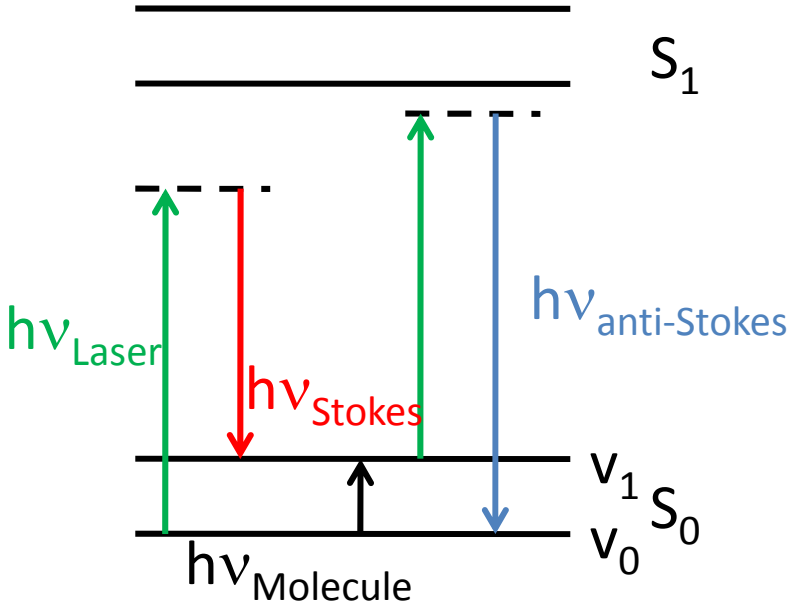
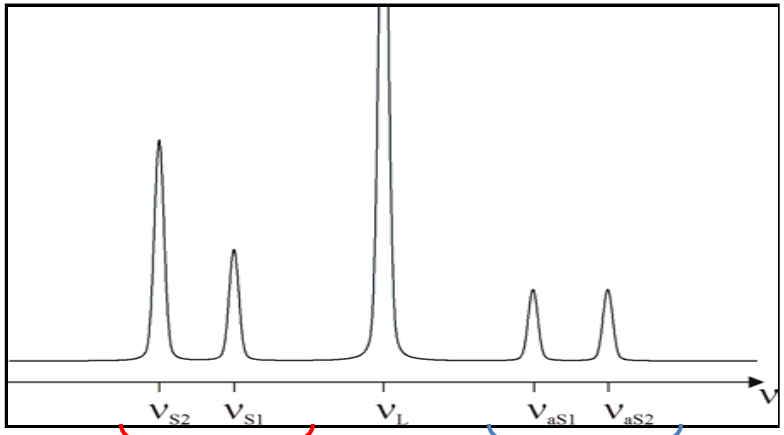
Raman of plant tissues

Janina Kneipp

Raman scattering and **resonant** Raman scattering





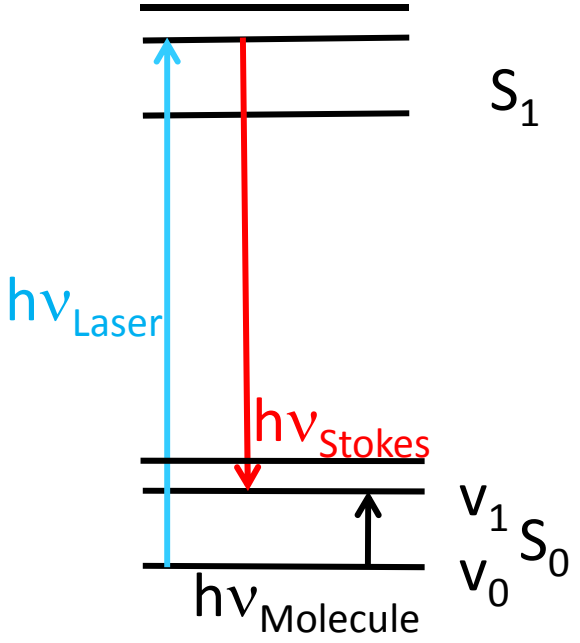
Stokes

$\nu_{Laser} - \nu_{Molecule}$

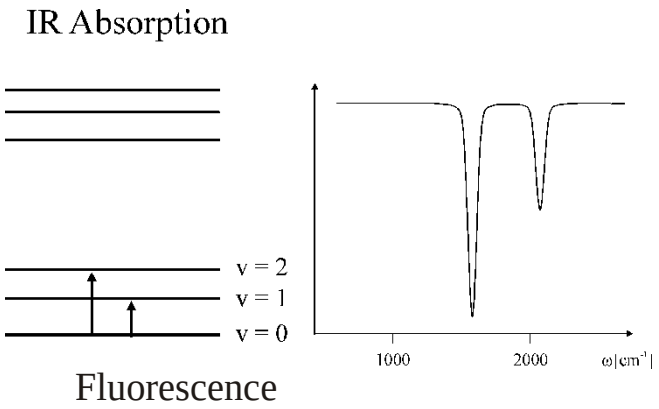
anti-Stokes

$\nu_{Laser} + \nu_{Molecule}$

resonant Raman scattering

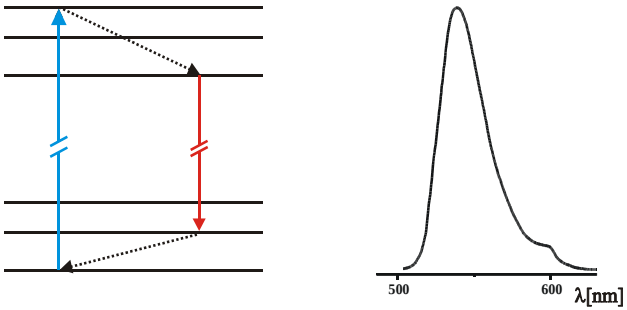


Cross-sections considered



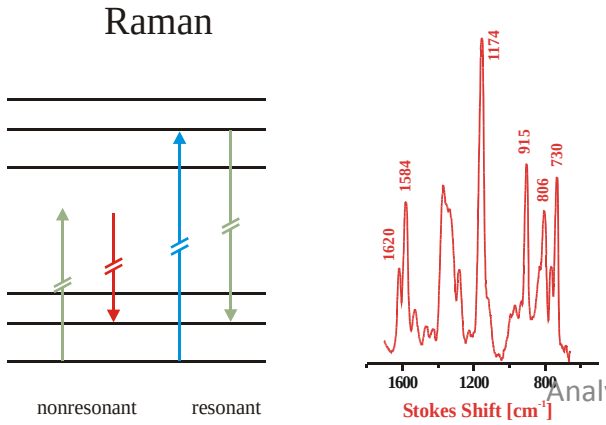
IR absorption

$\sigma_{abs} \approx 10^{-21} - 10^{-18} cm^2$



Fluorescence

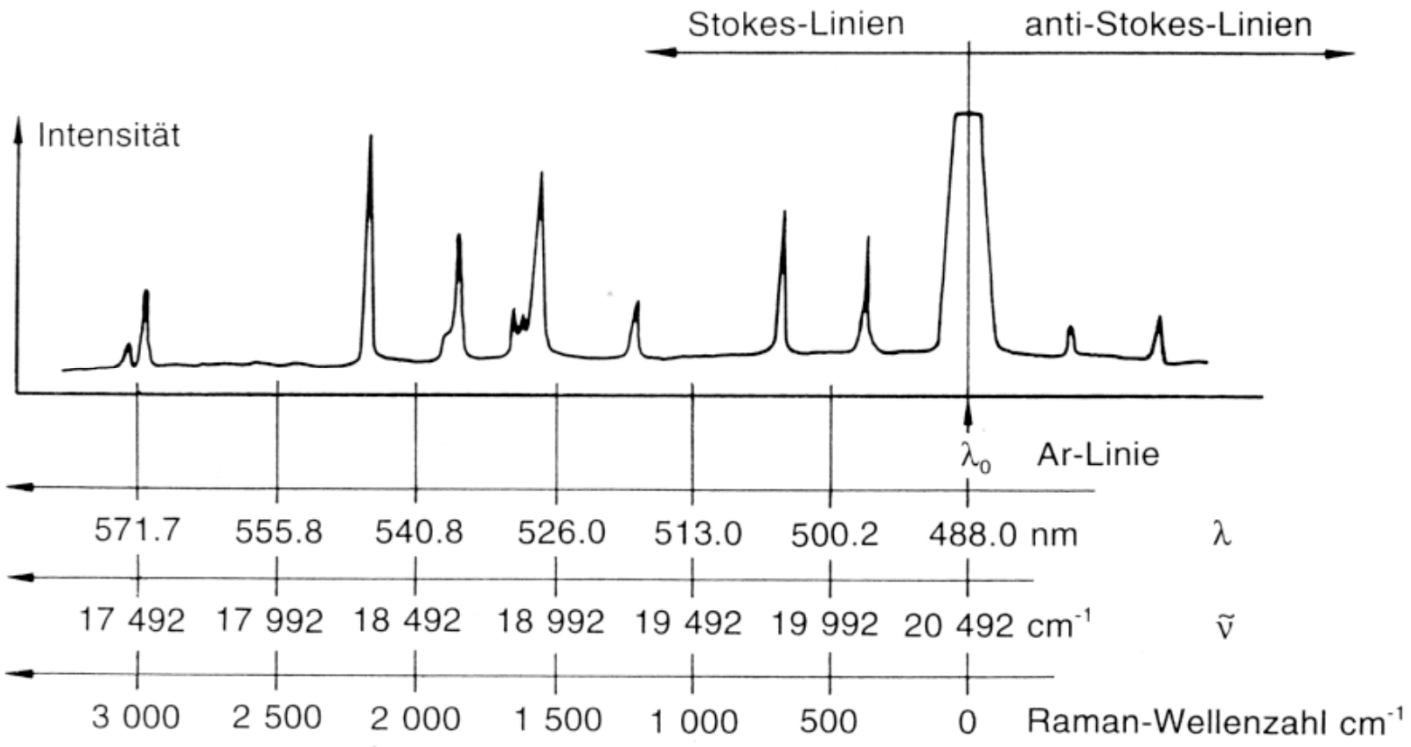
$\sigma_{FL} \approx 10^{-17} - 10^{-16}\ cm^2$



Raman scattering

$\sigma_{RS} \approx 10^{-30} - 10^{-26}\ cm^2$

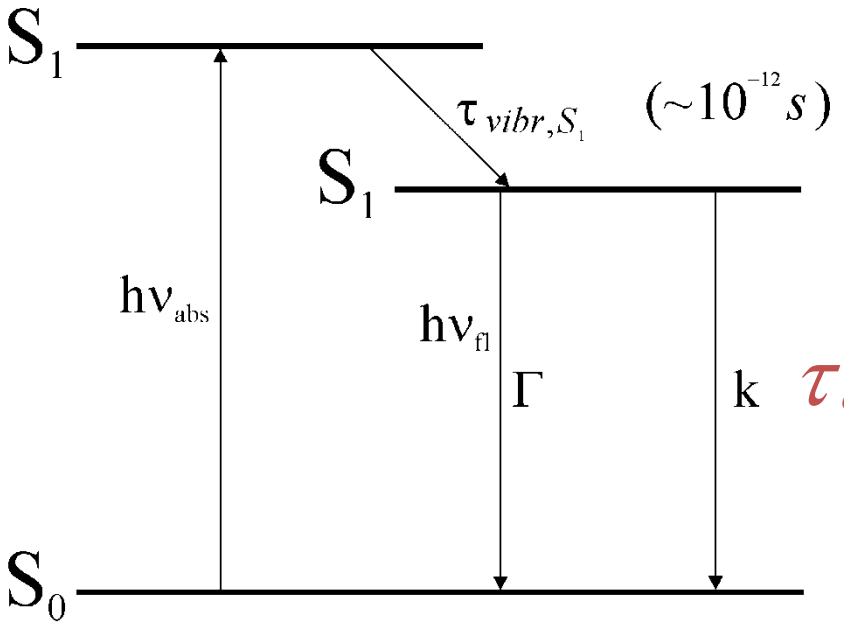
Raman shift with respect to λ_{ex} = 488 nm



Raman shift (cm^{-1})

Richard L. McCreery “Raman Spectroscopy for Chemical Analysis” Copyright © 2000 John Wiley & Sons, Inc.

