Optimization of advanced Wiener estimation methods for Raman reconstruction from narrow-band measurements in the presence of fluorescence background

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Abstract: Raman spectroscopy has shown great potential in biomedical applications. However, intrinsically weak Raman signals cause slow data acquisition especially in Raman imaging. This problem can be overcome by narrow-band Raman imaging followed by spectral reconstruction. Our previous study has shown that Raman spectra free of fluorescence background can be reconstructed from narrow-band Raman measurements using traditional Wiener estimation. However, fluorescence-free Raman spectra are only available from those sophisticated Raman setups capable of fluorescence suppression. The reconstruction of Raman spectra with fluorescence background from narrow-band measurements is much more challenging due to the significant variation in fluorescence background. In this study, two advanced Wiener estimation methods, i.e. modified Wiener estimation and sequential weighted Wiener estimation, were optimized to achieve this goal. Both spontaneous Raman spectra and surface enhanced Raman spectra were evaluated. Compared with traditional Wiener estimation, two advanced methods showed significant improvement in the reconstruction of spontaneous Raman spectra. However, traditional Wiener estimation can work as effectively as the advanced methods for SERS spectra but much faster. The wise selection of these methods would enable accurate Raman reconstruction in a simple Raman setup without the function of fluorescence suppression for fast Raman imaging.

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acquisition speed is improved significantly, this method causes field-curvature artifacts and coupled device (CCD). While spatial information is acquired along the laser line, the spectral phenomena especially in biological samples.

Another approach for Raman imaging is based on acousto-optic tunable filter (AOTF) [7] or its actual speed is limited by the requirement of autofocusing prior to data acquisition [6].

Raman spectroscopy has been widely used in biomedical and clinical applications [2, 3]. However, due to inherently weak Raman signals, Raman data acquisition is generally slow, which prohibits Raman spectroscopic imaging from being used to investigate fast changing phenomena especially in biological samples.

Several Raman imaging techniques have been developed to overcome this limitation. Line scanned Raman imaging [4, 5] collects both spatial and spectral information along a line simultaneously. Laser light is shaped into a line using cylindrical optics or a scanning mechanism and Raman spectra are collected by a two-dimensional detector array, i.e. charge-coupled device (CCD). While spatial information is acquired along the laser line, the spectral information is collected in the dimension perpendicular to the laser line. Although the data acquisition speed is improved significantly, this method causes field-curvature artifacts and its actual speed is limited by the requirement of autofocusing prior to data acquisition [6].

Another approach for Raman imaging is based on acousto-optic tunable filter (AOTF) [7] or...
on liquid crystal tunable filter (LCTF) [8]. This approach benefits from the capability of transmitting a selectable wavelength of light using tunable filters. However, the disadvantage of this method is long data acquisition when the required spectral resolution is high. Fiber array Raman imaging [9, 10] is also a technique which could speed up Raman acquisition significantly. Both spatial and spectral information can be collected at the same time with a fiber array by rearranging two-dimensional optical fibers array at the sample end to one-dimensional array on the detector end. However, the number of pixels in the fiber array is limited by the amount of fibers that could be mapped onto the CCD and the spatial resolution is limited by the fiber size.

Reconstruction of Raman signal from a few narrow-band measurements at each pixel is a potential way to realize fast Raman spectroscopic imaging. Only a few narrow-band Raman images would be required and the full Raman spectrum at each pixel can be reconstructed. Due to the small number of images required, the traditional spectral imaging setup, i.e. using multiple filters in front of a CCD, would work well and high spatial resolution and high spectral resolution could be achieved at the same time. Data acquisition would be much faster than most current Raman imaging setups based on laser scanning. In our previous study [11], we have successfully reconstructed Raman spectra from multiple narrow-band measurements of the spectra assuming zero fluorescence background in the measurements. However, fluorescence background always exists and its magnitude is often significant compared to the Raman signal unless sophisticated techniques, such as shifted excitation Raman difference spectroscopy [12], Fourier transformed Raman spectroscopy [13] or temporal gating [14], are employed to suppress fluorescence. Therefore, it is important to develop a simple and practical Raman spectroscopic imaging technique that works in the presence of fluorescence background without sacrificing reconstruction accuracy. Compared with our previous study, the reconstruction of Raman spectra in the presence of fluorescence background from narrow-band measurements is much more challenging due to the fact that significant variation in fluorescence background degrades the reconstruction accuracy, especially in biological samples.

To address this challenge, two advanced Wiener estimation (WE) methods, i.e. modified Wiener estimation [15] and sequential weighted Wiener estimation [16], previously developed by our group were optimized to facilitate Raman reconstruction in the presence of fluorescence background. Traditional Wiener estimation was also evaluated for the purpose of comparison. The optimal value of the key parameter in each advanced method, including the number of synthetic filters in modified WE and the number of iterations in sequential weighted WE, were identified. The reconstruction methods were evaluated on both spontaneous Raman data and SERS data, in which the former data represented the case of high fluorescence background while the latter data represented the case of low fluorescence background. Our results show the high feasibility of accurate reconstruction of Raman spectra using the advanced Wiener estimation methods even when significant fluorescence background is present. Thus the methods described in this paper will be applicable to a simple Raman setup without the function of fluorescence suppression for fast Raman imaging in most Raman spectroscopy based applications.

2. Materials and methods

2.1 Measurements of Raman Spectra

Spontaneous Raman data were collected from live, apoptotic and necrotic leukemia cells using a micro-Raman system (inVia, Renishaw, UK) coupled to a microscope (Alpha 300, WITec, Germany) in a backscattering setup. Ten Raman spectra from each group were collected over a range from 600 to 1800 cm\(^{-1}\). The excitation wavelength was 785 nm and the spectral resolution was 2 cm\(^{-1}\). The purpose for collecting spectra of live, necrotic and apoptotic cells was to reproduce the variance commonly seen in Raman spectra measured from cells in various conditions.
Surface enhanced Raman spectroscopy (SERS) data were measured from blood serum samples collected from 50 patients with nasopharyngeal cancer in Fujian Tumor hospital, Fuzhou, Fujian Province, China. Blood serum samples were obtained by centrifugation at 2,000 rpm for 15 minutes in order to remove blood cells and then mixed with silver colloidal nanoparticles at a size of 34 nm. The mixture was incubated for two hours at 4°C before measurements. A confocal Raman micro-spectrometer (inVia, Renishaw, UK) with 20 × objective was used to measure Raman spectra over a range from 600 to 1800 cm\(^{-1}\) (2 cm\(^{-1}\) spectral resolution) from human blood serum. The excitation wavelength was 785 nm and the integration time was 10s. The details of sample preparation have been described elsewhere [17, 18].

