

Raman spectroscopy, a vibrational spectroscopic technique, provides details about the chemical composition and structure of molecules within tissues and cells[1, 2]. Because of its non-invasive characteristics, Raman spectroscopy has become a powerful tool in the field of biomedical optics, particularly as a diagnostic tool for various diseases recently[3, 4]. However, those applications have been limited not only by weak Raman signals in tissue samples but also relatively slow acquisition[5]. Several alternative methods have been developed to overcome this problem. Huang et al. [6] developed a rapid near-infrared Raman spectroscopy system, which achieved a real-time in vivo skin measurement. However, the data acquisition speed is still slow when Raman spectra measured from multiple locations are acquired because only point-by-point measurement can be performed with fiber-optic probe. Another alternative way is to acquire Raman data corresponding to only a few Raman peaks. However, it often leads to inaccurate analysis because of insufficient information and Raman bands shared by multiple biochemical components [7]. The entire Raman spectral with adequate spectral resolution is important for implementing diagnosis based on Raman spectroscopy. Therefore, it is critical to develop a cost-effective and fast Raman acquisition system without sacrificing the spectral resolution.

Reconstruction of optical spectra from a few narrow-band measurements is one potential method to solve this problem, because of fast acquisition for a small number of narrow bands. Quantitative studies have carried out previously to recover reflectance spectra from a few narrow band measurements, e.g. pseudo-inverse[8], finite-dimensional modeling[9], Wiener estimation[10] etc. Wiener estimation is one of the most frequently used method because of fast data processing[11]. Several methods based on Wiener estimation, e.g. adaptive Wiener estimation[11] and modified Wiener estimation[12], have been developed for improving the reconstruction accuracy.

In this paper, we developed a fast method for the reconstruction of Raman spectra based on Wiener estimation. Measurements in a few narrow bands instead of the entire Raman spectra were performed, which enables fast acquisition. We evaluated the feasibility of Raman reconstruction on cell spectra using both traditional Wiener estimation and modified Wiener estimation, for four different sets of filter and two different numbers of filters. According to simulated results, reconstructed spectra are very close to original spectra, which demonstrates the feasibility of this approach.
2. MATERIALS AND METHODS

Two estimation methods are used to reconstruct Raman spectra in this paper for comparison, i.e. traditional Wiener estimation and modified Wiener estimation. Both methods involve two steps: the calibration step and the test step. In the calibration step, both Raman spectra and simulated narrow-band measurements are needed in order to create a Wiener matrix to transform narrow-band measurements to Raman spectra. In the test step, only narrow-band measurements are needed.

2.1 Sample preparation and measurement

Raman spectra used in this study were measured from human chronic myelogenous leukemia cells (K562 cell line), which were purchased from American Type Culture Collection (Manassas, VA, US). More details about sample preparation have been described elsewhere [7]. All spectra were acquired by a micro-Raman system (inVia, Renishaw, UK) coupled to a microscope (Alpha 300, WITec, Germany) in a backscattering geometry, in which the spectral resolution is 2 cm⁻¹. Thirty cell spectra were measured over a range of 600 cm⁻¹ to 1800 cm⁻¹ with an excitation wavelength of 785 nm generated by a diode laser and. A pre-processing was performed to remove fluorescence background. First, fluorescence background was estimated by using the fifth order polynomial fitting[13, 14] for each spectrum and subtracted from the original spectrum. Then, each spectrum was smoothed by Savitzky-Golay smoothing algorithm and normalized by dividing each data point by the maximum intensity in each spectrum.

2.2 Traditional Wiener estimation

As the original spectrum information S is a \( p \times n \) matrix, in which p means the number of variables and n means the number of the observations. When sampled with m filters F (\( m \times p \) matrix), simulated narrow-band measurements C (\( m \times n \) matrix) becomes

\[
C = FS
\]

Wiener estimation is to find a Wiener matrix W (\( p \times m \) matrix), which is used to transform narrow-band measurements C (\( m \times n \) matrix) into the corresponding Raman spectra R (\( p \times n \) matrix),

\[
R = WC
\]

so that the mean square error between the original and reconstructed spectra is minimized. In Wiener estimation, the transform matrix W is

\[
W = K_p F^T (F K_p F^T + K_n)^{-1}
\]

where \( K_p \) and \( K_n \) are the autocorrelation matrices of the spectra and noise, respectively.

2.3 Modified Wiener estimation

The modified Wiener estimation developed previously by our group [12], based on the traditional Wiener estimation, provides additional information by synthesizing new narrow-band measurements in the calibration step. The modified Wiener estimation consists of two stages and one postprocessing step, i.e. the calibration stage, the test stage and selection step, which are briefly reiterated below.

In the calibration stage, the relations for narrow-band correction, which are the second order polynomial relation and color difference relation, are established. The modified Wiener matrix, which is created by the combination of the synthesized and original narrow-band measurements, is computed for the reconstruction of Raman spectra in the test stage.

In the test stage, new narrow-band measurements are synthesized and corrected by the relations for narrow-band correction obtained in the calibration stage. Then, the corrected new narrow-band measurements and the original narrow-band measurements can be jointly used to reconstruct Raman spectra accurately by applying the modified Wiener matrix.

