Towards Stand-Off Resonance Raman Spectroscopy

Alison J. Hobro, Bernhard Zachhuber and Bernhard Lendl

Institute of Chemical Technologies and Analytics, Vienna University of Technology, Getreidemarkt 9/164-AC, A-1060 Vienna, Austria. alison.hobro@tuwien.ac.at

Instrumentation

- Nd:YAG laser operating at 355 and 532 nm
- 6” Schmidt-Cassegrain telescope
- Long pass filters for rejection of Rayleigh light
- Imaging spectrograph
- Gated ICCD camera.

Stand-Off Resonance Raman of Chromophore Solutions

Absorbance Spectra

Above are the absorbance spectra of each of the three chromophores and their structures, along with the laser wavelengths used in this study (represented by arrows).

Stand-Off Raman at 355 nm

FAD and FMN exhibit resonance Raman bands, e.g. at ~1584 cm⁻¹ from N=N=C stretching and ~1526, 1160 and 1006 cm⁻¹ from C-C stretching) and 1527 cm⁻¹ (methyl rocking, 1160 cm⁻¹ (methyl rocking), 1011 cm⁻¹ (methyl rocking), 870 cm⁻¹ (C=C stretching). Flavins do not exhibit any Raman bands in this region. The Raman intensity of flavins is shown to be much lower than that of the carotenoids, with FAD and FMN showing fluorescence and poorly defined Raman bands.

Stand-Off Raman at 532 nm

FAD and FMN show no evidence of resonance enhancement.

β-carotene exhibits resonance Raman bands at 968 and 1011 cm⁻¹ (methyl rocking) and 1527 cm⁻¹ (C=C stretching).

Why Stand-Off Resonance Raman?

Stand-Off Raman

Advantages
- Operator and instrumentation distanced from sample
- Useful where samples are dangerous, fragile or difficult to access
- No potential contamination of sample from measurement process

Disadvantages
- Raman signals are relatively weak and the stand-off distance exacerbates this.

Resonance Raman

Advantages
- Orders of magnitude increase in Raman signal
- Selective for the chromophore

Disadvantages
- Careful selection of laser excitation wavelength is essential.

Funding

The research leading to these results has received funding from the European Community’s Seventh Framework Programme (FP7/2007-2013) under Grant Agreement No 218037.

Experimetnal Procedures

Absorbance spectra of dilute solutions of the three chromophores were recorded using a Hewlett Packard 8452A diode array spectrophotometer. The concentration of the chromophore was 10⁻⁶ mol/l for FAD and 10⁻⁸ mol/l for FMN, diluted in deionised water and C67T. The spectrum was recorded using a grating with 2400 grooves/mm. A 300 grooves/mm 500 nm blaze grating was used. Each spectrum was the result of 50 pulses per exposure and 15 exposures. For measurements at 355 nm the laser power was reduced to 10 mJ/pulse. For 532 nm excitation the laser power was set to 20 mJ/pulse at the sample, the delay between the laser firing and data collection starting was adjusted to 117 ns and the gate width of the iCCD 4 ns.

Towards Stand-Off Resonance Raman Spectroscopy

Stand-Off Resonance Raman of Leaves

Above are the Raman spectra obtained from four different species of leaf. All four species reflect the resonance Raman spectrum of carotenoids, specifically β-carotene. The specific positions of the bands at 1526, 1160 and 1006 cm⁻¹ would indicate that it is found predominantly in the all-trans conformation. This is consistent with the fact that the majority of the carotenoids in the photosystem II complex are β-carotene (79%) and that these have been shown previously, with conventional Raman spectroscopy, to be in the all-trans conformation.

Conclusion

- The increased signal associated with resonance Raman can compensate for the weak signals associated with stand-off Raman.
- Stand-off resonance Raman can be used as a selective probe of chromophores at a distance.
- The choice of laser excitation wavelength determines the chromophore detected.
- This can be used to measure chromophores and provide information regarding their conformation, in a complex matrix, such as the measurements of β-carotene in leaves.