2.2 Simulation of narrow-band measurements and methods of reconstruction and evaluation

The simulation of narrow-band measurements and methods of reconstruction and evaluation were similar to those in the previous studies, which are briefly reiterated below. Since a filter is fully characterized by its transmission spectrum, it is reasonable to expect that the result shown here faithfully mimics the real situation in which Raman spectra are acquired by using these filters. The narrow-band measurement \(c\) was simulated from the measured Raman spectra and the transmittance spectra of the selected narrow-band filters according to Eq. (1).

\[
c = Fs
\]

where \(s\) (\(m \times 1\) matrix, in which \(m\) is the number of wavenumbers) is the Raman spectrum with fluorescence background, \(F\) (\(n \times m\) matrix, in which \(n\) is the number of filters) represents the transmission spectra of the filters.

![Fig. 1. General procedure for Wiener estimation.](image)

Wiener estimation was used to reconstruct Raman spectra from simulated narrow-band measurements, which was performed in two stages as shown in Fig. 1. In the calibration stage, Wiener matrix was constructed, which relates narrow-band measurements to the original Raman spectra measured from samples in the calibration set. In the test stage, Wiener matrix was applied to narrow-band measurements from an unknown sample to reconstruct its Raman spectrum. The Wiener matrix [19] \(W\) is defined in Eq. (2), in which the noise term is ignored.

\[
W = E(xc^T)[E(cc^T)]^{-1}
\]

where \(E(\cdot)\) denotes the ensemble average, the superscript “\(T\)” denotes matrix transpose and the superscript “\(^{-1}\)” denotes matrix inverse.

Modified Wiener estimation, which is based on traditional Wiener estimation, improves reconstruction accuracy by synthesizing new narrow-band measurements with an additional set of filters. In the calibration stage, the modified Wiener matrix was computed by the combination of original narrow-band measurements and the synthesized narrow-band
measurements. In addition, two strategies were used to find the correction relations for synthesizing new narrow-band measurements. In the test stage, new narrow-band measurements were synthesized and corrected by the correction relations obtained in the calibration stage. The modified Wiener matrix was then applied and Raman spectra were reconstructed accurately because the new synthesized narrow-band measurements can provide additional information. A final selection step was needed to select a better one from the results of reconstructed generated using two correction relations. More details about modified Wiener estimation have been described elsewhere [15].

Sequential weighted Wiener estimation, which is also based on traditional Wiener estimation, improves reconstruction accuracy by optimizing the calibration data set. The ensemble average in Eq. (2) is replaced by the weighted average as shown in Eq. (3).

\[ W = \sum_{i=1}^{n} w_i s_i e_i^T \sum_{j=1}^{n} (w_j e_j e_j^T)^{-1} \]  

where \( w_i \) or \( w_j \) is the weights for the \( i \)-th or \( j \)-th set of calibration data. This weight is calculated according to the following equation:

\[ w_i = \frac{d_i^{-1}}{\sum_{i=1}^{n} d_i^{-1}} \]  

where \( d_i \) is the difference between the Raman spectrum estimated from the test data (after the removal of fluorescence background) and the Raman spectrum in the \( i \)-th set of calibration data (after the removal of fluorescence background). More details about sequential weighted Wiener estimation have been described elsewhere [16].

In order to evaluate the accuracy of a reconstructed Raman spectrum, the reconstructed Raman spectrum was first preprocessed to remove fluorescence background by using the fifth-order polynomial fitting [20]. Then the relative root mean square error (RMSE) of the reconstructed Raman spectrum after the removal of fluorescence background, relative to the measured Raman spectrum in which fluorescence background was also removed in the same manner, was computed as in Eq. (5).

\[ \text{Relative RMSE} = \left( \frac{\sum_{i=1}^{N} [R_i(\lambda_i) - R_m(\lambda_i)]^2}{N \times \max[R_i(\lambda_i)^2]} \right)^{1/2} \]  

where \( R_i \) and \( R_m \) are the reconstructed Raman spectrum and the measured Raman spectrum (both after fluorescence background removed), respectively, \( \lambda_i \) is the \( i \)-th wavenumber (\( i \) is varied from 1 to \( N \)) and the function, \( \max[\] \), returns the maximum intensity of the input spectrum.

2.3 Filter optimization

2.3.1 Filters

Four different categories of filters were examined, which include commercial filters, Gaussian filters, PCs based filters and non-negative PCs based filters. Each category was introduced individually as follows.

A total of 37 commercial filters from five manufacturers were investigated as shown in Table 1. The transmittance spectra of these filters (not shown due to limited space) at least partially overlap with the range of 600 to 1800 cm\(^{-1}\) at an excitation wavelength of 785 nm.

