# **Research article**

# **Evaluating Noise Reduction Methods for Raman Spectroscopy in Transmission and Reflection Configurations**

Wasunun Sassuvun<sup>1</sup>, Prathan Buranasiri<sup>1</sup>, Surawut Wicharn<sup>2</sup>, Chokchai Puttharugsa<sup>2</sup>, Puenisara Limnonthakul<sup>2</sup>, Saksorn Limwichean<sup>3</sup> and Suwan Plaipichit<sup>2</sup>\*

<sup>1</sup>Physics Department, School of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand

<sup>2</sup>Department of Physics, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand <sup>3</sup>National Electronics and Computer Technology Center (NECTEC), National Science and Technology Development Agency, PathumThani, Thailand

Curr. Appl. Sci. Technol. 2024, Vol. 24 (No. 4), e0259042; https://doi.org/10.55003/cast.2024.259042

Received: 13 June 2023, Revised: 9 August 2023, Accepted: 30 November 2023, Published: 20 March 2024

## Abstract

Keywords	This work involved comparing Raman signals obtained from two
	different Raman spectroscopy configurations, using two distinct
boxcar averaging;	noise reduction methods. The excitation light source was a laser
	diode with a wavelength of 532 nm. A long-pass filter and focusing
Vancouver Raman	lens were utilized to block the excited light from the source and
algorithm;	concentrate the Raman signals due to their weaker nature
pharmacy;	compared to the excited light signals. Light of 532 nm wavelength
	was blocked during green laser diode illumination using a long-
Raman spectroscopy	pass filter. Two configurations were studied: transmission Raman
1 12	spectroscopy (TRS) and reflection Raman spectroscopy (RRS).
	Raman signals from both configurations were compared, and the
	boxcar averaging and Vancouver Raman algorithm (VRA) noise
	reduction methods were investigated and compared. The results
	showed that Raman signals from the transmission configuration
	were higher than those from the reflection configuration, and noise
	signals were effectively reduced using both the boxcar averaging
	and VRA methods.

### 1. Introduction

Raman spectroscopy, which is an analytical technique used to measure inelastically scattered light from matter, has been widely used in physics [1, 2], chemistry [3], medicine [4], pharmaceutical analysis [5], biological tissues, and nanoscience [6, 7]. A real-time measurement technique was used to reduce the time needed for Raman signal collection when investigating various materials [8]. A

<sup>\*</sup>Corresponding author: Tel.: (+66) 26495598

E-mail: suwanp@g.swu.ac.th

simple and low-cost Raman spectroscope for educational purposes was developed [9]. Moreover, a portable Raman spectroscope was proposed for use outside the laboratory [10].

Raman spectroscopy was applied in the pharmaceutical field, and samples were investigated without physically and chemically damage them. There was a report about the investigation of the paracetamol tablets using Raman spectroscopy [11]. In a transmission Raman spectroscopy configuration, excitation laser light is shone through a sample and the Raman signals that emit from the back of the sample are then collected. This system cannot be used to collect Raman signals when the thickness of a sample is more than 2 mm. On the other hand, there are no thickness limits when a reflection Raman spectroscopy configuration is used [12]. However, Raman spectroscopy system that is set up with a simplified design, has higher level of noise than a commercial system. Methods of noise reduction in such Raman system have attracted research interest.

In this paper, we compared noise reduction techniques for transmission and reflection Raman spectroscopy systems. Boxcar averaging (BA) was used for high-frequency modulation of Raman noise [13]. Vancouver Raman algorithm (VRA), which is simple and effective, is a technique used to reduce fluorescence noise [14]. Three parameters of the boxcar averaging technique consisting of boxcar width order, integration time, and scan average, were used to find the optimum condition that provided good signals. Finally, the boxcar averaging and VRA noise reduction methods were investigated and compared.