Lifetimes and time resolved detection

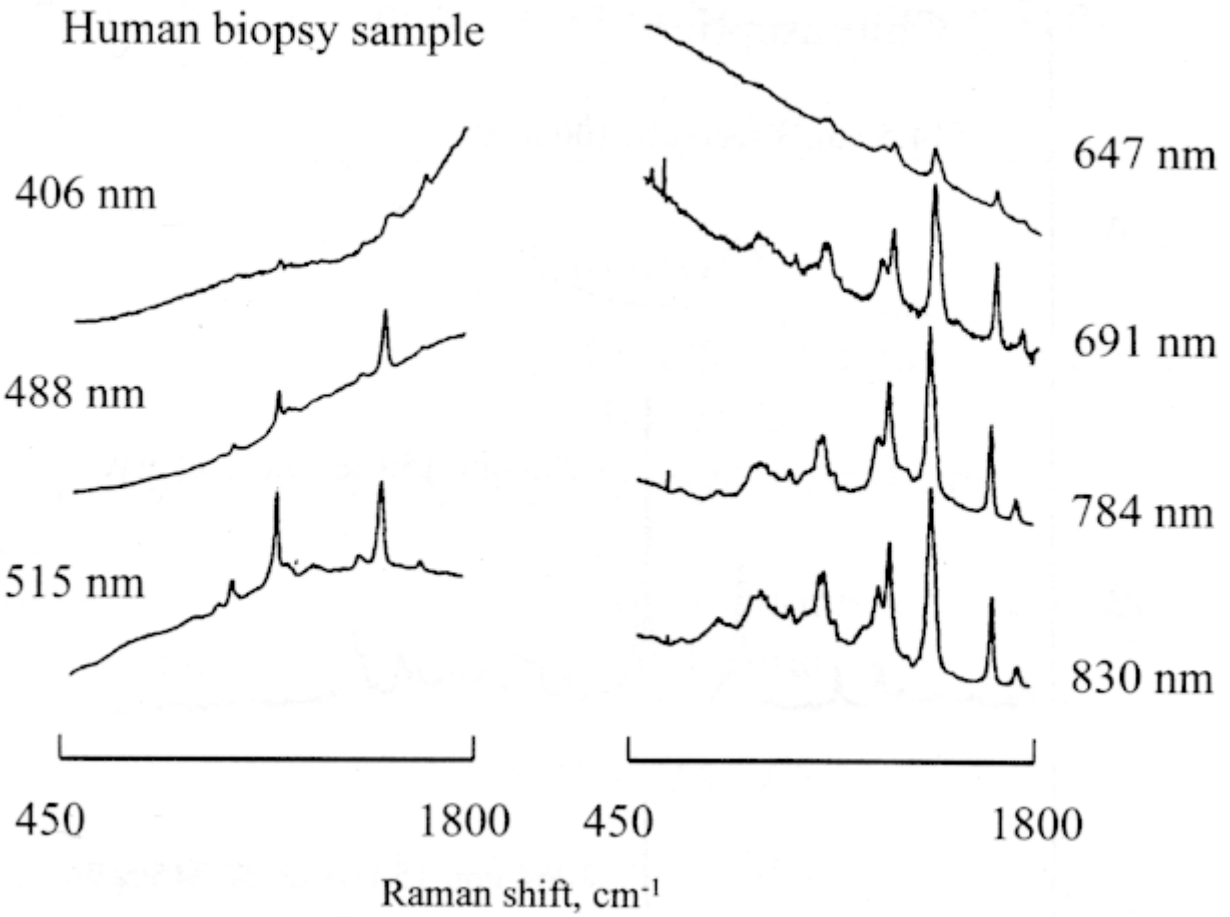


$\tau_{el} \sim ns$

$$\tau = \frac{1}{\Gamma + k}$$

It is possible to separate Raman from fluorescence photons using a „gate“!

What is a good excitation wavelength?



fluorescence

pre-resonant RS

resonant RS

normal RS

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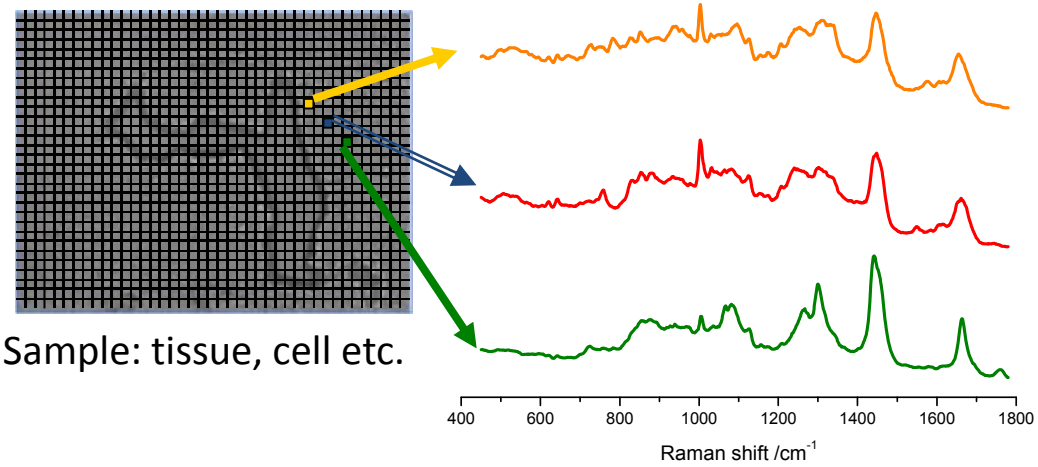
Correlating spectral with spatial information: Microspectroscopic Mapping and Imaging

(1) Microspectroscopic mapping



Sample: tissue, cell etc.

(2) Spectral analysis

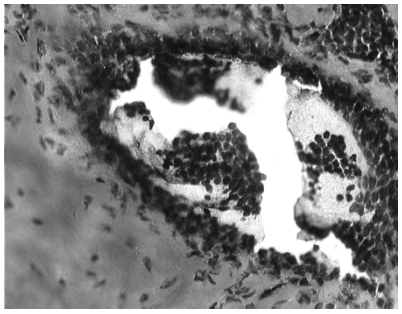
Single spectral parameters

- intensities, ratios, band correlations
- vibrational frequency & shifts

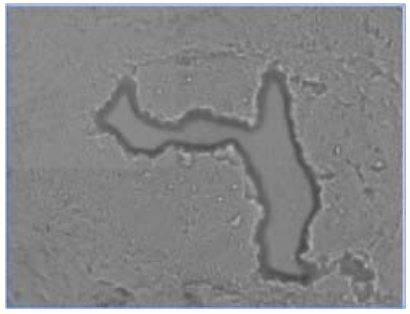
Multivariate information

- Spectrum = vector, pattern
- Principal components, cluster analysis, artificial neural networks

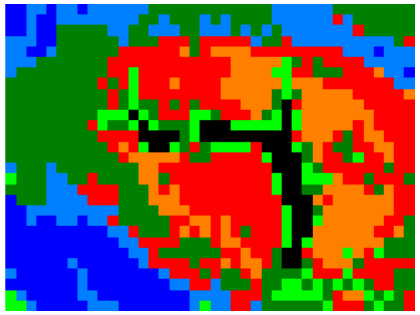
(3) Molecular Image: Combination of spectral parameters and spatial coordinates



H&E

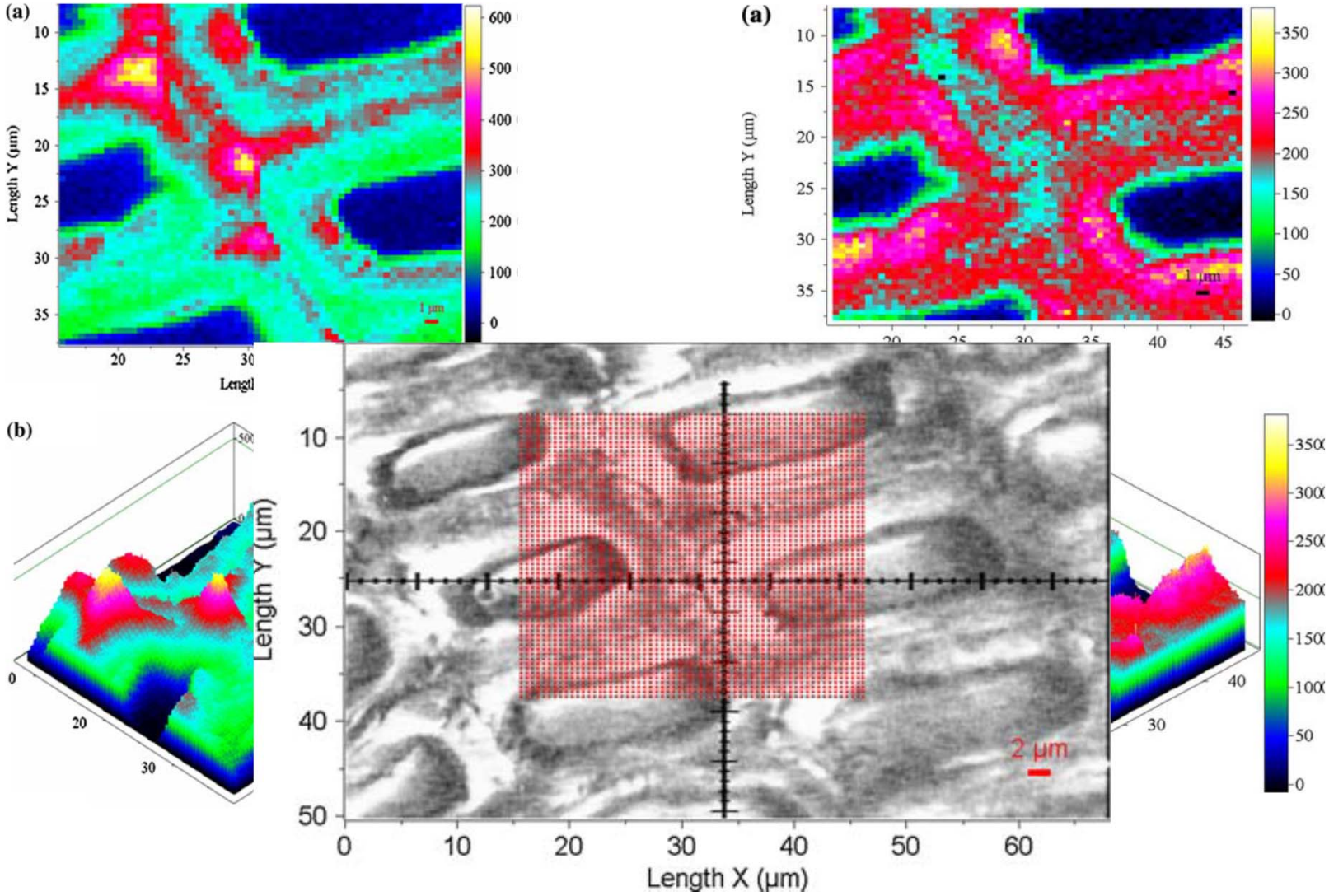


unstained



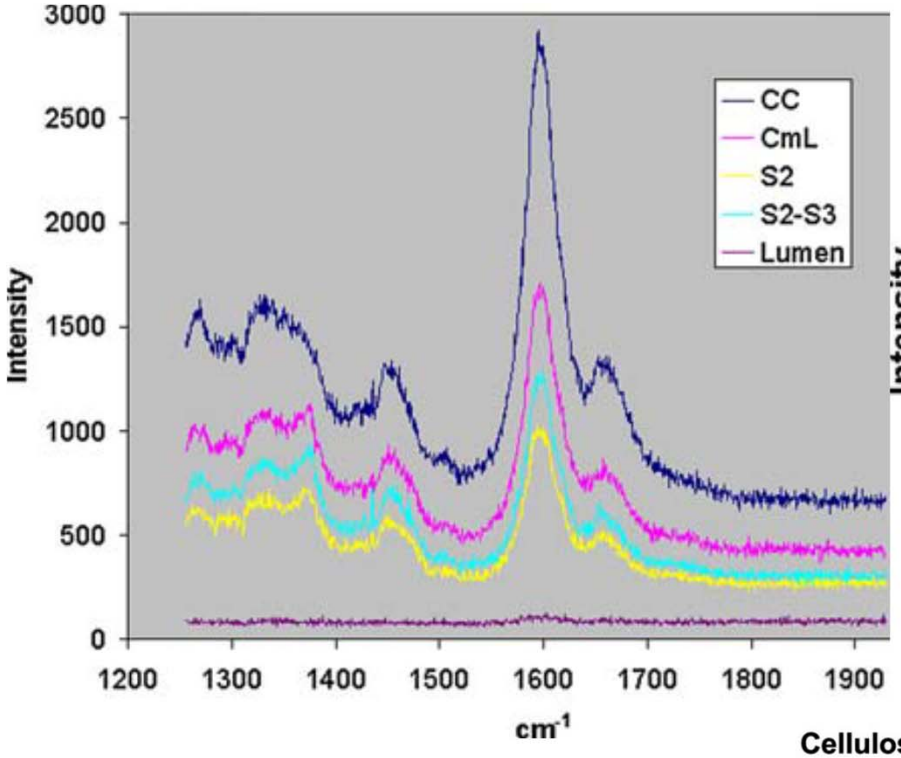
Raman image (e.g. K-means cluster)