A collection of 72 Gaussian filters were synthesized numerically in this study. A Gaussian filter was defined as
$G(\lambda) = \exp\left(\frac{-(\lambda - \mu)^2}{2\sigma^2}\right)$

where $G(\lambda)$ denotes the transmittance at the wavelength $\lambda$, $\mu$ represents the central wavelength and $\sigma$ represents the standard deviation. The central wavelength was varied over a range from 830 nm to 910 nm and the increment was 10 nm. The standard deviation was varied over a range from 2.5 nm to 20 nm and the increment was 2.5 nm.

| Manufacturer (Manufacturer) | Product numbers of commercial filters |
|----------------------------|-------------------------------------|
| Chroma Technique (Bellows Falls, VT, US) | D850/20m, D850/40m |
| Edmund Optics (Barrington, NJ, US) | NT 84-790, NT 84-791 |
| Omega Filters (Brattleboro, VT, US) | 3RD850LP, 3RD900LP, XB 142, XB 143, XB 146, XB 149, XF 3308, XL 19, XL 40, XLK 18, XLK 20 |
| Semrock (Rochester, NY, US) | FF 01-830/2-25, FF 01-832/37-25, FF 01-835/70-25, FF 01-840/12-25, FF 01-857/30-25, FF 01-910/5-25 |
| Thorlabs (Newton, NJ, US) | FB 830-10, FB 840-10, FB 850-10, FB 850-40, FB 860-10, FB 870-10, FB 880-10, FB 880-40, FB 890-10, FB 900-40, FB 910-10, FL 830-10, FL 850-10, FL 880-10, FL 905-10, FL 905-25 |

Both PCs based filters and non-negative PCs based filters were derived using the principle component analysis (PCA) method. For example, the transmittance spectrum of the i-th PC based filter was equivalent in shape to the i-th loading vector obtained from the PCA of the Raman spectra with fluorescence background in the calibration data set. The transmittance spectra of non-negative PCs based filters were generated using the same method as in the published paper [21].

2.3.2 Genetic algorithm

Genetic algorithm is usually used to generate useful solutions for optimization and search problems [22], which is based on the evolution, i.e. the survival of the fittest strategy [23]. In this study, the genetic algorithm was used to find the optimal combination of Gaussian filters and that of commercial filters to achieve a minimal relative RMSE in reconstructed Raman spectra. The optimization methodology proceeded in the following manner. Firstly, a population of filter combination was initialized randomly. Secondly, Wiener estimation was applied to reconstruct Raman spectra and the mean accuracy of the reconstructed Raman spectra was evaluated. Thirdly, a new population of filter combination was generated according to the mean accuracy of the reconstructed Raman spectra, in which the filter combination yielding higher reconstruction accuracy is more likely to become the parent for the generation of the new population. The crossover rate was 0.9 and the mutation rate was 0.1. The second and third steps were repeated iteratively until an optimized combination of filters was found. The optimization method was coded and run in Matlab (MATLAB R2010b, MathWorks, Natick, MA, US).

2.4 Leave-one-out method

The leave-one-out method [24] was used for cross-validation in our study to fully utilize each sample in an unbiased manner. The measurement from one sample was used as the test data each time and the measurements from the rest of samples were used as the calibration data set. This procedure was repeated until the measurement from every sample has been tested once. For Gaussian and commercial filters, a new set of optimal filters and Wiener matrix were generated from the calibration data set by the genetic algorithm in each round of the cross-validation and then applied to the test data. For PCs based filters and non-negative PCs based filters, the filters were fixed, thus it was not necessary to find optimal filters. Only the Wiener matrix was generated from the calibration data set in each round and then applied to the test data.
3. Results

The original spontaneous and SERS Raman spectra with fluorescence background are shown in Fig. 2. Compared with SERS spectra from blood serum sample in Fig. 2(b), the spontaneous Raman data from leukemia cells in Fig. 2(a) show higher variance in fluorescence background, which will affect the accuracy of reconstructed spectra as shown next.

![Fig. 2](image)

**Fig. 2.** Raw measured (a) spontaneous Raman spectra and (b) SERS spectra both with fluorescence background.

Table 2 shows the cumulative contribution ratio [25] of different PC numbers for spontaneous Raman spectra and SERS spectra. The cumulative contribution ratio refers to the ratio of the sum of eigenvalues corresponding to PCs of interest to the sum of all eigenvalues. By using up to six filters, a high percentage of 99.99% is reached in both sets of Raman spectra. Therefore, we test the filter number from three to six, which should be sufficient for Raman reconstruction with high accuracy.

| PC number | Spontaneous Raman spectra (%) | SERS spectra (%) |
|-----------|-----------------------------|-----------------|
| 2         | 99.69                       | 99.96           |
| 3         | 99.89                       | 99.98           |
| 4         | 99.95                       | 99.99           |
| 5         | 99.99                       | 99.99           |
| 6         | 99.99                       | 99.99           |
| 7         | 99.99                       | 100.00          |

Traditional Wiener estimation was investigated to reconstruct Raman spectra with high spectral resolution from the narrow-band Raman measurements with fluorescence background and the results are shown in Tables 3-4 and Fig. 3–6. In addition, the methods of modified Wiener estimation and sequential weighted Wiener estimation developed previously by our group were compared to traditional Wiener estimation for both spontaneous Raman spectra and SERS spectra as shown in Tables 5-8. The optimal values for the key parameter in each advanced method, including the number of synthetic filters in modified WE and the number of iterations in sequential weighted WE, were identified.

3.1 Traditional Wiener estimation

Table 3 shows the comparison in the mean relative RMSE of spontaneous Raman spectra (after fluorescence background removed) reconstructed from narrow-band measurements using different types and numbers of filters. The percentage values of reduction in the mean relative RMSE from three to four filters were 12.5%, 2.1%, 14.6% and 14.6% for commercial filters, Gaussian filters, PCs based filters and non-negative PCs based filters, respectively. The percentage values of reduction from four to five filters were 18.3%, 13.8%, 42.1% and
42.1% and the reduction from five to six filters were 13.0%, 19.0%, 17.7% and 17.7%, respectively. Figure 3 shows the comparison between the measured spontaneous Raman spectra and the spontaneous Raman spectra reconstructed by traditional Wiener estimation as well as the transmittance spectra of the best combination of six commercial filters corresponding to the typical case, i.e. FB 860-10, NT 84-791, FB 900-40, FF 01-857, XLK 20 and XB 143. Note that fluorescence background has been removed in both sets of spectra to facilitate comparison in Raman features. The typical case was the reconstructed spontaneous Raman spectrum with a relative RMSE close to the mean relative RMSE, while the best case and worst case were the reconstructed spontaneous Raman spectra with the minimum relative RMSE and maximum relative RMSE. The relative RMSEs were $1.57 \times 10^{-2}$, $3.29 \times 10^{-2}$, $6.69 \times 10^{-2}$ in the best case, the typical case and the worst case, respectively. Figure 4 shows the comparison between the measured spontaneous Raman spectra and the spontaneous Raman spectra reconstructed by traditional Wiener estimation with the first six non-negative PCs based filters. The relative RMSEs were $1.16 \times 10^{-2}$, $2.79 \times 10^{-2}$, $6.26 \times 10^{-2}$ in the best case, the typical case and the worst case, respectively.