For Raman spectroscopy illustrated in Figure 1, when light interacts on molecules, there are three types of scattering that can occur. First, the most common kind of scattering event involves molecular energy being unchanged after the incident photon interacts with the molecule. The wavelength of the scattered photon is equal to the incident photon. This is called elastic or Rayleigh scattering. The second type is inelastic scattering which involves photons losing energy and thus increasing in wavelength. This is called Stokes Raman scattering. The last type, which is called anti-Stokes Raman scattering, is inelastic scattering which involves decrease of wavelength.



Figure 1. Three types of scattering processes can occur when light interacts with a molecule

Equation 1 explains a Raman shift [15], where  $\Delta f$  is the Raman shift expressed in wavenumber,  $\lambda_0$  is the excitation wavelength, and  $\lambda_1$  is the wavelength of the scattered light. The Raman spectrum is often converted to a report in wavenumber and unit using centimeters (cm<sup>-1</sup>).

$$\Delta f = \left(\frac{1}{\lambda_0(\text{nm})} - \frac{1}{\lambda_1(\text{nm})}\right) \times \frac{10^7 \text{ nm}}{\text{cm}}$$
(1)

BA is a data treatment method that enhances the signal-to-noise ratio [16]. This noise reduction technique was proposed in early 1960 to improve signal quality in experiments

investigating nuclear magnetic resonances [17, 18]. BA, which features the use of proper integration time in order to obtain effective signals, is used for high-frequency modulation of Raman noise [19]. In the early work, BA was created by electric components. BA technique uses a digital procedure for smoothing irregularities in a waveform [19, 20].

VRA is an iterative algorithm used to correct background due to fluorescence in Raman spectra. VRA is a technique used to reduce noise and reduce Raman signal simultaneously. VRA procedure starts with input of raw Raman signal:  $O_0(v)$  into an iteration loop, where v is the Raman shift in cm<sup>-1</sup>. Then, at  $i^{th}$  iteration, a single polynomial fitting function:  $P_i(v)$ , which represents background noise, is generated using the raw Raman signal curve. The residual  $R_i(v)$  and its standard deviation  $\sigma$  can be calculated as follows:

and

$$R_i(\nu) = O_{i-1}(\nu) - P_i(\nu), \tag{2}$$

$$\sigma_i = \sqrt{\frac{(R_i(\nu_1) - \bar{R})^2 + (R_i(\nu_2) - \bar{R})^2 + \dots + (R_i(\nu_N) - \bar{R})^2}{N}},$$
(3)

where N is the number of data points on the spectral curve. Meanwhile, the average residual at each Raman shift is written as:

$$\bar{R} = \frac{R(\nu_1) + R(\nu_2) + \dots + R(\nu_N)}{N}$$
(4)

Here, the SUM of the fitting function and the value of standard deviation, called SUM, is compared with the ex-Raman signal:  $O_{i-1}(v)$ . SUM is designated as a Raman signal to lessen polynomial fitting distortion. If the values of SUM are greater than  $O_{i-1}(v)$ , they will be regarded as Raman signals. A model of Raman signal:  $O_i(v)$  is constructed for making the polynomial fitting function of the next iteration. The iterative polynomial fitting procedure is stopped when it meets criteria:  $|(\sigma_i - \sigma_{i-1})/\sigma_i| < 5\%$ . The final polynomial fitting function:  $P_f(v)$  is considered as the fluorescence background. The pure Raman spectra: RS(v) is derived from the input raw spectra:  $O_0(v)$  by subtracting the final fitting function, as in following equation

$$RS(\nu) = O_0(\nu) - P_f(\nu)[21].$$
(5)

The Raman signal measurement often includes fluorescence effect. So, the large Raman signals are obtained due to a lot of noise. Therefore, we applied BA and VRA techniques to smoothen irregularities in a waveform and to reduce fluorescence noise.

