Chemical Analysis of Complex Biological Systems by Raman Spectroscopy

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OUTLINE

A. RAMAN SPECTROSCOPY
B. CAROTENOIDs
C. RAMAN MICROSCOPY
D. PHOTobleaching
E. POLARIZATION
F. MAPPING
G. OTHER METHODS
PRINCIPLE OF INELASTIC AND ELASTIC SCATTERING

- Low probability event
- 1 in $10^8$ photons inelastically scattered
- Insensitive technique

PRINCIPLE OF INELASTIC AND ELASTIC SCATTERING


Li and Church, Journal of Food and Drug Analysis, 29-48, 2014
TECHNOLOGICAL ADVANCES

- Efficient laser sources: Diode lasers, gas-based lasers, pulsed or continuous-wave

- Low-noise detectors: Charge-coupled devices (CCDs), electron-multiplying CCDs

- Effective Rayleigh filters: Dielectric edge filters, notch filters, Single or multistage monochromators

- High-throughput optics
RAMAN SPECTROSCOPIC MICROSCOPE SYSTEM

CAROTENOIDs

CAROTENOIDES

FT-RAMAN SPECTRA OF PURE CAROTENOID STANDARDS

β-carotene

α-carotene

Lutein

C=C

C—C

- CH₃

Schulz, H., et al. *Biopolymers*, 212-221, 2005
CAROTENOIDS ANALYSIS: \textit{IN-SITU}

Schulz, H., et al. \textit{Biopolymers}, 212-221, 2005
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**FLUORESCENCE**

- Sample dependent
- Use of near-IR source
- 4 picosecond optical Kerr shutter


MICROSCOPY + RAMAN SPECTROSCOPY = NON-DESTRUCTIVE ANALYSIS

C=C : 1525 cm⁻¹

Schulz, H., et al. Biopolymers, 212-221, 2005
RESOLUTION: SPATIAL

\[ \Delta x = 0.61\lambda/\text{NA} \]

- shorter wavelength and high-magnification optics

Spectral Resolution

- Higher excitation wavelength

\[ \text{C=C} : 1525 \text{ cm}^{-1} \]

Depends on the scientific question:
- molecular information
  OR
- localized information

Schulz, H., et al. *Biopolymers*, 212-221, 2005
PENETRATION DEPTH

- Shorter wavelength, higher energy but more scattered
- Hence, ideal for studying the surface of a tomato sample
- But, need longer wavelength if information needed about the carotenoid composition of a tomato core

PHOTOBLEACHING

- Changes in the electronic structure
- Important for **samples containing** carotenoids
- Carotenoids signal steadily decreases.

PHOTOBLEACHING

- The variance affects **Reproducibility**
- Orders of magnitude stronger

DEALING WITH COMPOUNDS SUSCEPTIBLE TO PHOTOBLEACHING:

Post-acquisition data treatment:

- Omitting carotenoid spectral regions

Most variation in 1157 cm$^{-1}$ and 1525 cm$^{-1}$

DEALING WITH COMPOUNDS SUSCEPTIBLE TO PHOTOBLEACHING:

- Elimination of these peaks does not eliminate variation.
- The width of the carotenoid bands force to discard other spectral features hidden beneath.

DEALING WITH COMPOUNDS SUSCEPTIBLE TO PHOTOBLEACHING:

**Spectra processing:**

**EMSC:** background signal correction using Extended Multiplicative Scatter Correction

**EMSC-SIS:** Extended Multiplicative Scatter Correction and Spectral Interference Subtraction

DEALING WITH COMPOUNDS SUSCEPTIBLE TO PHOTOBLEACHING:

Photodecomposition:

- Before data acquisition
- Long time 30-60 min
  - a) 10min
  - b) 30min
  - c) 50min
  - d) 70min

The difference spectra can be used to study the carotenoids and their interactions with the biological matrix.

POLARIZATION:

The Raman spectrum depends on the orientation and polarization of light.

Intensities vary depending on the angle between polarizability tensor of a specific molecular vibration and the exciting source.

Polarized Raman spectroscopy: information about structure and orientation

**B**: cellulose parallel along the fiber from 2774 to 3026 cm$^{-1}$

**C**: cellulose oriented with a high angle in respect to the fiber from 1067 to 1106 cm$^{-1}$ (orientation-sensitive cellulose band 1097 cm$^{-1}$)

Cross-section of wood *L. Procera*
MAPPING:

Large data sets that require computational processing

- Pre-processing (minimizing variability):
  - Cosmic rays
  - Fluorescence
  - Poor signal to noise ratio
  - Baseline correction (polynomial fitting, derivative spectra)
  - Truncating the spectra
- Feature extraction: PLS or PCA
- Classification: HCA or PCA
MAPPING:
A better understanding on structure, chemical composition of plant cells, tissues and organs.

Raman spectrum is a combination of the spectra of the single compounds.

OTHER METHODS:

- **HPTLC/AMD**

![Graph showing carotenoids](image)


- **HPLC** is the most used method for quantification, but carotenoids need to be extracted (destructive)
SUMMARY:

- Sample preparation
- Choose instrumentation
- Resonant vs non resonant
- Photobleaching
- Polarization
- Mapping
- Supporting methods
Thank you for your attention!