### Table 3. Comparison in the mean relative RMSE of spontaneous Raman spectra (after fluorescence background removed) reconstructed from narrow-band measurements using different types and numbers of filters

| Commercial filters | Gaussian filters | PCs based filters | Non-negative PCs based filters |
|--------------------|-----------------|------------------|-------------------------------|
| 3 filters          | $5.61 \times 10^{-2}$ | $5.18 \times 10^{-2}$ | $7.10 \times 10^{-2}$ |
| 4 filters          | $4.91 \times 10^{-2}$ | $5.07 \times 10^{-2}$ | $6.06 \times 10^{-2}$ |
| 5 filters          | $4.01 \times 10^{-2}$ | $4.37 \times 10^{-2}$ | $3.51 \times 10^{-2}$ |
| 6 filters          | $3.49 \times 10^{-2}$ | $3.54 \times 10^{-2}$ | $2.89 \times 10^{-2}$ |

Fig. 3. Comparison between the measured spontaneous Raman spectrum and the spontaneous Raman spectrum reconstructed by traditional Wiener estimation with the best combination of six commercial filters in (a) the best case, (b) the typical case, (c) the worst case. (d) shows the transmittance spectra of the best combination of six commercial filters corresponding to the typical case. Note that fluorescence background has been removed in both sets of spectra to facilitate comparison in Raman features.
Fig. 4. Comparison between the measured spontaneous Raman spectrum and the spontaneous Raman spectrum reconstructed by traditional Wiener estimation with the best combination of six non-negative PCs based filters in (a) the best case, (b) the typical case, (c) the worst case. (d) shows the filters’ transmittance spectra. Note that fluorescence background has been removed in both sets of spectra to facilitate comparison in Raman features.

Fig. 5. Comparison between the measured SERS spectrum and the SERS spectrum reconstructed by traditional Wiener estimation with the best combination of six commercial filters in (a) the best case, (b) the typical case, (c) the worst case. (d) shows the transmittance spectra of the best combination of six commercial filters corresponding to the typical case. Note that fluorescence background has been removed in both sets of spectra to facilitate comparison in Raman features.

Table 4 shows the comparison in the mean relative RMSE of reconstructed SERS spectra (after fluorescence background removed) from narrow-band measurements using different types and numbers of filters. The percentage values of reduction in the mean relative RMSE from three to four filters were 2.7%, 2.7%, 3.5% and 3.5% for commercial filters, Gaussian filters, PCs based filters and non-negative PCs based filters, respectively. The percentage
values of reduction from four to five filters were 1.2%, 3.9%, 15.0% and 15.0% and the percentage values of reduction from five to six filters were 15.0%, 10.1%, 2.9% and 2.9%, respectively. Figure 5 shows the comparison between the measured SERS spectrum and the SERS spectrum reconstructed by traditional Wiener estimation and the transmittance spectra of the best combination of six commercial filters corresponding to the typical case, i.e. FL 840-10, FF 01-857, FL 905-10, FB 850-40, FF 01-840 and XB 149. The relative RMSEs were $9.1 \times 10^{-3}$, $2.13 \times 10^{-2}$ and $5.76 \times 10^{-2}$ in the best case, the typical case and the worst case, respectively. Figure 6 shows the comparison between the measured SERS spectra and the SERS spectra reconstructed by traditional Wiener estimation with the first six non-negative PCs based filters. The relative RMSEs were $9.3 \times 10^{-3}$, $2.09 \times 10^{-2}$, $4.58 \times 10^{-2}$ in the best case, the typical case and the worst case, respectively.

| Commercial filters | Gaussian filters | PCs based filters | Non-negative PCs based filters |
|--------------------|------------------|------------------|-------------------------------|
| 3 filters          | $2.65 \times 10^{-2}$ | $2.64 \times 10^{-2}$ | $2.55 \times 10^{-2}$        |
| 4 filters          | $2.57 \times 10^{-2}$ | $2.57 \times 10^{-2}$ | $2.46 \times 10^{-2}$        |
| 5 filters          | $2.54 \times 10^{-2}$ | $2.47 \times 10^{-2}$ | $2.09 \times 10^{-2}$        |
| 6 filters          | $2.16 \times 10^{-2}$ | $2.22 \times 10^{-2}$ | $2.03 \times 10^{-2}$        |
3.2 Optimization of modified Wiener estimation

Table 5. Comparison in the mean relative RMSE of spontaneous Raman spectra (after fluorescence background removed) reconstructed from narrow-band measurements with the best combination of three filters between traditional Wiener estimation and modified Wiener estimation involving different number of synthetic filters

| Number of synthetic filters | Commercial filters | Gaussian filters | PCs based filters | Non-negative PCs based filters |
|----------------------------|--------------------|------------------|-------------------|-------------------------------|
| Relative RMSE for traditional WE | N.A. | $5.61 \times 10^{-2}$ | $5.18 \times 10^{-2}$ | $7.10 \times 10^{-2}$ | $7.10 \times 10^{-2}$ |
| Percent change in relative RMSE for Modified WE* | 1 | $-3.7\%$ | $+0.6\%$ | $+4.4\%$ | $+9.6\%$ |
| 2 | $-18.2\%$ | $-12.7\%$ | $+2.7\%$ | $+4.7\%$ |
| 3 | $-14.1\%$ | $-12.2\%$ | $+1.8\%$ | $+3.8\%$ |
| 4 | $-10.7\%$ | $+533.2\%$ | $+1.0\%$ | $+2.4\%$ |
| 5 | $+88.9\%$ | $+2074.7\%$ | $+0.8\%$ | $+2.1\%$ |
| 6 | $-25.7\%$ | $+525.5\%$ | $+1.0\%$ | $+1.5\%$ |
| 7 | $+482.9\%$ | $+7070.5\%$ | $+0.7\%$ | $+1.4\%$ |
| 8 | $+2600.5\%$ | $+11510.0\%$ | $+11.3\%$ | $+2.1\%$ |