#### 2. Materials and Methods

The TRS system is shown in Figure 2. A laser diode with a wavelength of 532 nm and power of 200 mW was used as the excitation light source. The laser beam was incident on an objective lens  $(OL_1)$  to focus the excitation light onto a paracetamol tablet. The Raman signals which were emitted from the sample were magnified with another objective lens  $(OL_2)$  and then the fluorescence signal was blocked by a long-pass filter. The long-pass filter blocks fluorescence signals only allowing Raman signals to transmit through. The filter was selected to block wavelengths above 532 nm. After that, the Raman signals were focused and collected using a focusing lens (FL) and spectrometer.



Figure 2. Transmission Raman spectroscopy (TRS) system

The RRS system is shown in Figure 3. A laser diode with a wavelength of 532 nm was used as the excitation light source. Light from the laser passed through a beam splitter, which is an optical device that splits a beam of light into two beams, and then focused on the sample by microscope objective lens (OL). The reflected light composed of an excited signal from the light source and Raman signal from the sample was amplified by the objective lens. Then the excited light source and Raman signals were filtered out as they had been in the transmission configuration. The existing Raman signals were focused and distinguished by focusing lens and spectroscopy, respectively.



Figure 3. Reflection Raman spectroscopy (RRS) system

The parameters of the Raman signals collected, which were integration time, boxcar average and scan average, were studied to find the optimum condition that produced a good signalto-noise ratio. Boxcar width is a function that is widely used in spectroscopy systems to reduce noise signals. The integration time is defined as the period of signal collection. The scan average is the exposure indicator quantity used for calculating the average signal level. Both TRS and RRS systems used the same parameters for signal collection. Integration time was varied from 1-10 s while the boxcar average and scan average were fixed to 1 and one time, respectively. After that, an optimum integration time was selected to investigate the boxcar average that provided clear signals. Raman shift signals with Boxcar width order equal to 1, integration time equal to 10 s and scan average equal to one time. It was ascertained that VRA could reduce noise signals. Thus, BA and VRA techniques were compared.

#### 3. Results and Discussion

#### 3.1 Reflection Raman spectroscopy (RRS)

Figure 4(a) compares Raman shift signals obtained from the paracetamol sample that were collected using various integration times, while BA and scan average were fixed to 1 and one time, respectively. The Raman signals at integration times of 1 s were very small and could not be used to identify the sample. The Raman signals increased with integration time increase. The Raman signals of paracetamol sample were dominant when the integration time was more than 5 s. Moreover, Raman signals were of maximum intensity when the integration time was 10 s (maximum integration time of detector).

Figure 4(b) shows a comparison of Raman shift signals obtained from the paracetamol samples that were collected using various BA, when the integration time and scan average were fixed to 10 s and one time, respectively. BA is a function for reducing noise signals. The BA equal to 1 provided sharp and high Raman shift signals and noise signals. An increase of BA reduced the sharp peaks of noise signals. However, an extreme BA might reduce the sharp peaks both of Raman shift signals and noise signals, as can be seen for BA equal to 10 (pink line). The Raman shift peaks of paracetamol could not be identified. As illustrated in the blue line, the BA of 5 provide low noise signals and clear Raman shift signals.

The comparison of both noise reduction methods is shown in Figure 4 (c). The VRA has lower noise than the 5<sup>th</sup> order of BA. Although the Raman shift identity of paracetamol was moderately lost compared to the original, the main peak of the Raman shift signals for paracetamol remained.

Signal to noise ratio is the ratio between the power of a desired signal and an undesired signal or noise signal. Figure 5(b) compares Raman signals for various integration times when the BA and scan average were fixed to 1 and one time, respectively. The Raman signal at integration time of 10 s had the highest signal-to-noise ratio. However, there were some peaks that had low signal to noise ratios because long integration time might increase the temperature of the sensor. Sensor with higher temperature produce higher thermal noise. In contrast to the RRS, the Raman signals at the integration time of 10 s is not as good as the TRS. This is because the Raman signals of the TRS do not pass through the beam splitter. Beam splitting causes signal loss in the reflected Raman system which is signal loss by stimulation and reflection. Therefore, long integration time gave both high Raman signals and a lot of noise signals.