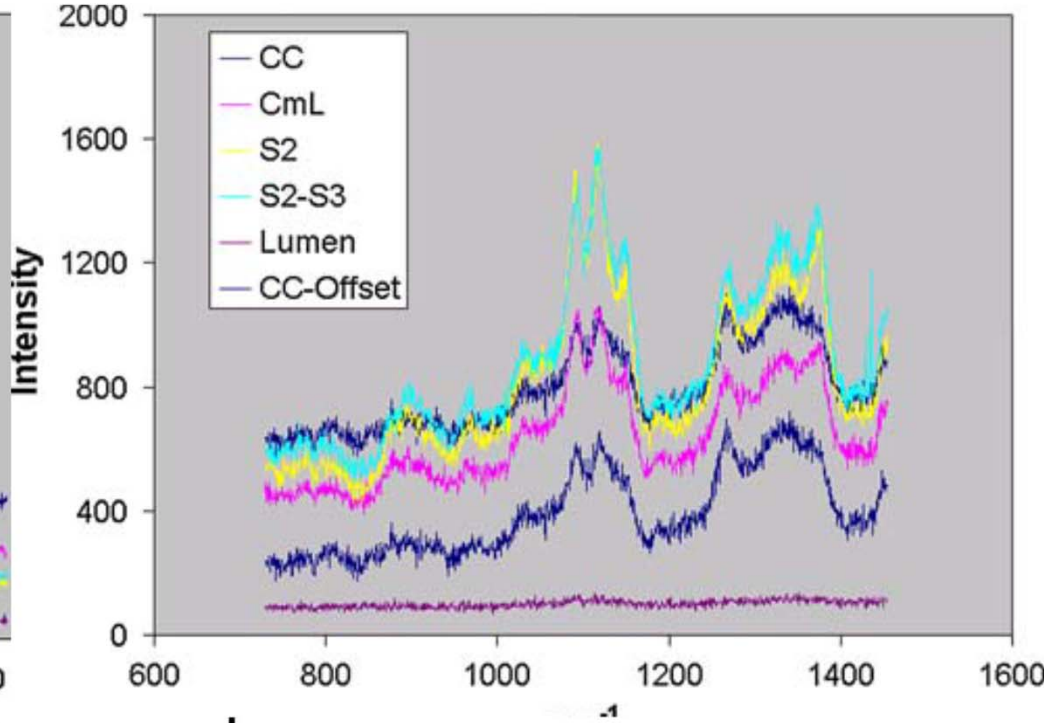
Lignin and cellulose distribution



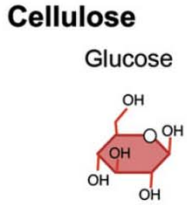
Agarwal, U. P. (2006). Raman imaging to investigate ultrastructure and composition of plant cell walls: distribution of lignin and cellulose in black spruce wood (Picea mariana). *Planta, 224(5), 1141-1153.*

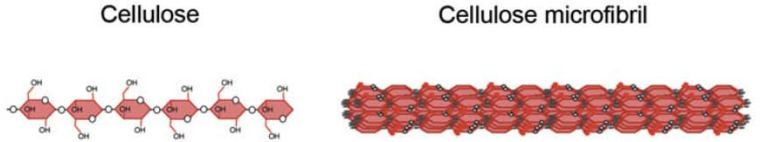
Lignin and cellulose spectra

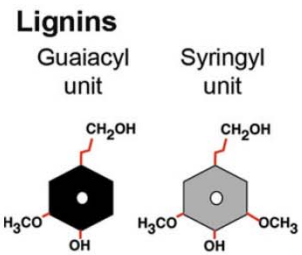


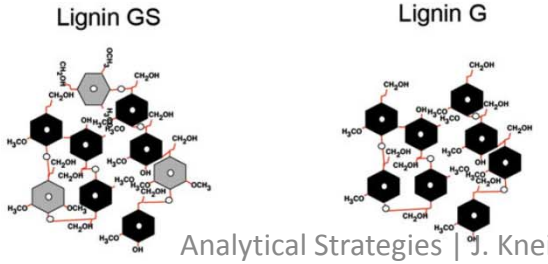


Agarwal, U. P. (2006) *Planta, 224(5), 1141-1153.*





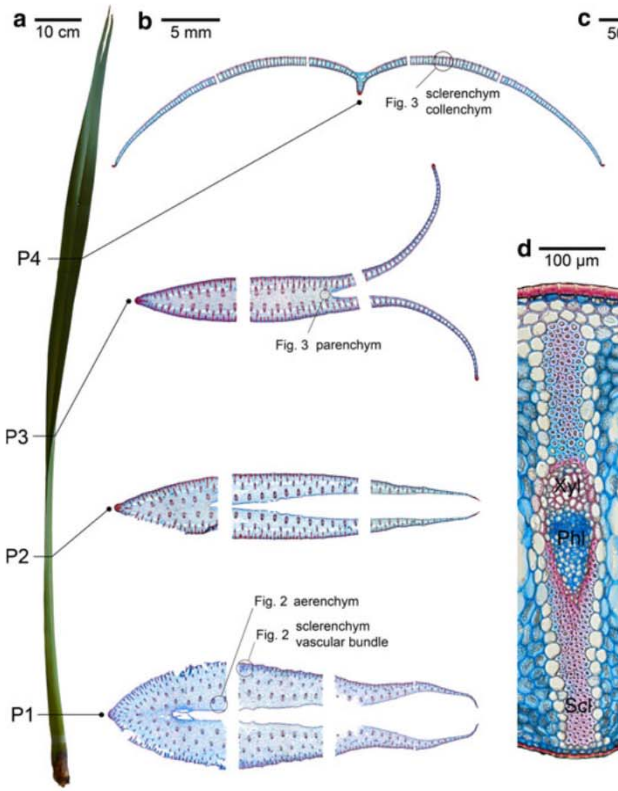


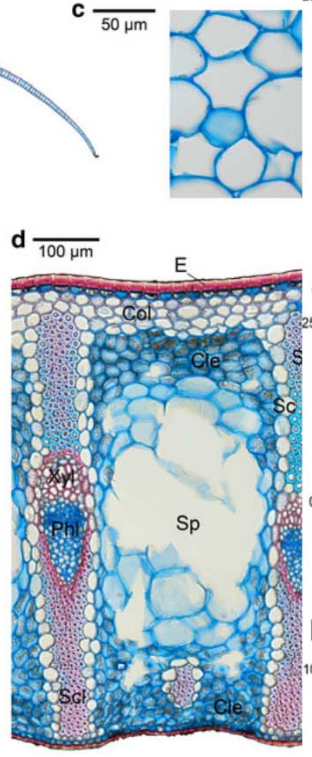


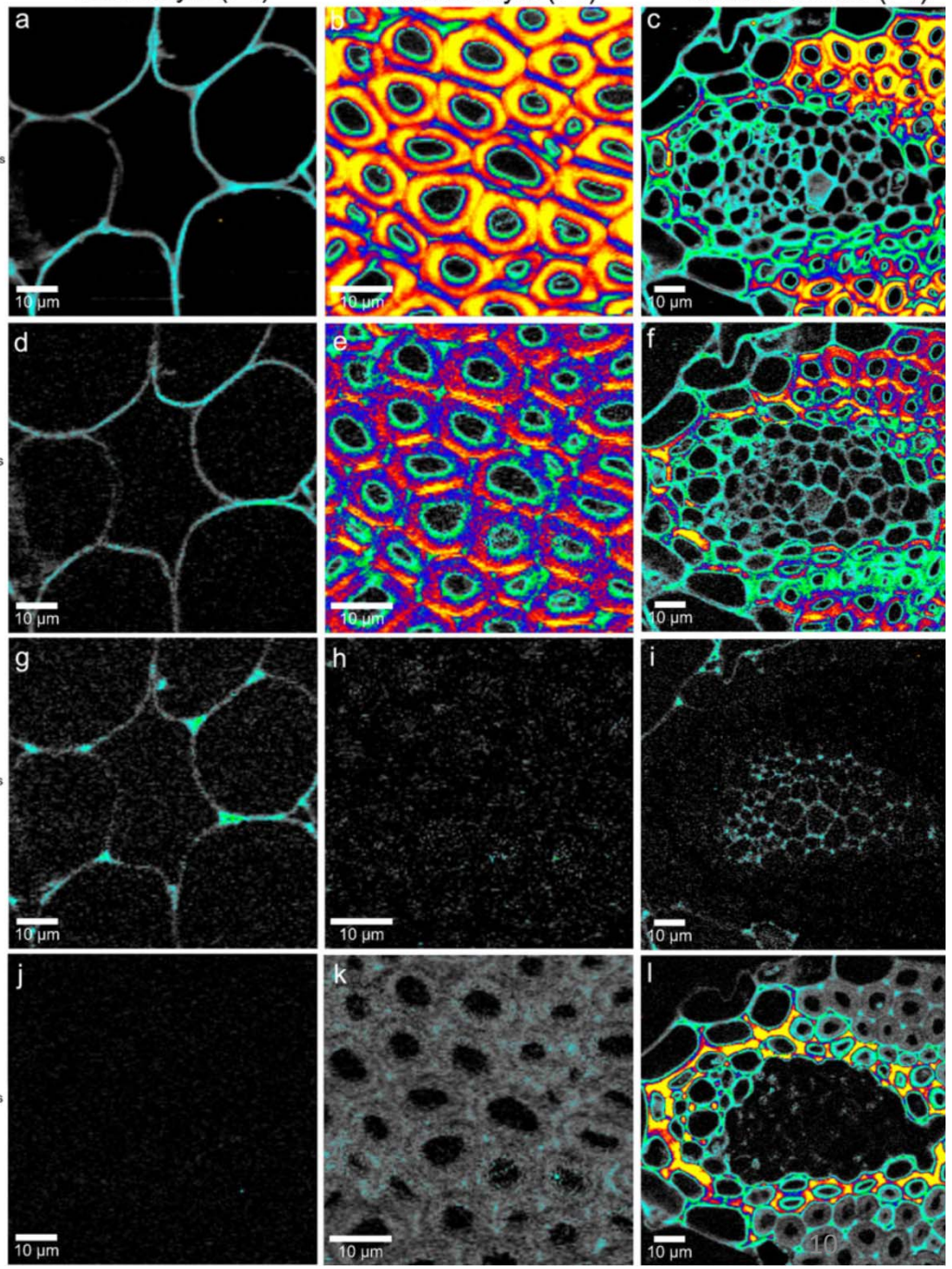
Sarkar, P.. (2009) *Journal of Experimental Botany, 60, 3615-3635.*