* Percent change in relative RMSE for modified WE = 100% relative RMSE for modified WE - relative RMSE for traditional WE / relative RMSE for traditional WE

Table 5 shows the comparison in the mean relative RMSE of spontaneous Raman spectra (after fluorescence background removed) reconstructed from narrow-band measurements with the best combination of three filters between traditional Wiener estimation and modified Wiener estimation involving different numbers of synthetic filters. For commercial and Gaussian filters, the optimal reconstruction results of modified WE show significant improvement compared with traditional WE, in which the maximum percent reduction in the mean relative RMSE are 25.7% (6 synthetic filters) and 12.7% (2 synthetic filters), respectively. However, for PCs based filters and non-negative PCs based filters, there are always small increases in the mean relative RMSE from traditional Wiener estimation to modified Wiener estimation for PCs based filters and non-negative PCs based filters.

Table 6. Comparison in the mean relative RMSE of SERS spectra (after fluorescence background removed) reconstructed from narrow-band measurements with the best combination of three filters between traditional Wiener estimation and modified Wiener estimation involving different number of synthetic filters

| Number of synthetic filters | Commercial filters | Gaussian filters | PCs based filters | Non-negative PCs based filters |
|----------------------------|--------------------|------------------|-------------------|-------------------------------|
| Relative RMSE for traditional WE | N.A. | $2.65 \times 10^{-2}$ | $2.64 \times 10^{-2}$ | $2.55 \times 10^{-2}$ | $2.55 \times 10^{-2}$ |
| Percent change in relative RMSE for Modified WE* | 1 | $0\%$ | $0\%$ | $0\%$ | $0\%$ |
| 2 | $-1.9\%$ | $-0.4\%$ | $0\%$ | $0\%$ |
| 3 | $-2.6\%$ | $+0.4\%$ | $+0.4\%$ | $+0.4\%$ |
| 4 | $-5.3\%$ | $-1.1\%$ | $0\%$ | $0\%$ |
| 5 | $-5.2\%$ | $-1.5\%$ | $+0.8\%$ | $+0.8\%$ |
| 6 | $-5.2\%$ | $-1.5\%$ | $0\%$ | $0\%$ |
| 7 | $-5.2\%$ | $-0.8\%$ | $+0.8\%$ | $+0.8\%$ |
| 8 | $-5.2\%$ | $-0.4\%$ | $+1.2\%$ | $+1.2\%$ |

* Percent change in relative RMSE for modified WE = 100% relative RMSE for modified WE - relative RMSE for traditional WE / relative RMSE for traditional WE

Table 6 shows the comparison in the mean relative RMSE of SERS spectra (after fluorescence background removed) reconstructed from narrow-band measurements with the best combination of three filters between traditional Wiener estimation and modified Wiener estimation involving different number of synthetic filters. For commercial and Gaussian filters, the optimal reconstruction results of modified WE show significant improvement compared with traditional WE, in which the maximum percent reduction in the mean relative RMSE are 25.7% (6 synthetic filters) and 12.7% (2 synthetic filters), respectively. However, for PCs based filters and non-negative PCs based filters, there are always small increases in the mean relative RMSE from traditional Wiener estimation to modified Wiener estimation for PCs based filters and non-negative PCs based filters.
estimation involving different numbers of synthetic filters. For commercial and Gaussian filters, the modified WE shows slight improvement in reconstruction accuracy compared with traditional WE. However, for PCs based filters and non-negative PCs based filters, the modified WE shows no improvement in reconstruction accuracy compared with traditional WE.

3.3 Optimization of sequential weighted Wiener estimation

Table 7 shows the comparison in the mean relative RMSE of spontaneous Raman spectra (after fluorescence background removed) reconstructed from narrow-band measurements with the best combination of filters between traditional Wiener estimation and sequential weighted Wiener estimation involving different numbers of iterations. For all types of filters and all numbers of filters, the reconstruction results of sequential weighted WE (the 1st iteration) show significant improvement compared with traditional WE. However, there’s only tiny improvement in reconstruction accuracy from the 1st iteration to the 2nd iteration in sequential weighted WE.

| Number of filters | Commercial filters | Gaussian filters |
|-------------------|--------------------|------------------|
|                   | Relative RMSE for Traditional WE | Percent change in Relative RMSE for Sequential weighted WE* | Relative RMSE for Traditional WE | Percent change in Relative RMSE for Sequential weighted WE* |
| 3                 | $5.61 \times 10^{-2}$ | -20.7% | $5.18 \times 10^{-2}$ | -14.7% |
| 4                 | $4.91 \times 10^{-2}$ | -7.7% | $5.07 \times 10^{-2}$ | -20.7% |
| 5                 | $4.01 \times 10^{-2}$ | -12.0% | $4.37 \times 10^{-2}$ | -20.6% |
| 6                 | $3.49 \times 10^{-2}$ | -10.0% | $3.54 \times 10^{-2}$ | -14.1% |

PCs based filters

| Number of filters | Commercial filters | Gaussian filters |
|-------------------|--------------------|------------------|
|                   | Relative RMSE for Traditional WE | Percent change in Relative RMSE for Sequential weighted WE* | Relative RMSE for Traditional WE | Percent change in Relative RMSE for Sequential weighted WE* |
| 3                 | $7.10 \times 10^{-2}$ | -14.9% | $7.10 \times 10^{-2}$ | -14.9% |
| 4                 | $6.06 \times 10^{-2}$ | -13.4% | $6.06 \times 10^{-2}$ | -13.4% |
| 5                 | $3.51 \times 10^{-2}$ | -16.8% | $3.51 \times 10^{-2}$ | -16.8% |
| 6                 | $2.89 \times 10^{-2}$ | -17.3% | $2.89 \times 10^{-2}$ | -18.0% |