The comparison between Raman signals and signal-to-noise ratio obtained from the paracetamol samples that were collected using various BA, while integration times and scan average were fixed at 10 s for one time. The Raman signal Boxcar width order 3<sup>rd</sup> provided the best signal-to-noise ratio when compared with the Boxcar width order at 1<sup>st</sup>, 5<sup>th</sup>, 10<sup>th</sup>, as shown in Figure 6(b). The intensity of TRS signal is better than the RRS signal. To obtain an increased Raman signal, the noise signals was also increased. The RRS had a low signal. The noise and Raman signal were also reduced when the higher order of BA was applied. This was related to the integration time component that was previously discussed.

#### **3.2 Transmitted Raman spectroscopy (TRS)**

Figure 7(a) shows a comparison of Raman shift signals that were collected at various integration times obtained from the paracetamol sample, with the BA and scan average fixed to 1 and one time, respectively. As in the case of the RRS configuration, using integration of 1 s provided small Raman shift signals. The identity of Raman shift signals of paracetamol could not be verified because the



Figure 4. The result of reflection for the paracetamol sample (a) various integration time of Raman signal (b) various boxcar width order of Raman signal (c) comparison of Raman signals with Raman signals in VRA



Figure 5. Comparison between Raman signals (a) various integration time of Raman signal (b) various integration time of signal-to-noise ratio of Raman signal



Figure 6. Comparison of Raman signals (a) various boxcar width order of Raman signal (b) various boxcar width order of signal-to-noise ratio of Raman signal

magnitude of Raman signals was not different from the noise signals. Higher integration time produced higher Raman signals. When the integration time was greater than 5 s, the Raman signals tended to dominate. Moreover, overall Raman signals of the TRS configuration resulted in larger Raman signals than the RRS configuration because there was no power loss from the beam splitter as in the previous studies [19].

Figure 7(b) shows a comparison of Raman shift signals obtained from the paracetamol sample collected at various BA, with integration time and scan average fixed to 1 s and one time, respectively. As in the case of the RRS configuration, BA equal to 1 provided highly sharp signals. The noise signal peaks were smaller when the BA was increased. Unlike the RRS configuration, the identity of paracetamol could be slightly identified when the BA was equal to 10, as can be seen in the pink line. The BA equal to 5 provided low noise signals and clear Raman shift signals as in the case of the RRS configuration. Moreover, there was an absence of signals at Raman shift from 0 cm<sup>-1</sup> to 596 cm<sup>-1</sup> because of the different wavelengths between the excitation light source and the long-pass filter. This filter permits light with a wavelength of more than 550 nm to pass through and light in the wavelength from 532 nm to 550 nm cannot pass. By calculating with equation (2), this



**Figure 7.** The result of transmission for the paracetamol sample (a) various integration time of Raman signal (b) various boxcar width order of Raman signal (c) comparison of Raman signals with Raman signal in VRA

is equivalent to a Raman shift from 0 to 596 cm<sup>-1</sup>. Likewise, in the RRS system, the 5<sup>th</sup> order of BA has clear Raman signals when compared with the others but the VRA provides broad Raman shift signals and flat noise signals.

Figure 8(b) compares Raman signals with various integration times when BA and scan average were fixed to 1 and one time, respectively. Increasing integration time produced a better signal-to-noise ratio. The integration time of 10 s provided the best signal-to-noise ratio when compared with the integration times at 1 s, 5 s, and 8 s.