Raman

Lignin staining

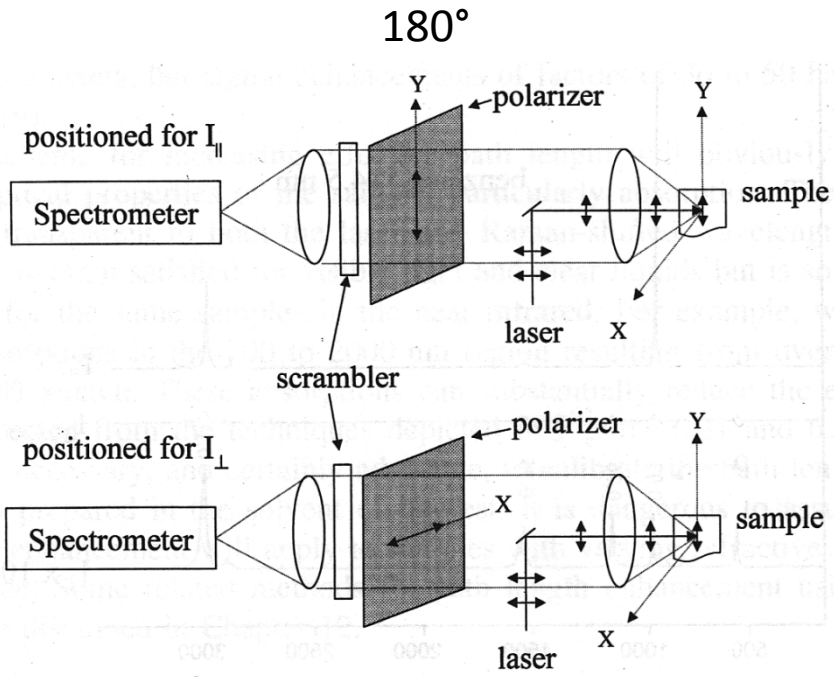


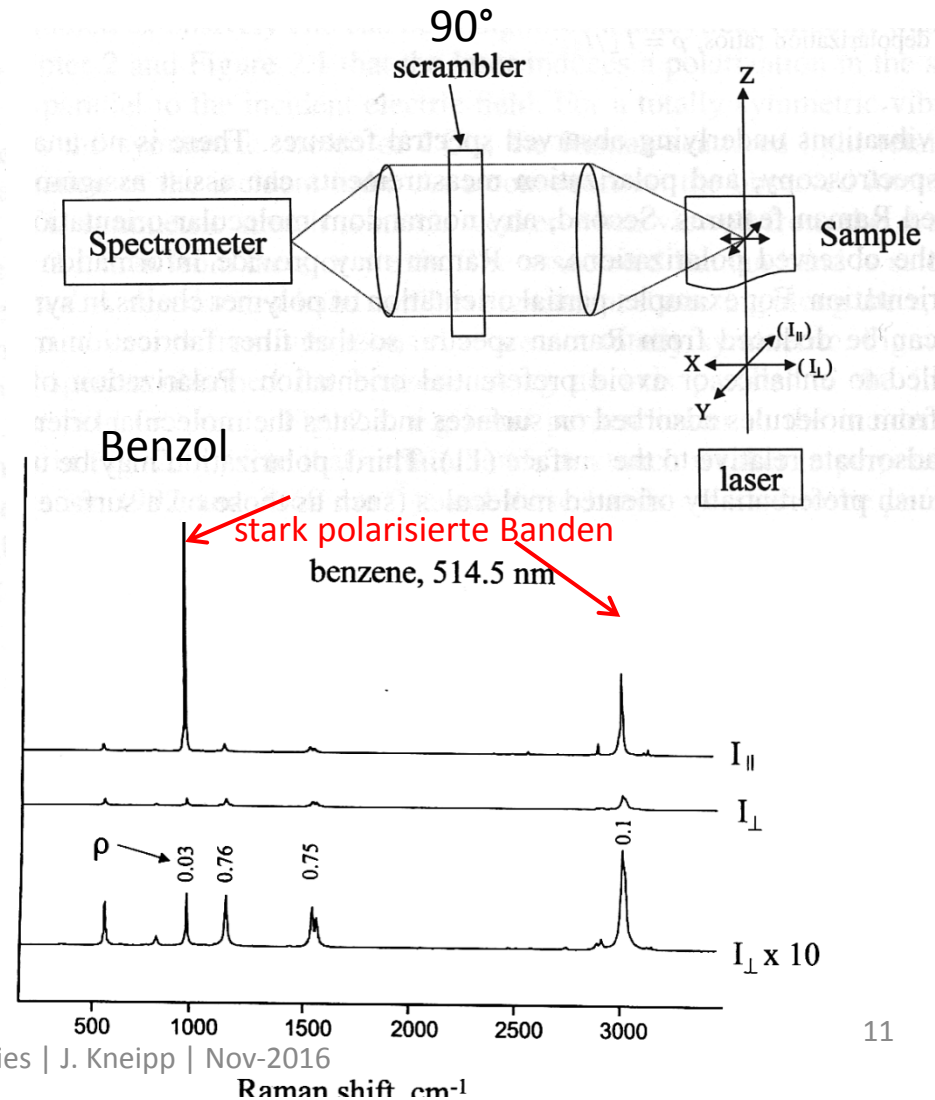




Richter, S., Mussig, J., & Gierlinger, N. (2011). Functional plant cell wall design revealed by the Raman imaging approach. *Planta, 233(4), 763-772.*

Polarization dependence of Raman scattering

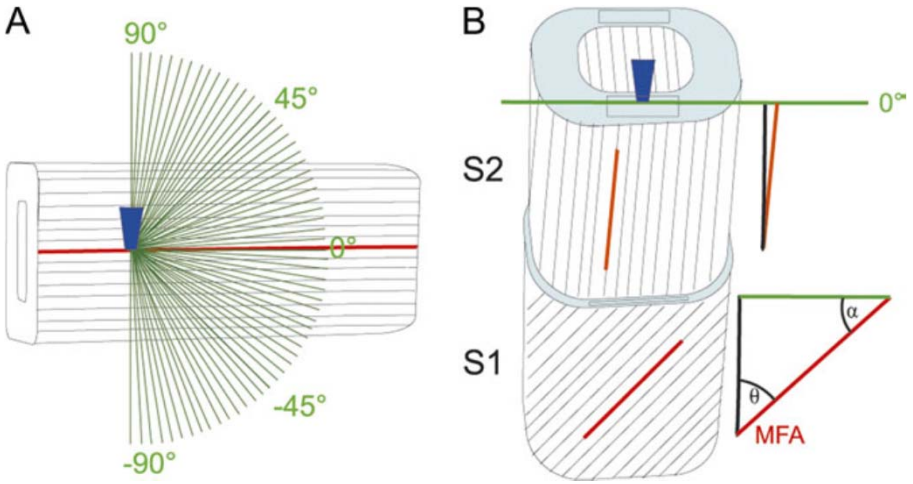


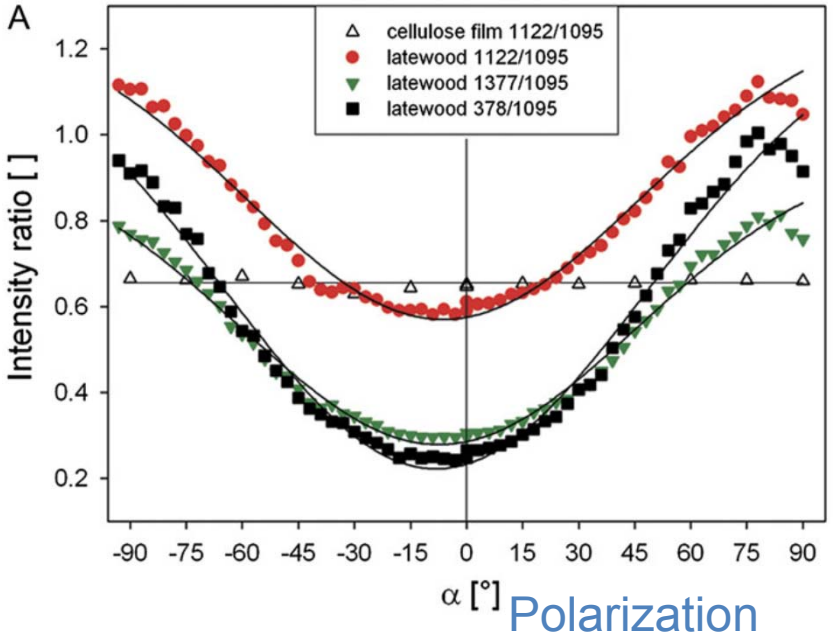


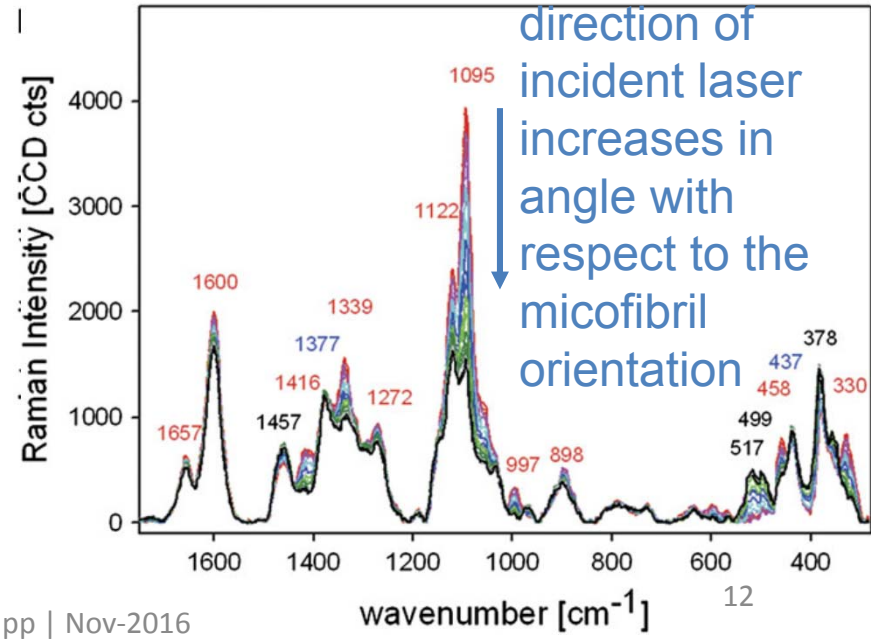
Depolarisationsgrad ρ

$$\rho = \frac{I_{\perp}}{I_{\parallel}}$$

Information on cellulose orientation







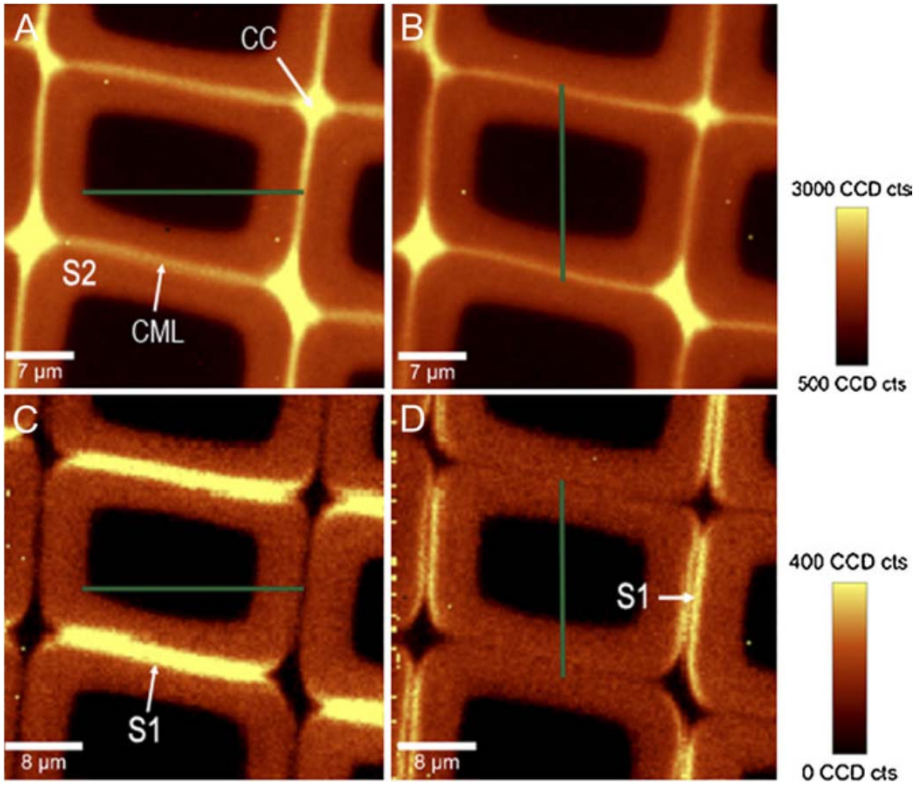
Polarization direction of incident laser increases in angle with respect to the micofibril orientation

Gierlinger, N., Luss, S., Konig, C., Konnerth, J., Eder, M., & Fratzl, P. (2010). Cellulose microfibril orientation of Picea abies and its variability at the micron-level determined by Raman imaging. *Journal of Experimental Botany, 61(2), 587-595.*