Non-negative PCs based filters

| Number of filters | Commercial filters | Gaussian filters |
|-------------------|--------------------|------------------|
|                   | Relative RMSE for Traditional WE | Percent change in Relative RMSE for Sequential weighted WE* | Relative RMSE for Traditional WE | Percent change in Relative RMSE for Sequential weighted WE* |
| 3                 | $7.10 \times 10^{-2}$ | -14.9% | $7.10 \times 10^{-2}$ | -14.9% |
| 4                 | $6.06 \times 10^{-2}$ | -13.4% | $6.06 \times 10^{-2}$ | -13.4% |
| 5                 | $3.51 \times 10^{-2}$ | -16.8% | $3.51 \times 10^{-2}$ | -16.8% |
| 6                 | $2.89 \times 10^{-2}$ | -17.3% | $2.89 \times 10^{-2}$ | -18.0% |

* Percent change in relative RMSE for sequential weighted WE = \( \frac{\text{relative RMSE for sequential weighted WE} - \text{relative RMSE for traditional WE}}{\text{relative RMSE for traditional WE}} \times 100\%

Table 8 shows the comparison in the mean relative RMSE of SERS spectra (after fluorescence background removed) reconstructed from narrow-band measurements with the best combination of filters between traditional Wiener estimation and sequential weighted Wiener estimation involving different numbers of iterations. For all types of filters and all numbers of filters, the reconstruction results of sequential weighted WE (1st iteration and 2nd iteration) are close to the traditional WE. There is neither significant improvement nor reduction observed.

| Number of filters | Commercial filters | Gaussian filters |
|-------------------|--------------------|------------------|
|                   | Relative RMSE for Traditional | Percent change in Relative RMSE for Sequential weighted WE* | Relative RMSE for Traditional | Percent change in Relative RMSE for Sequential weighted WE* |
| 3                 | $7.10 \times 10^{-2}$ | -14.9% | $7.10 \times 10^{-2}$ | -14.9% |
| 4                 | $6.06 \times 10^{-2}$ | -13.4% | $6.06 \times 10^{-2}$ | -13.4% |
| 5                 | $3.51 \times 10^{-2}$ | -16.8% | $3.51 \times 10^{-2}$ | -16.8% |
| 6                 | $2.89 \times 10^{-2}$ | -17.3% | $2.89 \times 10^{-2}$ | -18.0% |

Table 8. Comparison in the mean relative RMSE of SERS spectra (after fluorescence background removed) reconstructed from narrow-band measurements with the best combination of filters between traditional Wiener estimation and sequential weighted Wiener estimation involving different numbers of iterations

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ability of PCs based filter to capture more variance, i.e. information, compared with Gaussian filters and commercial filters. In addition, we also verified the importance of capturing both Raman signal and fluorescence background information. We tested PCs based filters that were generated from Raman signal or fluorescence background alone. For PCs based filters generated from the Raman signal, the relative RMSEs were $3.92 \times 10^{-2}$ and $2.38 \times 10^{-2}$ for three, four, five and six filters, respectively. For PCs based filters generated from fluorescence background, the relative RMSEs were $4.04 \times 10^{-2}$, $3.26 \times 10^{-2}$, $2.93 \times 10^{-2}$ and $2.30 \times 10^{-2}$ for three, four, five and six filters, respectively. Both sets of relative RMSEs were considerably larger than those obtained using PCs based filters generated from Raman spectra with fluorescence background as shown in Table 4, which implies that information from both Raman signal and fluorescence background is important for reconstruction. For spontaneous Raman spectra, the mean values of reconstruction accuracy using Gaussian filters and commercial filters were better than PCs based filters when only three or four filters were used. This could be attributed to the fact that fluorescence background was much larger in spontaneous Raman spectra compared to that in SERS spectra. In this case, the variance for fluorescence background in a spontaneous Raman spectrum was considerably larger than the Raman signal in magnitude. Based on the characteristics of PCA, the first three or four PCs, from which the transmittance spectra of these PCs based filters were derived, capture most information from smooth fluorescence background and less information from the Raman signal on top of the fluorescence background. Interestingly, PCs based filters showed better reconstruction accuracy than Gaussian filters and commercial filters when five or six filters were used. Moreover, the improvement in accuracy for PCs based filters from four to five filters was significant, which means that more information about Raman signal was collected by the additional PCs based filters as sufficient information about fluorescence background has been collected by the first four PCs based filters. We also tested the PCs based filters that were generated from the

| Numb of filters | PCs based filters | Non-negative PCs based filters |
|----------------|------------------|-------------------------------|
|                | Relative RMSE for Traditional WE | Percent change in Relative RMSE for Sequential weighted WE* | Relative RMSE for Traditional WE | Percent change in Relative RMSE for Sequential weighted WE* |
|                | 1st iteration | 2nd iteration | | 1st iteration | 2nd iteration | | 1st iteration | 2nd iteration |
| 3              | $2.55 \times 10^{-2}$ | $-1.6\%$ | $-1.6\%$ | $2.55 \times 10^{-2}$ | $-1.6\%$ | $-1.6\%$ | $2.55 \times 10^{-2}$ | $-1.6\%$ | $-1.6\%$ |
| 4              | $2.46 \times 10^{-2}$ | $+0.8\%$ | $+0.8\%$ | $2.46 \times 10^{-2}$ | $+0.8\%$ | $+0.8\%$ | $2.46 \times 10^{-2}$ | $+0.8\%$ | $+0.8\%$ |
| 5              | $2.09 \times 10^{-2}$ | $+2.4\%$ | $+2.4\%$ | $2.09 \times 10^{-2}$ | $+2.4\%$ | $+2.4\%$ | $2.09 \times 10^{-2}$ | $+2.4\%$ | $+2.4\%$ |
| 6              | $2.03 \times 10^{-2}$ | $+0.5\%$ | $0\%$ | | | | | | |

* Percent change in relative RMSE for sequential weighted WE - relative RMSE for traditional WE

4. Discussions

We have demonstrated that full Raman spectra can be reconstructed by Wiener estimation from a few narrow-band measurements in the presence of fluorescence background. Compared to the previous study [11], in which Raman spectra were free of fluorescence background and this can be only achieved with sophisticated Raman setups, the results shown in this study prove the feasibility of applying this method in common Raman measurements that most Raman applications involve. This opens the possibility of performing fast Raman imaging in a simple Raman setup without considerably sacrificing the accuracy of Raman measurements.