Figure 9(b) compares Raman signals with various BA when the integration time and scan average were fixed at 10 and one time, respectively. The Raman signal in the BA of 5<sup>th</sup> order exhibited a high signal-to-noise ratio making it a superior signal. The TRS signal was noteworthy for its effectiveness in reducing noise. The BA was optimized to eliminate excessive values because high BA produces low noise signals and low Raman shift signals. Therefore, order 5<sup>th</sup> is a suitable value for BA. The BA at order 5<sup>th</sup> gave a good result. For the BA at 1<sup>st</sup>, 3<sup>rd</sup> and 10<sup>th</sup> order, there was little difference in the signal-to-noise ratio. Elevating the boxcar width order to 10 did not enhance the signal-to-noise ratio as it decreases the Raman signal in addition to reducing noise. Additionally, the 1<sup>st</sup> order of BA offers large Raman signals with a lot of noise.

The comparison between the RAW (data that had not been processed for use) Raman signals and Raman signals after applying both of noise reduction methods is shown in Figure 10. Both of BA and VRA methods produced signal to noise ratio that were greater than RAW Raman signals. Moreover, the signals to noise ratios of the BA method was greater than the VRA method.

Finally, the Raman signals of our system were compared with a commercial system. As shown in Figure 11, the Raman signals obtained from both TRS and RRS matched with commercial systems, which used excitation wavelengths at 532 nm and 785 nm, caused the Raman shift more than 596 cm<sup>-1</sup>.



**Figure 8.** Comparison of Raman signals (a) various integration time of Raman signal (b) various integration time of signal-to-noise ratio of Raman signal



Figure 9. Comparisons of Raman signals (a) various boxcar width order of Raman signal (b) various boxcar width order of signal-to-noise ratio of Raman signal



Figure 10. Comparison of signal-to-noise ratios of the Raw Raman signal, Vancouver Raman algorithm and boxcar averaging (a) RRS system (b) TRS system



**Figure 11.** Comparison of our Raman spectroscopy with commercial Raman spectroscopy. Pink line and blue line are Raman signals from our system while red line and black line are Raman signals from the Thermo Almega XR 532 nm and Scientific Instruments Center, respectively.

#### 4. Conclusions

In this research paper, we proposed both transmitted and reflected Raman spectroscopy systems and subsequently reduced and compared the noise signals. The intensity of the transmission Raman spectroscopy (TRS) signals was higher than that of the reflection Raman spectroscopy (RRS) signals. Increasing the integration time increased the overall signals, but it might also led to an increase in noise signals. The BA function was found to be satisfactory in reducing noise signals; however, the sharpness of signal peaks decreased as the BA increased. The Vancouver Raman algorithm (VRA) performed well in reducing noise, but it was not effective when dealing with signals with closely spaced peaks. Our system's Raman signal peaks aligned well with those of the commercial system. The absence of Raman shift in the range of 0-596 cm<sup>-1</sup> was attributed to the difference in the excitation light source and the long-pass filter used.