Lignin signals are not sensitive to polarization

Lignin signals

Cellulose S1 layer with high microfibril angle



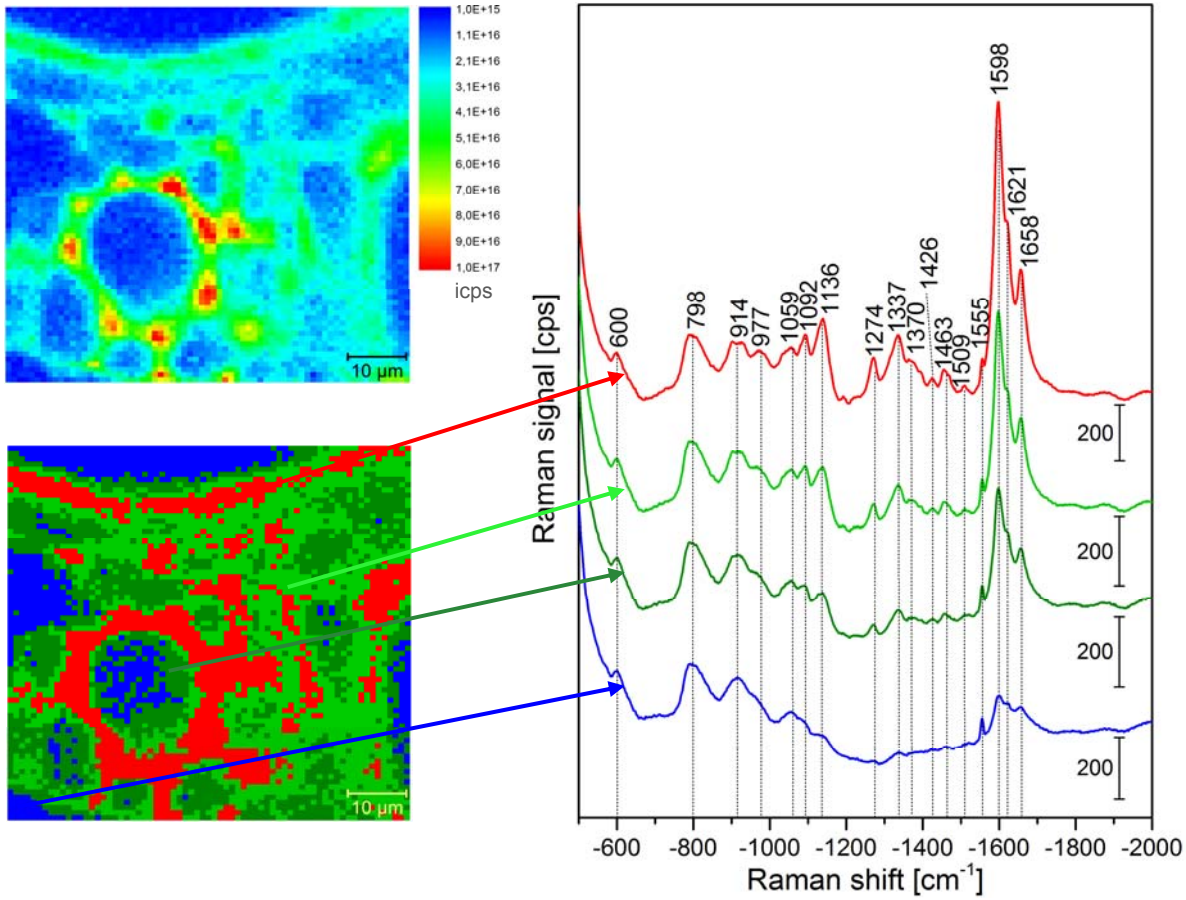
Gierlinger, N., Luss, S., Konig, C., Konnerth, J., Eder, M., & Fratzl, P. (2010). Cellulose microfibril orientation of Picea abies and its variability at the micron-level determined by Raman imaging. *Journal of Experimental Botany, 61(2), 587-595.*

JK1

Ongoing work: Raman spectroscopic imaging of cucumber root section

Chemical map of lignin distribution

Hyperspectral map, obtained using information from all molecules



excitation laser wavelength: 532 nm

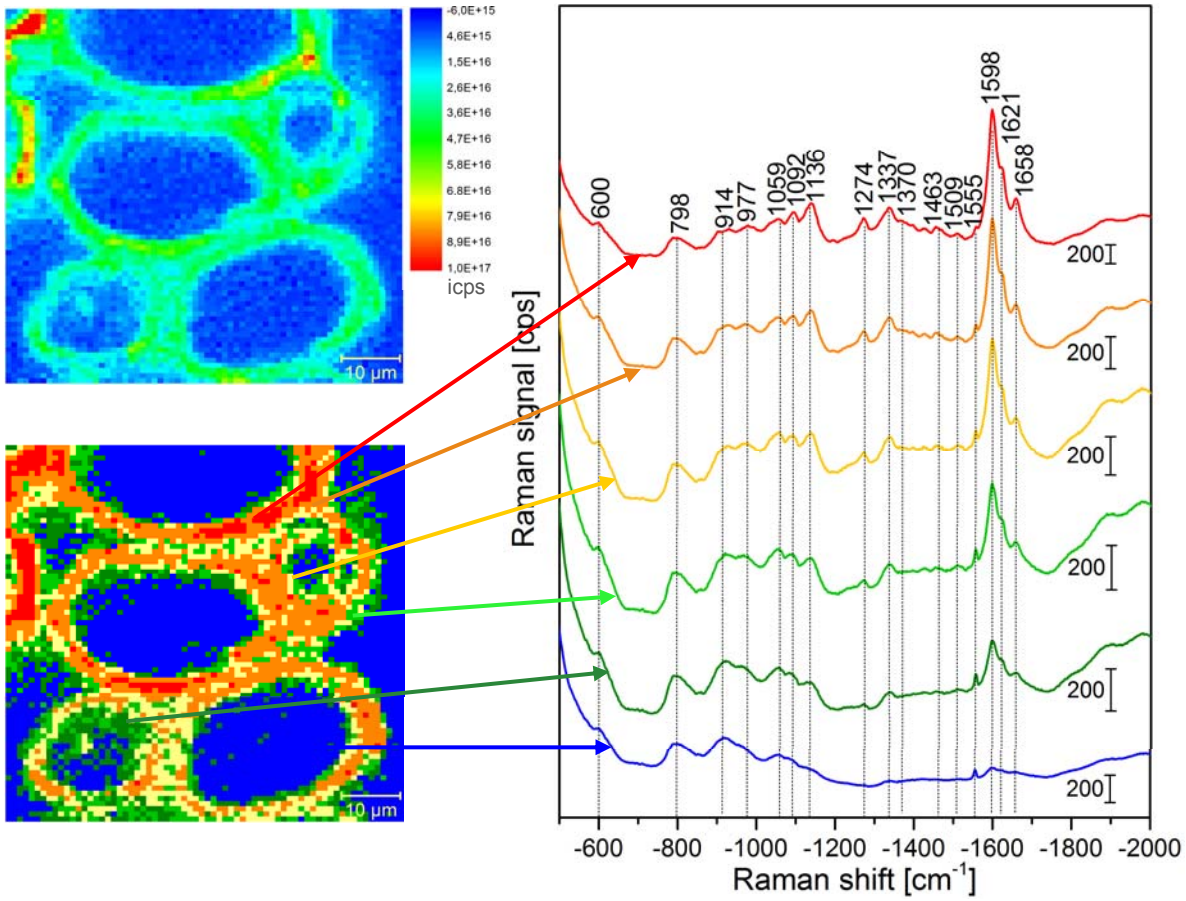
I. Zeise, Zs. Heiner *et al.*, in preparation

Ongoing work: Raman spectroscopic imaging of cucumber stem section

Characteristic Raman spectra

Chemical map of lignin distribution

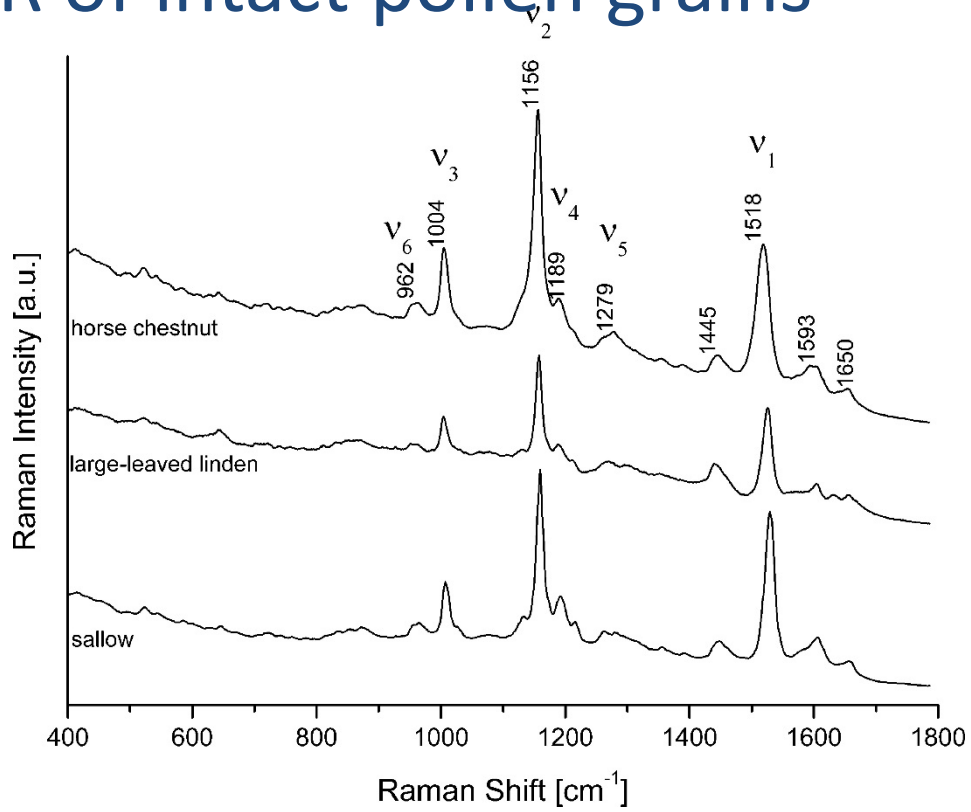
Hyperspectral map, obtained using information from all molecules



I. Zeise, Zs. Heiner *et al.*, in preparation

excitation laser wavelength: 532 nm

RR of intact pollen grains



Raman spectra of individual untreated pollen grains from horse chestnut, large-leaved linden, and sallow, excited with 785 nm (10^6 W/cm2, acquisition time 1 s).

Published in: Franziska Schulte; Jens Mäder; Lothar W. Kroh; Ulrich Panne; Janina Kneipp; *Anal. Chem.* **2009,** 81, 8426-8433.
DOI: 10.1021/ac901389p
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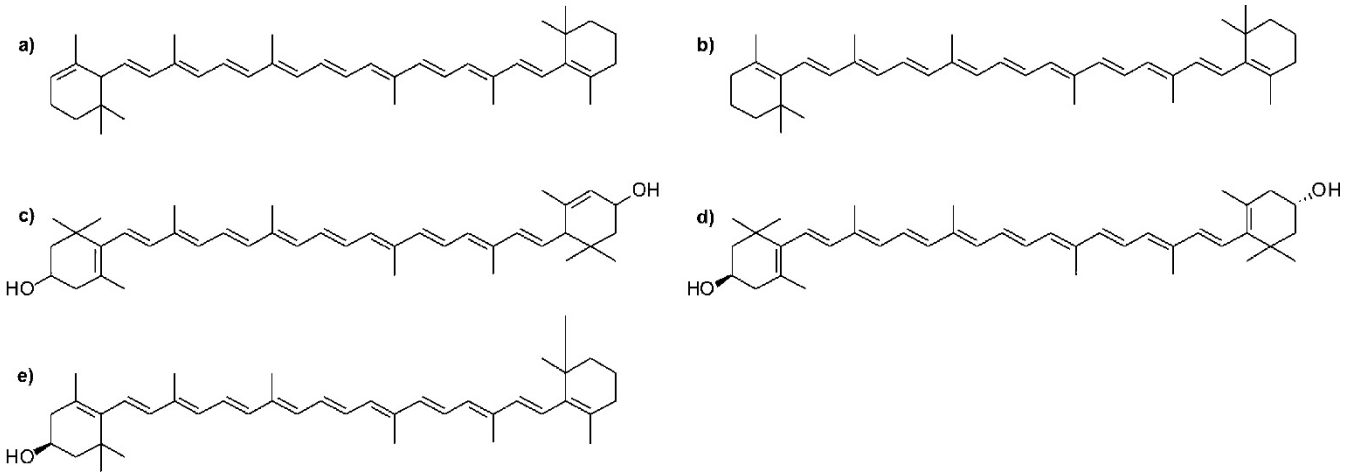
Similar molecules, similar spectra?



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

Similar molecules, similar spectra?