In traditional Wiener estimation, for SERS spectra, Gaussian filters and commercial filters showed worse accuracies compared with PCs based filters. This could be attributed to the ability of PCs based filter to capture more variance, i.e. information, compared with Gaussian filters and commercial filters. In addition, we also verified the importance of capturing both Raman signal and fluorescence background information. We tested PCs based filters that were generated from the Raman signal or fluorescence background alone. For PCs based filters generated from Raman signal, the relative RMSEs were $3.92 \times 10^{-1}$, $2.90 \times 10^{-1}$, $2.50 \times 10^{-2}$ and $2.38 \times 10^{-2}$ for three, four, five and six filters, respectively. For PCs based filters generated from fluorescence background, the relative RMSEs were $4.04 \times 10^{-2}$, $3.26 \times 10^{-2}$, $2.93 \times 10^{-2}$ and $2.30 \times 10^{-2}$ for three, four, five and six filters, respectively. Both sets of relative RMSEs were considerably larger than those obtained using PCs based filters generated from Raman spectra with fluorescence background as shown in Table 4, which implies that information from both Raman signal and fluorescence background is important for reconstruction. For spontaneous Raman spectra, the mean values of reconstruction accuracy using Gaussian filters and commercial filters were better than PCs based filters when only three or four filters were used. This could be attributed to the fact that fluorescence background was much larger in spontaneous Raman spectra compared to that in SERS spectra. In this case, the variance for fluorescence background in a spontaneous Raman spectrum was considerably larger than the Raman signal in magnitude. Based on the characteristics of PCA, the first three or four PCs, from which the transmittance spectra of these PCs based filters were derived, capture most information from smooth fluorescence background and less information from the Raman signal on top of the fluorescence background. Interestingly, PCs based filters showed better reconstruction accuracy than Gaussian filters and commercial filters when five or six filters were used. Moreover, the improvement in accuracy for PCs based filters from four to five filters was significant, which means that more information about Raman signal was collected by the additional PCs based filters as sufficient information about fluorescence background has been collected by the first four PCs based filters. We also tested the PCs based filters that were generated from the
Raman signals or fluorescence background alone. For PCs based filters generated from the Raman signals, the relative RMSEs were $1.67 \times 10^{-3}$, $1.38 \times 10^{-1}$, $7.46 \times 10^{-2}$ and $6.35 \times 10^{-2}$ for three, four, five and six filters, respectively. For PCs based filters generated from fluorescence background, the relative RMSEs were $9.03 \times 10^{-2}$, $8.48 \times 10^{-2}$, $9.11 \times 10^{-2}$ and $8.17 \times 10^{-2}$ for three, four, five and six filters, respectively. These values were much larger than those obtained using PCs based filters generated from Raman spectra with fluorescence background as shown in Table 3. This further proves the importance of deriving optimal filters from the summation of the Raman signals and fluorescence background. For both spontaneous Raman spectra and SERS spectra, additional filters can improve the reconstruction accuracy significantly. Therefore, a tradeoff between the accuracy and cost needs to be made in the choice of number of filters. Compared with spontaneous Raman spectra, the reconstruction accuracy of SERS spectra was much better when the same number of filters were used as shown in Tables 3 and 4. This observation could be explained by two factors. One is that SERS spectra contain smaller fluorescence background compared to spontaneous Raman spectra, which lowers down the requirement on the effectiveness of the filter set in capturing most information. The other is that SERS spectra exhibit higher signal-to-noise ratio, which reduces the influence of noise on reconstruction. Therefore, using sophisticated methods, e.g. shifted excitation Raman difference spectroscopy [12], Fourier transformed Raman spectroscopy [13], and temporal gating [14], to suppress fluorescence background and/or improve the signal-to-noise ratio of Raman signals would further improve the reconstruction accuracy in this method.

In Table 5, the maximum percent reduction in the relative RMSE in modified Wiener estimation for commercial filters and Gaussian filters were 25.7% (6 synthetic filters) and 12.7% (2 synthetic filters), respectively, compared with the traditional Wiener estimation. In contrast, there were small increases in the mean relative RMSE from traditional Wiener estimation to modified Wiener estimation for PCs based filters and non-negative PCs based filters. These observations could be explained as follows. While additional information was provided in modified Wiener estimation by synthesized narrow-band measurements, error was also induced by the correction step in this method. The final reconstruction accuracy was the result of competition between the gain (the additional information) and the loss (the induced error). For commercial and Gaussian filters, the additional filters created in the modified Wiener estimation were the first several PCs based filters. Different from that, the additional filters started from the forth PCs based filters for PCs based filters and non-negative PCs based filters because the first three PCs filters have been applied. The first three PCs based filters with relatively smooth shapes were likely to capture additional useful information (mainly from slow changing fluorescence background), which outweighed the error induced (mainly from sharp Raman signals) in the correction process. In comparison, those PCs based filters after the first three ones with sharp peaks were likely to induce larger errors in the correction step because it can only capture additional information mainly from similarly sharp Raman peaks, which were more difficult to correct. In SERS spectra, which contained much weaker fluorescence background than the spontaneous Raman spectra, shown in Table 6, there was no considerable difference in terms of the relative RMSE between modified Wiener estimation and traditional Wiener estimation. Therefore, it is expected that the method of modified Wiener estimation would show more significant improvement over traditional Wiener estimation in Raman spectra with intense fluorescence background. What's more, in both spontaneous Raman spectra and SERS spectra, the relative RMSE usually followed a trend of first decreasing and then increasing for an increasing number of synthetic filters. This can be attributed to the same reason, i.e. the compromise between the useful information and error induced by the additional synthetic filters.