Because two relations for narrow-band correction yield different estimated Raman spectra, a simple selection step is used to find the more accurate result out of two. The two sets of reconstructed Raman spectra are multiplied by the original system matrix to find the estimated narrow-band measurements. The reconstructed Raman spectrum that yields narrow-band measurements closer to the original values will be selected as the final result.
3. RESULTS AND DISCUSSIONS

A total of 30 cell spectra from each group, i.e. control group, apoptosis group and necrosis group, were measured and pre-processed to remove fluorescence background. Narrow-band measurements were simulated by multiplying each pre-processed spectra by the transmission curve of the corresponding filters. A leave-one-out method was used to test the proposed method[15]. In this method, only one spectrum was picked as the test sample each time and the rest of data points were used as the calibration data set. The procedure was repeated until all the data points were tested. All the data processing were coded and run in Matlab (Version 7.6, MathWorks, Natick, MA, US).

In this paper, the parameters that are investigated include 1) the reconstruction algorithm, which involves traditional Wiener estimation and modified Wiener estimation; 2) different set of filters, which involves commercial filters, Gaussian filters and filters synthesized from principle component analysis (PCA); 3) the number of filters, either three or six. The accuracy of reconstruction is assessed by the mean root mean square error (RMSE) between the original spectra and reconstructed spectra.

Fig. 1 shows the original Raman spectrum without fluorescence background, the Raman spectrum reconstructed by the traditional Wiener estimation and the Raman spectrum reconstructed by the modified Wiener estimation in the cases of the best estimation, typical estimation and worst estimation. The results from the traditional Wiener estimation are also shown for comparison. Compared to the traditional Wiener estimation, the modified Wiener estimation method reduces the mean RMSE by 3.6%. According to the Table 1 and Fig. 1, the reconstructed Raman spectra are in general quite close to the original Raman spectra and the modified Wiener estimation has a better performance and higher accuracy.
Table 1 Comparison of the mean RMSE between the traditional Wiener estimation and modified Wiener estimation

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<th>Traditional Wiener estimation</th>
<th>Modified Wiener estimation</th>
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<td>Mean RMSE</td>
<td>$2.81 \times 10^{-2}$</td>
<td>$2.71 \times 10^{-2}$</td>
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</table>

Table 2 shows the comparison of the different filter sets, including Gaussian filters, commercial filters, PCA based filters and non-negative PCA based filters, in which the traditional Wiener estimation was used for all filter sets. All filter sets used in the comparison have three filters. Gaussian filters are determined by Gaussian functions with different center wavelength, i.e. 845nm, 870 nm and 895nm, and a standard deviation of 15 nm. Commercial filters are laser line filter (SKU: XL19, Omega Optical), BrightLine® single-band bandpass filter (FF01-857/30-25, Semrock) and laser line filter (FL905-10, Thorlabs). The transmission spectra of PCA based filters are equivalent to the first three PCs of the original Raman spectra without fluorescence background. The transmission spectra of non-negative PCA based filters are obtained in the same way using a published method [16]. According to Table 2, PCA based filters and non-negative PCA based filters shows the best result. This could be attributed that PCA based filters and non-negative PCA based filters captured most variance in the original spectra by its definition.

Table 2 Comparison in the mean RMSE among four filter sets obtained in the traditional Wiener estimation

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<th>Filter Sets</th>
<th>Mean RMSE</th>
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<td>Gaussian filters</td>
<td>$2.81 \times 10^{-2}$</td>
</tr>
<tr>
<td>Commercial filters</td>
<td>$2.62 \times 10^{-2}$</td>
</tr>
<tr>
<td>PCA based filters</td>
<td>$2.47 \times 10^{-2}$</td>
</tr>
<tr>
<td>Non-negative PCA based filters</td>
<td>$2.47 \times 10^{-2}$</td>
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The comparison in reconstructed Raman spectra between three and six Gaussian filters is made in Fig. 2, in which six Gaussian filters have center wavelengths of 835 nm, 850 nm, 865 nm, 880 nm, 895 nm and 910 nm, respectively, and a standard deviation 10 nm. The difference is visible at some wavenumbers but quite small. In Table 3, it can be seen that the use of six Gaussian filters reduces the mean RMSE by 7.1% because of more information provided by additional filters.

Table 3 Comparison in the mean RMSE between three and six Gaussian filters obtained in the traditional Wiener estimation

<table>
<thead>
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<th>Mean RMSE</th>
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<tbody>
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<td>3 Gaussian filters</td>
<td>$2.81 \times 10^{-2}$</td>
</tr>
<tr>
<td>6 Gaussian filters</td>
<td>$2.61 \times 10^{-2}$</td>
</tr>
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In conclusion, we have developed a fast method for the reconstruction of Raman spectra using Wiener estimation. A few narrow-band measurements instead of the entire Raman spectrum are needed, which enable fast data acquisition. According to simulation results, reconstructed spectra are very close to measured spectra. Therefore, this method represents a new direction to speed up Raman data acquisition in an imaging setup to investigate fast changing phenomena.

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REFERENCE