#### References

- [1] Lin, H., Liao, C.S., Wang, P., Kong, N. and Cheng, J.-X., 2018. Spectroscopic stimulated Raman scattering imaging of highly dynamic specimens through matrix completion. *Light Science and Application*, 7(5), 17179-17189.
- [2] Qiu, J., Qi, X., Li, X., Tang, Y., Lantu, J., Mi, X. and Bayan, H., 2018. Broadband transmission Raman measurements using a field-widened spatial heterodyne Raman spectrometer with mosaic grating structure. *Optics Express*, 26(20), 26106-26119.
- [3] Fan, L.-S., Constanin, L., Li, D. W., Liu, L., Keramatnejad, K., Azina, C., Huang, X., Golgir, H.R., Lu, Y., Ahmadi, Z., Wang, F., Shield, J., Cui, B., Silvain, J.-F. and Lu, Y.-F., 2018. Ultraviolet laser photolysis of hydrocarbons for nondiamond carbon suppression in chemical vapor deposition of diamond films. *Light Science and Application*, 7(4), 17177-17186.
- [4] Bonefacino, J., Tam, H.-Y., Glen, T.S., Cheng, X., Pun, C.-F.J., Wang, J., Lee, P.-H., Tse, M.-L.V. and Boles, S.T., 2018. Ultra-fast polymer optical fiber Bragg grating inscription for medical devices. *Light Science and Application*, 7, 17161-17171.
- [5] Griffen, J.A., Owen, A.W. and Matousek, P., 2015. Development of transmission Raman spectroscopy towards the in line, high throughput and non-destructive quantitative analysis of pharmaceutical solid oral dose. *Royal Society of Chemistry*, 140, 107-112.
- [6] Stone, N. and Matousek, P., 2008. Advanced transmission Raman spectroscopy: a promising tool for breast disease diagnosis. *American Association for Cancer Research*, 68(11), 4424-4430.
- [7] He, W., Li, B. and Yang, S., 2020. High-frequency Raman analysis in biological tissues using dual-wavelength excitation Raman spectroscopy. *Society for Applied Spectroscopy*, 74(2), 241-244.
- [8] Drescher, D. and Kneipp, J., 2012. Nanomaterial in complex biological systems: insight from Raman Spectroscopy. *Chemical Society Reviews*, 41, 5780-5799.
- [9] Lorigan, G.A., Patterson, B.M., Sommer, A.J. and Danielson, N.D., 2002. Cost-effective spectroscopic instrumentation for the physical chemistry laboratory. *Journal of Chemical Education*, 79(10), 1264-1267.
- [10] Cullum, B.M., Mobley, J., Chi, Z., Stokes, D.L., Miller, G.H. and Vo-Dinh, T., 2000. Development of a compact, handheld Raman instrument with no moving parts for use in field analysis. *Review of Scientific Instruments*, 71, 1602-1607.
- [11] Foster, M.J., Storey, J. and Zentile, M.A., 2017. Spatial-heterodyne spectrometer for transmission-Raman observations. *Optics Express*, 25(2), 1598-1604.

- [12] Matousek, P. and Parker, A.W., 2006. Bulk Raman analysis of pharmaceutical tablets. *Society for Applied Spectroscopy*, 60(12), 1353-1357.
- [13] Collier, J.L., Goddard, B.J., Goode, D.C., Marka, S. and Telle, H.H., 1996. A low-cost gated integrator boxcar averager. *Measurement Science and Technology*, 7, https://doi.org/10.1088/0957-0233/7/9/003.
- [14] León-Bejarano, F., Méndez, M.O., Ramírez-Elías, M.G. and Alba, A., 2019. Improved Vancouver Raman algorithm based on empirical mode decomposition for denoising biological samples. *Society for Applied Spectroscopy*, 73(12), https://doi.org/10.1177/0003702819860121.
- [15] Sankaranarayanan, D., 1996, Two lectures on Raman spectroscopy Fundamentals. *Conference on Refresher Course in Physics*. M.G. University, Kottayam, Kerala, India.
- [16] Ananthi, S., 2005. A Text Book of Medical Instruments. New Delhi: New Age International.
- [17] Blume, R.J., 1961. Boxcar integrator with long holding times, *Review of Scientific Instruments*, 32, 1016-1018.
- [18] Ware D. and Mansfield, P., 1966. High stability "Boxcar" integrator for fast NMR transients in solids. *Review of Scientific Instruments*, 37, 1167-1171.
- [19] Fimpel, P., Riek, C., Ebner, L., Leitenstorfer, A., Brida, D. and Zumbusch, A., 2018. Boxcar detection for high-frequency modulation in stimulated Raman scattering microscopy. *Applied Physics Letters*, 112, https://doi.org/10.1063/1.5022266.
- [20] Somerville, W.R.C., Ru, E.C.L., Northcote, P.T. and Etchegoin, P.G., 2010. High performance Raman spectroscopy with simple optical components. *American Journal of Physics*, 78, 671-677.
- [21] Zhao, J., Lui, H., McLean, D.I. and Zeng, H., 2007. Automated autofluorescence background subtraction algorithm for biomedical Raman spectroscopy. *Society for Applied Spectroscopy*, 61(11), 1225-1232.