Dual Wavelength Raman Spectroscopy

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Introduction

Raman spectroscopy is a valuable non-destructive and non-contact technique for analysis of a wide range of samples ranging from graphene to biological cells. Due to the difference in nature of the probed samples, the choice of laser excitation wavelength has a direct impact on the reliability and sensitivity of the Raman signals of specific samples. In general, the wavelength of a laser has direct effect on the following experimental capabilities:

1.Raman scattering cross-section of probed molecules. The Raman scattering intensity is $(1/\lambda)^4$ dependent, where λ is the laser wavelength. Hence, a visible excitation wavelength of 532nm yields (~ 5´) higher Raman scattering than a near infra-red laser of 785nm.

2. Spatial Resolution of Raman images. The theoretical diffraction limited spatial resolution, *d* is defined as $d = 1.22*\lambda/NA$; where NA is the numerical aperture of the microscope objective used. Hence, the longer the laser wavelength used, the worse spatial resolution is anticipated ($\propto \lambda$).

3.Detector sensitivity. Current CCD technology has higher quantum yield at the visible range than near IR region. Therefore, for NIR laser excitation, the Raman signal and sensitivity is reduced due to reduced signal collection at the NIR wavelengths.

4. Background fluorescence signals. In overall, shorter wavelengths, such as 488nm and 532nm, provide higher Raman scattering efficiency and better spatial resolution. However, short wavelengths overlap with the absorption range of most molecules, hence yield high background fluorescence signals. Nevertheless, these shorter excitation wavelengths can be useful for resonance Raman spectroscopy measurements of samples, such as graphene and beta carotene .

	Lower wavelengths	Higher wavelengths
Raman scattering cross-section	✓	X
Spatial resolution	✓	X
Detector sensitivity	✓	X
Background fluorescence signals	X	✓

Table 1 Summary of the pro and cons of the laser excitation wavelength.

For biological sample measurement, higher laser wavelengths are generally preferred. This is because longer wavelengths, such as 785nm and 830nm, produce lesser background fluorescence than visible wavelengths, and are able to penetrate deeper into the sample. For instance, it is more practical to use 785-nm laser for tissue measurement, because of the absorption of blood at low wavelength and the absorption of water is at higher wavelength. 785-nm wavelength falls within the "near-infrared window of the biological tissue".

Therefore, a single excitation wavelength cannot satisfy all the applications in Raman spectroscopy. Raman microscopy with multiples excitation wavelengths is a must for today multi- and inter-disciplinary research environment such as industries lab and research institute/center. uRaman by Technospex Pte. Ltd. provides a unique feature of multiple excitations wavelength and acquisition stacking system. It offers users a wide range of experimental capabilities and flexibility to investigate their samples. In this technical note, we demonstrate that uRaman can be used to interrogate various samples and the pros-and-cons of using different laser excitation wavelengths.

Experiment and Equipment

The uRaman-Ci microscope system is equipped with two separate modules, each equips with separate excitation wavelength via stacking system as shown in Figure 1. uRaman has 3 different laser wavelengths, i.e. 532, 633, and 785 nm. Any combination of 2 laser wavelengths is possible and Raman spectroscopy can be done individually or simultaneously. For example, two commonly used excitation wavelengths – 532 nm and 785 nm, are stacked as shown in Figure 1. Resonance Raman scattering measurements for samples such as graphene, beta carotenoids and cells activities can be performed using 532-nm excitation wavelength module (*532-TEC-Ci – Pol; Pol refers to polarization Raman enable*) due to higher scattering intensity and lower auto-fluorescence signal from the cells. When fluorescence from the sample becomes severe, 785 nm (*785-Ci-T-MAP*) excitation wavelength can be used for suppression of undesired fluorescent. 633 nm excitation

Figure 1 uRaman / uSight with dual laser excitation wavelength system, i.e. 532 - TEC - Ci - Pol (top) and 785-Ci-T-MAP (bottom), equipped with motorised stage and several objective

Results and Discussion

Lavender Essential Oil

Lavender essential oil is probed using dual excitation wavelengths at 532 nm and 785 nm (Figure 2). Stronger Raman signal is observed when measured using 532-nm excitation wavelength as compared to 785-nm excitation wavelength. The Raman intensity from 532-nm excitation wavelength is calculated to be ~4.8 times higher than the one at 785 nm. The results are likely contributed by higher Raman cross-section and higher quantum efficiency of the detector at the green wavelength region.

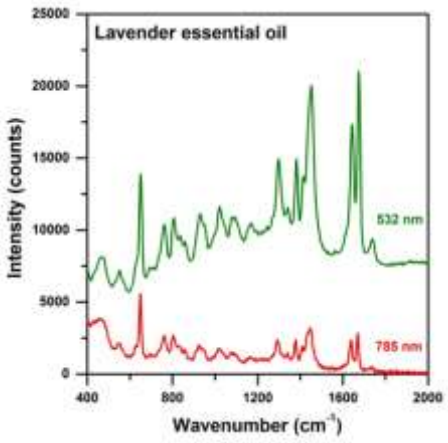

Figure 2. The Raman scattering spectra of lavender essential oil measured using 532-nm (3 mW and 10 s integration time) and 785-nm (50 mW and 10 s integration time) laser excitation wavelengths, respectively.