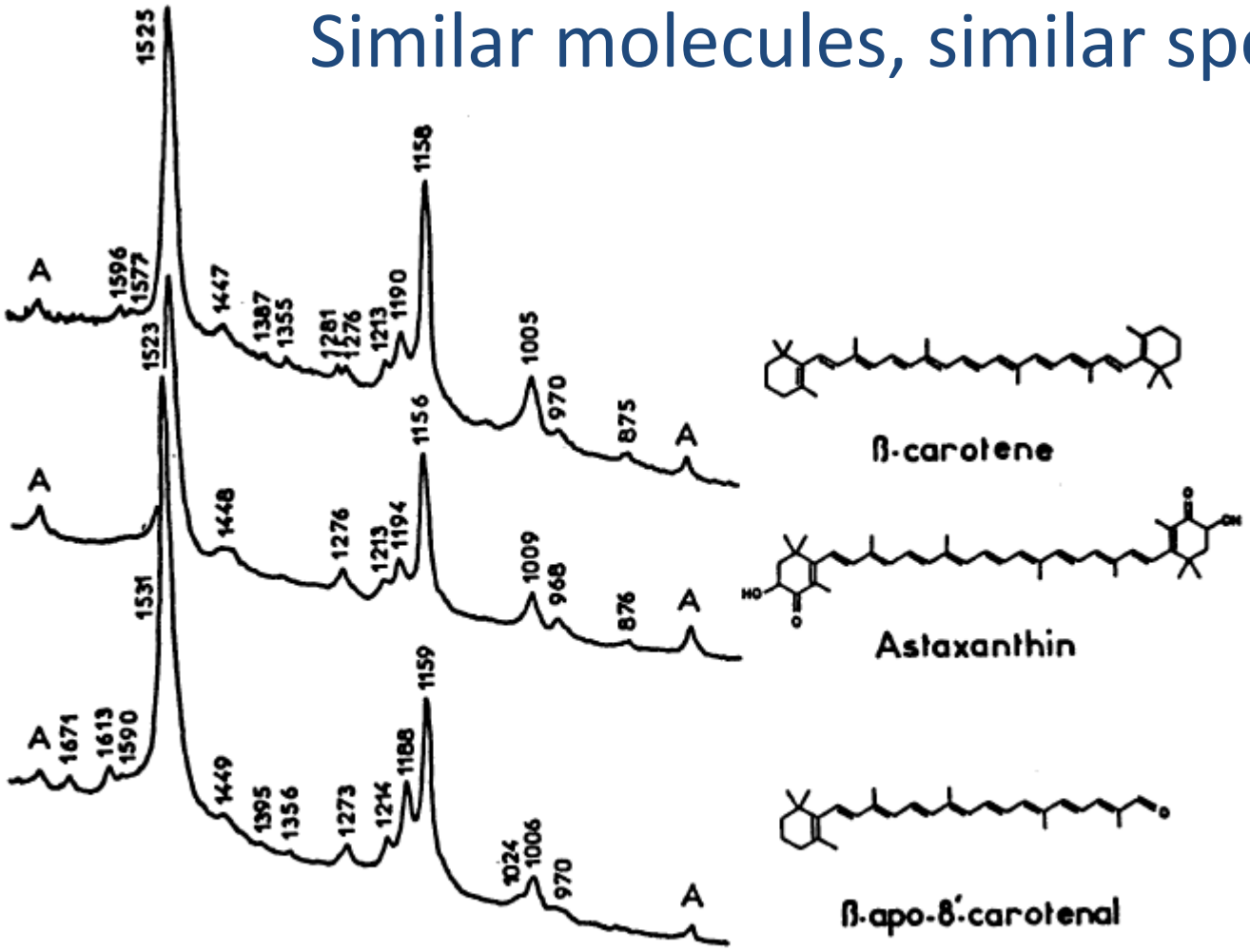


Fig. 1. Typical RR spectra of carotenoids in acetone with 488 nm excitation. Acetone bands are marked A.

J.C. Merlin *Pure & Appl. Chem.*, Vol. 57, No. 5, pp. 785–792, 1985.

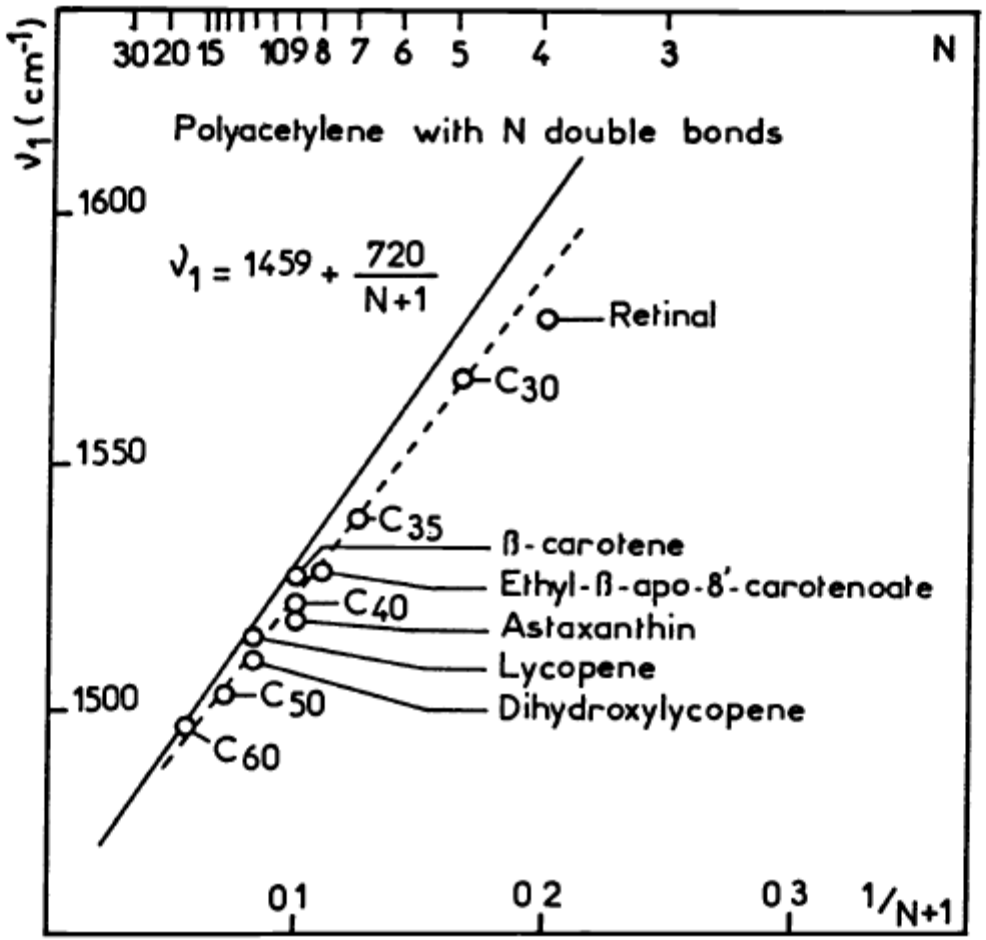


Fig. 2. Relation between C=C stretching frequency (ν_1) and the number of double bonds for polyacetylenes and carotenoids.

J.C. Merlin *Pure & Appl. Chem.*, Vol. 57, No. 5, pp. 785–792, 1985.

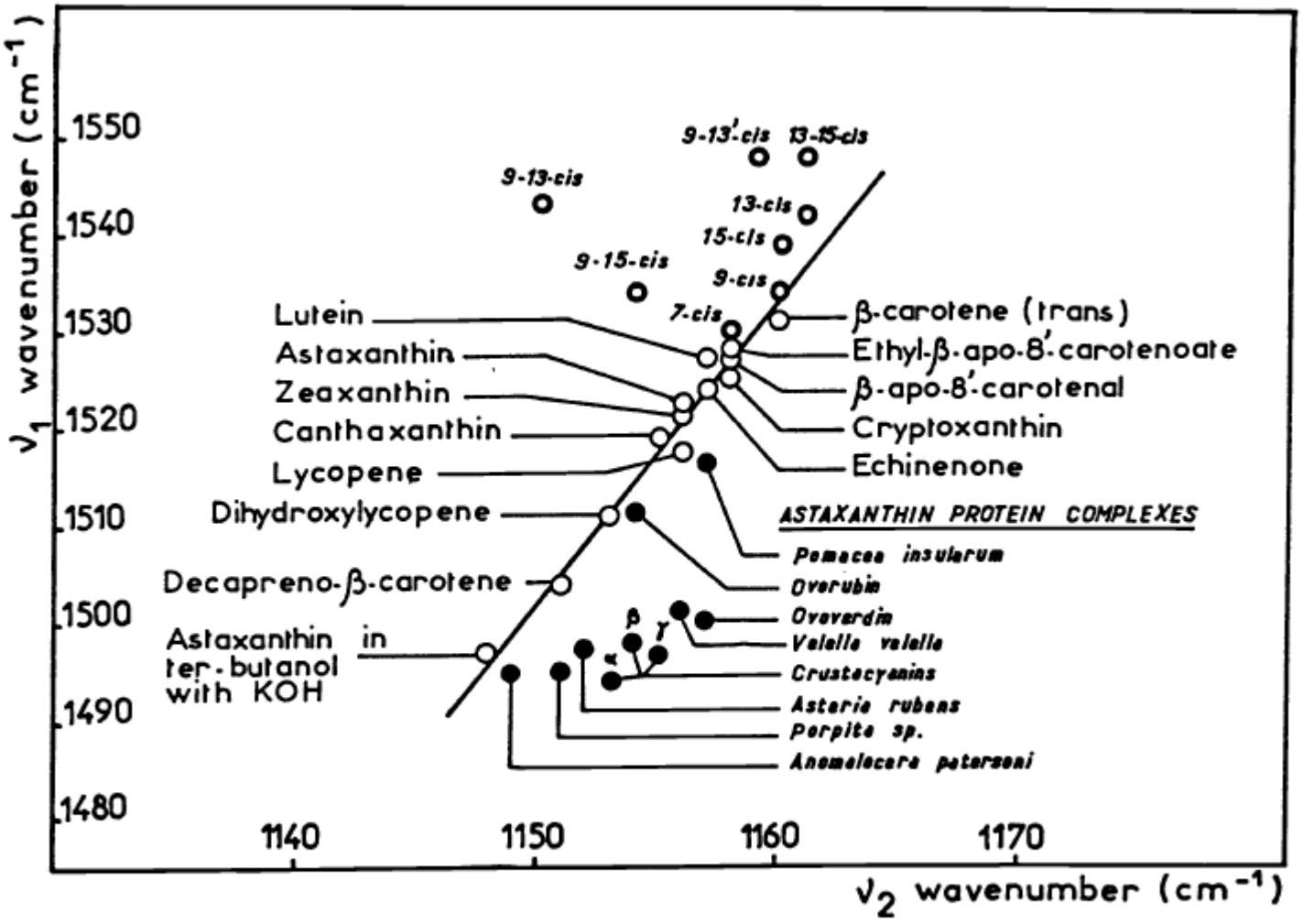


Fig. 3. Plot of C=C stretching frequency (ν_1) vs. C–C stretching frequency (ν_2). Values for β-carotene isomers provide from Ref. 20, values for astaxanthin-protein complexes are obtained from Ref. 25-29.

J.C. Merlin *Pure & Appl. Chem.*, Vol. 57, No. 5, pp. 785–792, 1985.

