Raman of plant tissues

Janina Kneipp
Raman scattering and **resonant** Raman scattering

\[
\begin{align*}
S_0 & \rightarrow h \nu_{\text{Molecule}} \\
S_0 & \rightarrow v_1 \\
S_1 & \rightarrow h \nu_{\text{Laser}} \\
S_1 & \rightarrow h \nu_{\text{Stokes}} \\
S_1 & \rightarrow v_1 \\
S_0 & \rightarrow h \nu_{\text{Laser}} \\
S_0 & \rightarrow h \nu_{\text{anti-Stokes}} \\
S_0 & \rightarrow v_0
\end{align*}
\]
Cross-sections considered

IR Absorption

Fluorescence

Raman

IR absorption

\( \sigma_{\text{abs}} \approx 10^{-21} - 10^{-18} \text{ cm}^2 \)

Fluorescence

\( \sigma_{\text{FL}} \approx 10^{-17} - 10^{-16} \text{ cm}^2 \)

Raman scattering

\( \sigma_{\text{RS}} \approx 10^{-30} - 10^{-26} \text{ cm}^2 \)
Raman shift with respect to $\lambda_{ex} = 488 \text{ nm}$

Lifetimes and time resolved detection

\[ \tau = \frac{1}{\Gamma + k} \]

It is possible to separate Raman from fluorescence photons using a „gate“!
What is a good excitation wavelength?

Human biopsy sample

- Fluorescence
- Pre-resonant RS
- Resonant RS

Raman shift, cm⁻¹

Correlating spectral with spatial information: Microspectroscopic Mapping and Imaging

(1) Microspectroscopic mapping

Sample: tissue, cell etc.

(2) Spectral analysis

Single spectral parameters
• intensities, ratios, band correlations
• vibrational frequency & shifts

Multivariate information
• Spectrum = vector, pattern
• Principal components, cluster analysis, artificial neural networks

(3) Molecular Image: Combination of spectral parameters and spatial coordinates

H&E | unstained | Raman image (e.g. K-means cluster)
Lignin and cellulose distribution

Lignin and cellulose spectra


Lignin staining

Polarization dependence of Raman scattering

180°

positioned for \( I_\parallel \)

\[ \rho = \frac{I_\bot}{I_\parallel} \]

90°

positioned for \( I_\bot \)

benzene, 514.5 nm

\( \text{stark polarisierte Banden} \)
Information on cellulose orientation

Lignin signals are not sensitive to polarization

Ongoing work: Raman spectroscopic imaging of cucumber root section

**Chemical map** of lignin distribution

**Hyperspectral map**, obtained using information from all molecules

Characteristic Raman spectra

excitation laser wavelength: 532 nm

I. Zeise, Zs. Heiner et al., in preparation
Ongoing work: Raman spectroscopic imaging of cucumber stem section

**Chemical map** of lignin distribution

**Hyperspectral map**, obtained using information from all molecules

Excitation laser wavelength: 532 nm

Characteristic Raman spectra

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RR of intact pollen grains

Raman spectra of individual untreated pollen grains from horse chestnut, large-leaved linden, and sallow, excited with 785 nm (10^8 W/cm^2, acquisition time 1 s).
Similar molecules, similar spectra?

Structure of (a) α-carotene, (b) β-carotene, (c) lutein, (d) zeaxanthine, (e) cryptoxanthine (all trans isomers).
Similar molecules, similar spectra?

Fig. 1. Typical RR spectra of carotenoids in acetone with 488 nm excitation. Acetone bands are marked A.

Fig. 2. Relation between C=C stretching frequency \( (\nu_1) \) and the number of double bonds for polyacetylenes and carotenoids.

Fig. 3. Plot of $\nu_1$ stretching frequency vs. $\nu_2$ stretching frequency. Values for $\beta$-carotene isomers are obtained from Ref. 20; values for astaxanthin-protein complexes are obtained from Ref. 25-29.

Fig. 4. Raman lines of \( \beta \)-carotene isomers in the 1100-1300 cm\(^{-1}\) region.
From Y. Koyama, T. Kakii, K. Saiki and K. Tsukida (Ref. 20)
In situ spectra of the carotenoid molecules contained in pollen grains from horse chestnut, large-leaved linden, and sallow. The spectra are differences of Raman spectra measured at the beginning and end of an irradiation period with 633 nm laser light (excitation intensity $10^6$ W/cm$^2$).

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Combination of resonance Raman with HPTLC
Same retention factor, different RR spectrum

Raman spectra of carotenoids extracted from horse chestnut, mahaleb cherry, large-leaved linden, and sallow pollen with a retention factor of 0.91 (excitation wavelength 488 nm, accumulation time 1 s, $\sim 10^2$ W/cm²).

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Pyrocystis lunula

Fig. 6. In vivo fluorescence and RR spectrum (frame insert) of single Pyrocystis lunula cell (right) with 457.9 nm excitation.
Euglena

Orientation of Carotenoid Molecules in the Eyespot of Alga: 
*In Situ* Polarized Resonance Raman Spectroscopy

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Fig. 2. Micrograph of a Euglena cell. At the left, a flagellum is seen.

Fig. 3. (A) Micrograph of the left half (with the flagellum edge) of a Euglena cell observed with an objective of 50×. The red spot at the upper-left portion of the cell is considered to be the eyespot. The 14 × 17 μm rectangle indicates the area subjected to our present Raman mapping. At each of the 270 points corresponding to 1 μm intervals across the y = 0 μm line, y = 1 μm line, y = 2 μm line, etc., to y = 17 μm line, the

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Chlamydomonas

Fig. 5. Polarized Raman spectra (xx), (yy), (xy), and (yx) of the same cell shown in Fig. 4, at the spot (x,y) = (11,5). Here, (xy) means, for example, that the electric vector of the exciting beam is placed along the x-axis, and the electric vector of the scattering beam is placed along the y-axis.

Fig. 7. Polarized Raman spectra (xx), (yy), (xy), and (yx) of the same cell shown in Fig. 5, at the spot (x,y) = (6,7). Here, (xy) means, for example, that the electric vector of the exciting beam is placed along the x-axis, and the electric vector of the scattering beam is placed along the y-axis.