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Resonance Raman Spectroscopy

Depending on the type of samples, visible laser such as 532 nm is generally ideal for resonance Raman spectroscopy experiments. This is especially true for carbon materials, which sp^2 bond bandgap overlaps with the visible laser excitation wavelength, resulting in resonance Raman scattering effect. Hence, we use resonance Raman spectroscopy at 532 nm laser excitation wavelength to identify and distinguish crystalline graphene layers and its defect (Figure 3). Using uRaman, the defects region from the single layer graphene is clearly observed with 532 nm excitation wavelengths but not with 785 nm laser.

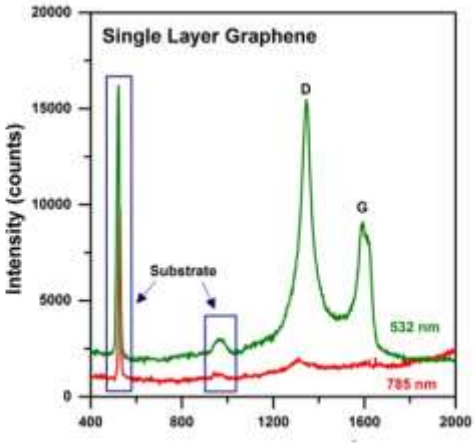

Figure 3. Raman spectra of a graphene layer with defect using 532nm and 785nm excitation wavelength, respectively. Integration time is 10s for both lasers.

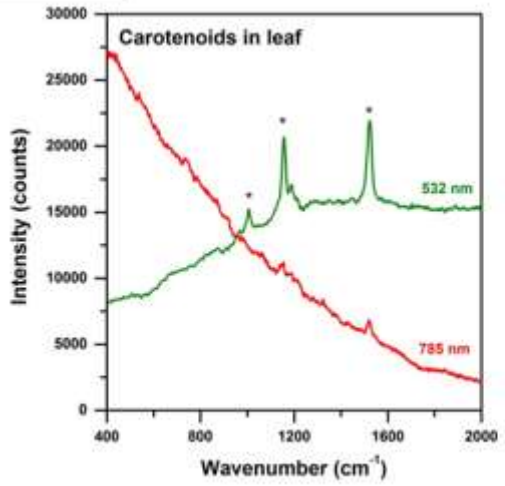

Figure 4. Raman spectra of a leaf using 532nm and 785nm excitation wavelength, respectively. Integration time for 532nm is 2s and 785nm is 20s, respectively.

Carotenoids are organic pigments that are commonly found in the chloroplasts and chromoplast of plants. Carotenoids consist of basic structure made up of isoprene units, which are joined end-to-end to give a conjugated chain structure. In 532 nm excitation wavelength, two intense Raman peaks, which are the characteristics of carotenoid pigments (see Figure 4), are clearly observed as compared to the weaker Raman signal measured using 785-nm laser excitation. These two peaks are the fingerprints of C=C stretching (the 1524 cm^{-1} band) and C-C single-bond stretching (at 1159 cm^{-1}), originating from conjugated chain in carotenoid structure.

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Royal Jelly

Royal Jelly is the diet of queen bees and newly hatched young bees. It is rich in proteins, vitamins, enzymes, sugar and amino acids; whereas honey mainly consists of glucose molecules. Due to royal jelly's much higher commercial value and nutritional functions, there are a lot of fake and counterfeited royal jelly using glucose as a replacement in the markets. Raman spectroscopy can be used to directly identify and authenticate royal jelly using the molecular signature of protein in the royal jelly.

Figure 5A illustrates the Raman spectra of royal jelly excited using 532-nm and 785-nm excitation wavelengths. At 532-nm excitation wavelength (Figure 5A top, green curve), the entire spectrum is overwhelmed with fluorescence background, severely suppressing the Raman signal. Such observation is due to the excitation of fluorescence, because there are most electronic transitions of molecules occur at UV and visible regions. In comparison, using 785-nm excitation, the fluorescence background is greatly reduced (Figure 5A bottom, red curve and Figure 5B), revealing the Raman peaks constituting of fructose, glucose (* in Figure 5B) and protein and collagen (° in Figure 5B).

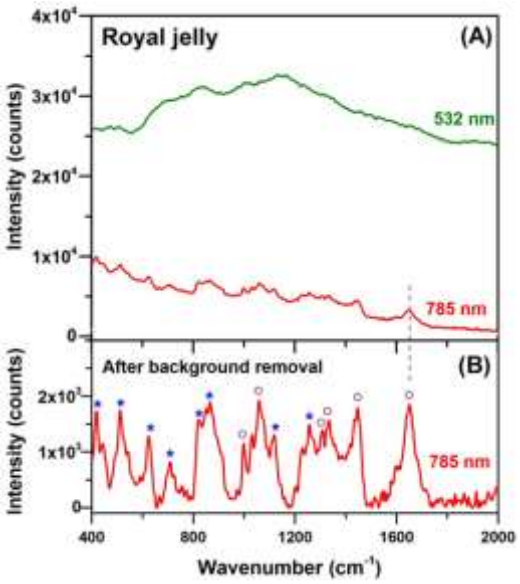

Figure 5. Raman spectra of Royal Jelly excited using 532 nm (6 mW and 3 s integration time) and 785 nm (50 mW and 60 s integration time) lasers, respectively. The (*) and (°) *inset in (B)* represent peaks form fructose, glucose (*) and protein, collagen (°), respectively.

Conclusion

Using uRaman's capability to stack multiple uRaman modules of different excitation wavelength, (532 nm and 785 nm), one is able to using multiple excitation wavelengths to investigate arbitrary analyte concurrently. uRaman provides researchers the opportunity to explore their samples in various condition with fraction of the cost as compared to other suppliers.

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