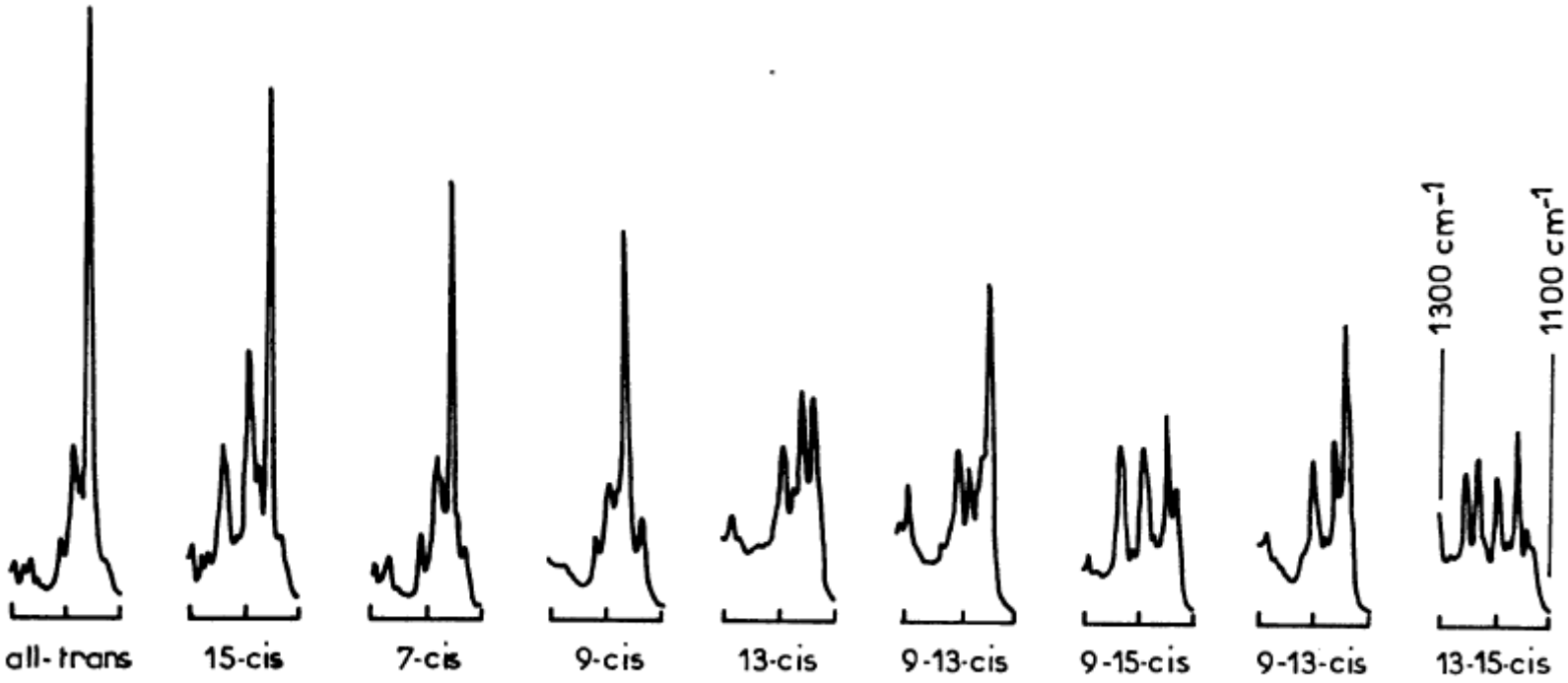
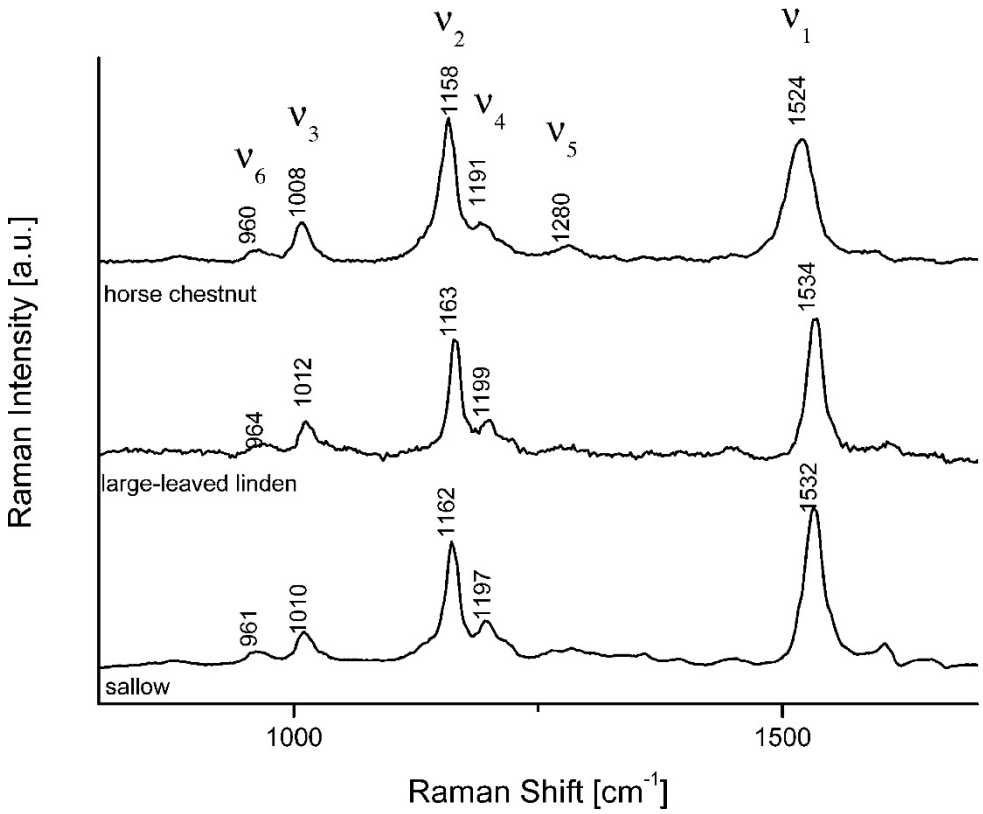


Fig. 4. Raman lines of β-carotene isomers in the 1100-1300 cm^{-1} region. From Y. Koyama, T. Kakii, K. Saiki and K. Tsukida (Ref. 20)

J.C. Merlin *Pure & Appl. Chem.*, Vol. 57, No. 5, pp. 785–792, 1985.

In situ difference spectra



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/cm2).

Published in: Franziska Schulte; Jens Mäder; Lothar W. Kroh; Ulrich Panne; Janina Kneipp; *Anal. Chem.* **2009,** 81, 8426-8433.
DOI: 10.1021/ac901389p
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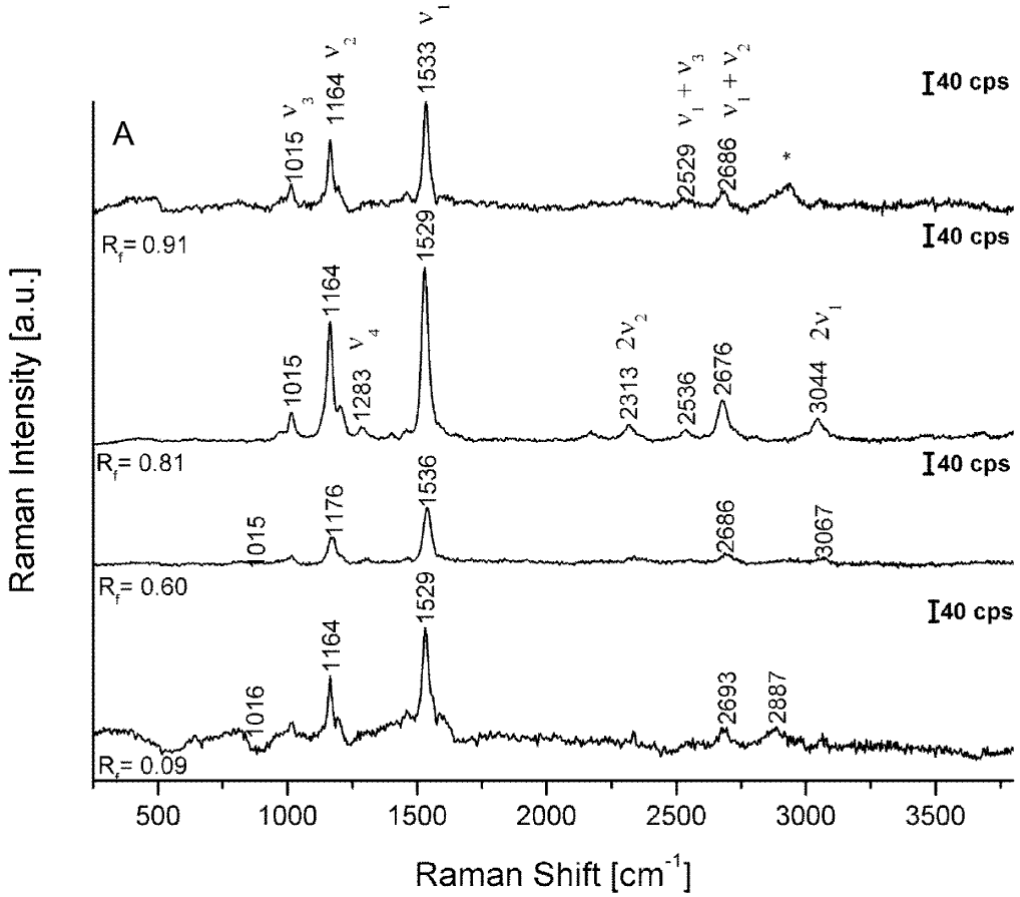
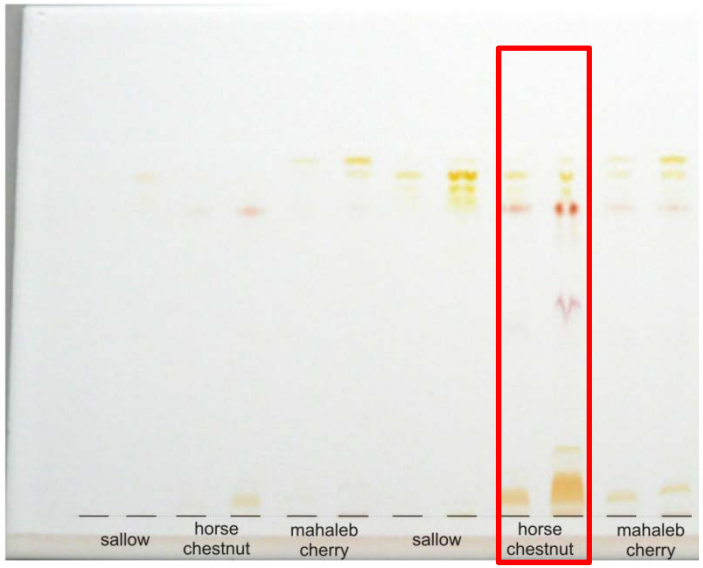
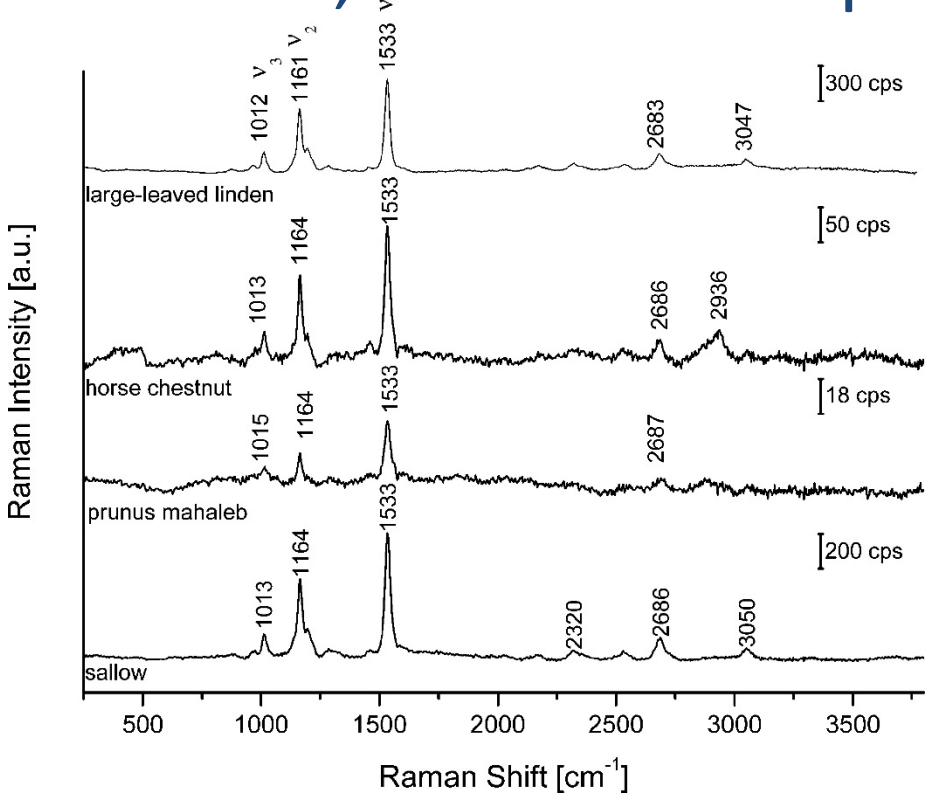
Combination of resonance Raman with HPTLC





F. Schulte et al. *Anal. Chem.,* **81** (2009) 8426-8433

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, ~10^2 W/cm2).