In contrast to modified Wiener estimation, the sequential weighted Wiener estimation improves reconstruction accuracy for all types of filters when applying to spontaneous Raman spectra as shown in Table 7. This observations could be attributed to the following factor. In sequential weighted Wiener estimation, the improvement in reconstruction stems from the fact that the calibration data are generated by minimizing the contribution from data very different
from the given test sample, which is independent of filter type or number in principle. Therefore, the sequential weighted Wiener estimation is applicable to all types and numbers of filters. In addition, there is no considerable improvement when performing two iterations, which suggests one iteration should be sufficient. For SERS spectra shown in Table 8, which contained much weaker fluorescence background compared to the spontaneous Raman spectra, there is no considerable difference in terms of the relative RMSE between sequential weighted Wiener estimation and traditional Wiener estimation. This is likely due to much lower variance in SERS spectra, i.e. there’s not much calibration data very different from test samples. Therefore, it is expected that the method of sequential weighted Wiener estimation would show more significant improvement over traditional Wiener estimation in Raman spectra with intense fluorescence background.

In a brief summary, both modified Wiener estimation and sequential weighted Wiener estimation show significant improvement in the reconstruction of spontaneous Raman spectra. The sequential weighted Wiener estimation is more advantageous than modified Wiener estimation in the case that more than three filters or PCs based filters were used. However, when there’s a limited choice of filters, modified Wiener estimation can still work better than others because more accurate Wiener matrices can be created by introducing more synthetic narrow-band measurements; whereas the sequential weighted Wiener estimation would degrade dramatically because the poor initial estimation would lead to an inaccurate calibration data set. In addition, the combination of those two advanced Wiener estimation methods could further improve performance because of the different principles in the two methods. However, this may not be true when using PCs based filters. Because the first three PCs filters have been applied, additional synthetic filters used in modified Wiener estimation have to be the PCs with higher orders. Those PCs with higher orders contain sharp peaks, which contain more significant noises and are likely to induce larger errors in the correction step. The computation time taken to reconstruct 50 SERS spectra measured from blood serum samples with 3 filters for each method are shown in Table 9, which was tested in a workstation with Intel(R) Xeon(R) CPU E5-2630 v2 2.60GHz, 16 G RAM and Windows 7 operating system. The significant difference is attributed to the fact that the traditional WE only needs to create the Wiener matrix once for all samples, while the modified WE and sequential weighted WE need to create the Wiener matrix for each individual sample. Therefore, although both advanced Wiener estimation methods show significant improvement in reconstruction of spontaneous Raman spectra, traditional Wiener estimation can be advantageous in the reconstruction of SERS spectra because of its similarly high reconstruction accuracy and fast computation.

### Table 9. Computation time taken to reconstruct 50 SERS spectra measured from blood serum samples with 3 filters for traditional WE, modified WE and sequential weighted WE (1st iteration)

| Method                      | Computation time |
|-----------------------------|------------------|
| Traditional WE with 3 filters | 6.71 × 10^{-4}s  |
| Modified WE with 3 filters | 0.29s            |
| Sequential weighted WE with 3 filters (1st iteration) | 0.14s |

In practice, Wiener matrix needs to be constructed again for a new set of calibration data when using a different type of samples. While this is the major limitation for this method, we believe that this method can still find a large number of biomedical applications, such as differentiation of cancer from normal samples [26] and classification of cell death mode [27] etc., in which most popular methods rely on multi-variate statistical analysis thus also requiring a set of data for training the classifier.

We anticipate that this method of reconstruction will be advantageous when employed in Raman imaging. Currently most Raman imaging techniques use point scanning or line scanning, in which every scan would involve the acquisition of Raman intensity at many wavenumbers. However, this way will yield Raman images with high spectral resolution but low spatial resolution. Wide-field Raman imaging using a CCD could be performed at each
wavenumber, this would require a filter with an extremely narrow pass band and tunable central wavelength or a number of filters with different central wavelengths. Moreover, it would be very time consuming given the number of wavenumbers involved (hundreds to thousands depending on spectral resolution required). Our Raman imaging approach consists of two steps, i.e. taking the narrow-band Raman images and then performing spectral reconstruction at each pixel of the Raman image. Therefore, our method requires only a few narrow-band filters with much larger bandwidths to get a few Raman images and the full Raman spectrum at each pixel can be reconstructed. The potential improvement in the speed will be dramatic just considering the difference in the number of Raman images required between traditional Raman imaging and the proposed strategy. The narrow-band Raman imaging system can be simply implemented by taking narrow-band Raman images sequentially by using a filter wheel loaded with different narrow-band filters. We are also developing a Raman imaging system to acquire multiple narrow-band images simultaneously.

5. Conclusions

In this study, two advanced methods for spectral reconstruction based on Wiener estimation, i.e. modified Wiener estimation and sequential weighted Wiener estimation, were optimized to facilitate the reconstruction of Raman spectra with high spectral resolution from narrow-band Raman measurements with fluorescence background for the first time. Traditional Wiener estimation was also evaluated for the purpose of comparison. The optimal value for the key parameter in each advanced method, including the number of synthetic filters in modified Wiener estimation and the number of iterations in sequential weighted Wiener estimation, were identified. The reconstruction methods were evaluated on both spontaneous Raman data and SERS data. Compared with traditional Wiener estimation, both advanced Wiener estimation methods showed significant improvement in the reconstruction accuracy of spontaneous Raman spectra, while the reconstruction accuracy values of SERS spectra were similar. Furthermore, it was found that sequential weighted Wiener estimation was more advantageous than modified Wiener estimation in the case that more than three filters or PC based filters were used, while modified Wiener estimation should be more advantageous when there’s a limited choice of filters. The traditional Wiener estimation can be advantageous in the reconstruction of Raman spectra with low variance in fluorescence background, e.g. SERS spectra, because of its fast computation. Compared with our previous study, the optimized advanced Wiener estimation methods can be used in a simple Raman setup without the function of fluorescence suppression for fast Raman imaging. Therefore, this method opens a new avenue for Raman imaging to investigate fast changing biomedical phenomena using a simple Raman imaging setup without the function of fluorescence suppression.

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