Published in: Franziska Schulte; Jens Mäder; Lothar W. Kroh; Ulrich Panne; Janina Kneipp; *Anal. Chem.* **2009,** 81, 8426-8433.
DOI: 10.1021/ac901389p
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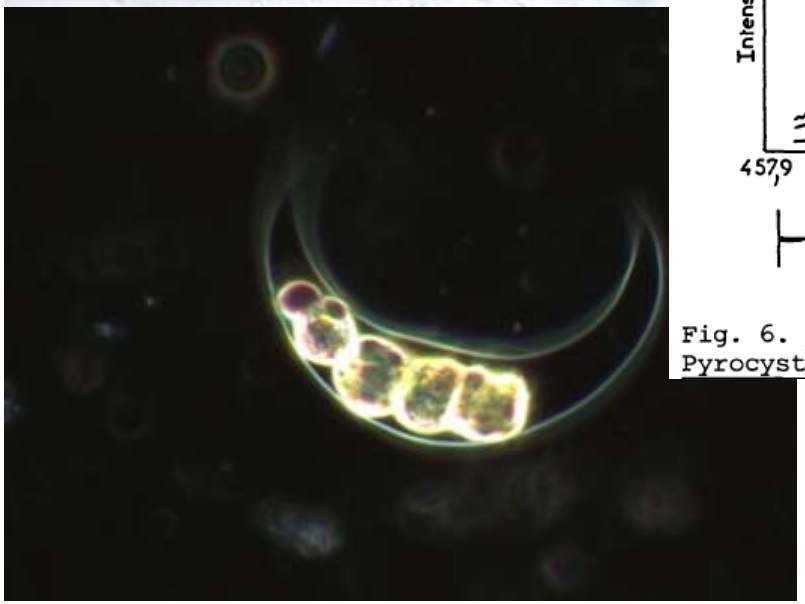
Pyrocystis lunula



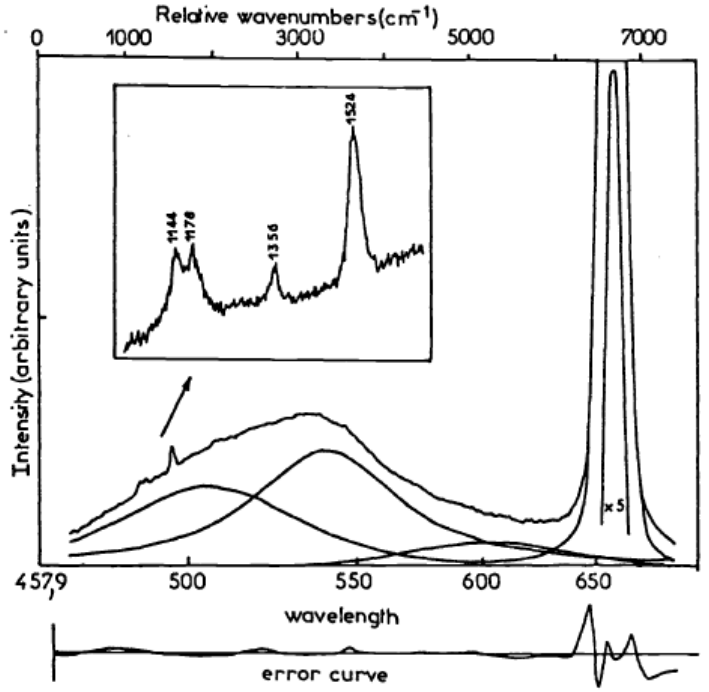


Fig. 6. In vivo fluorescence and RR spectrum (frame insert) of single Pyrocystis lunula cell (right) with 457.9 nm excitation.

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Euglena

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

YOSHIKO KUBO, TERUKI IKEDA,* SHI-YUAN YANG, and MASAMICHI TSUBOI

JASCO Corporation, 2967-5, Ishikawa-cho, Hachioji, Tokyo 192-8537, Japan (Y.K., T.I.); and Department of Fundamental Science, Iwaki-Meisei University, Iwaki, Fukushima 970-8551, Japan (S.-Y.Y., M.T.)

Applied Spectroscopy 2001

FIG. 2. Micrograph of a *Euglena* cell. At the left edge, a flagellum is seen.

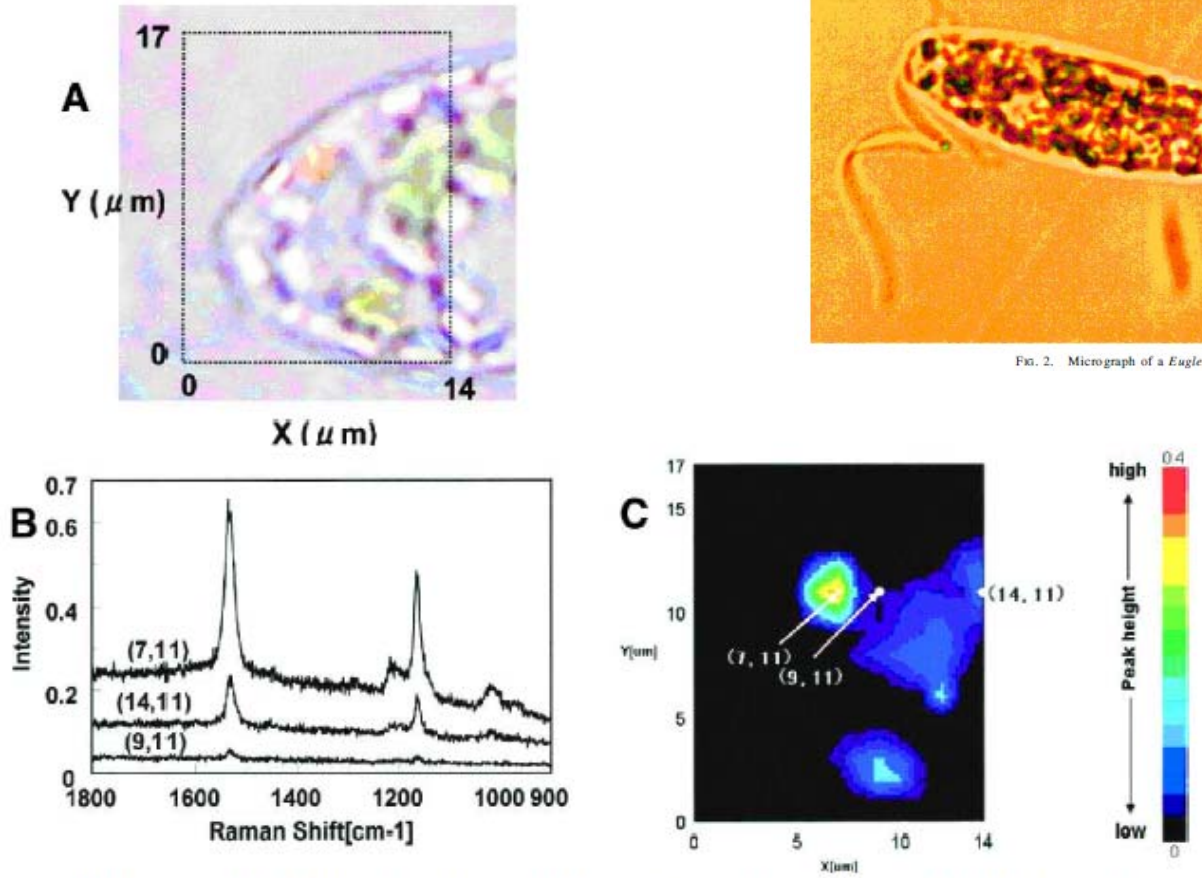

FIG. 3. (A) Micrograph of the left half (with the flagellum edge) of a *Euglena* cell observed with an objective of 50×. The red spot at the upper-left portion of the cell is considered to be the eyespot. The 14 × 17 μm rectangle indicates the area subjected to our present Raman mapping. At each of the 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

Chlamydomonas

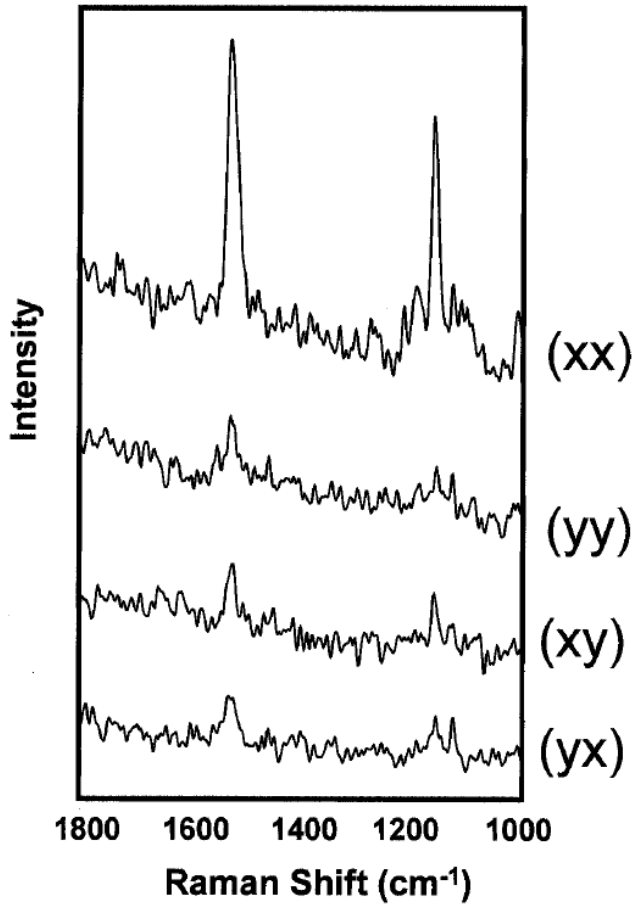

FIG. 7. Polarized Raman spectra (xx), (yy), (xy), and (yx) of the same cell shown in Fig. 5, at the spot (x,y) = (6,7). Here, (xy) means, for example, that the electric vector of the exciting beam is placed along the x-axis, and the electric vector of the scattering beam is placed along the y-axis.

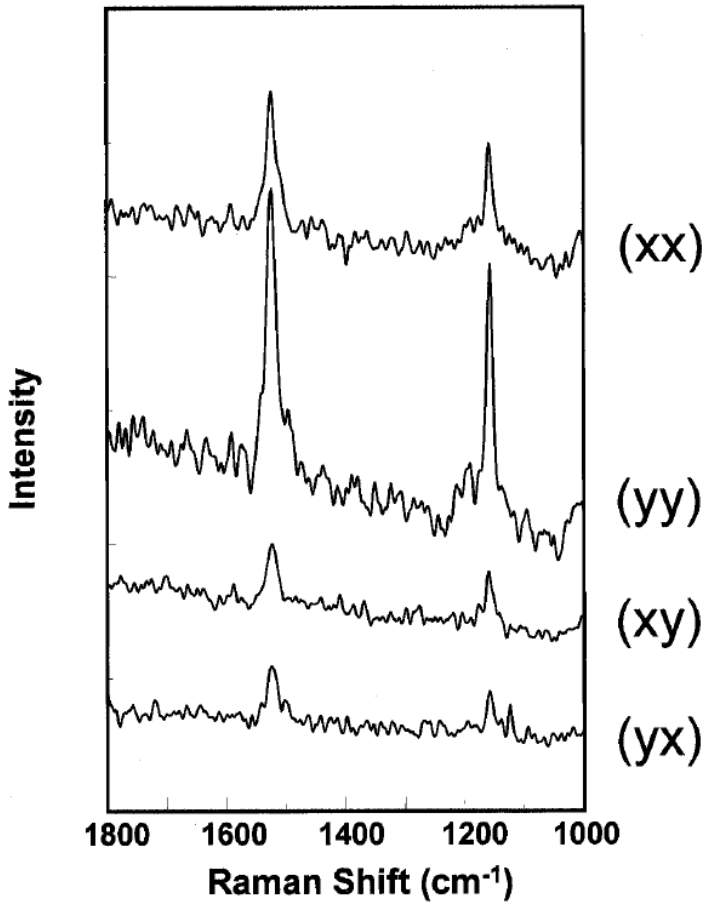

FIG. 5. Polarized Raman spectra (xx), (yy), (xy), and (yx) of the same cell shown in Fig. 4, at the spot (x,y) = (11,5). Here, (xy) means, for example, that the electric vector of the exciting beam is placed along the x-axis, and the electric vector of the scattering beam is placed along the y-axis.