Probing the molecular structure of the intact leaf cuticle by polarization modulation-infrared reflection-absorption spectroscopy

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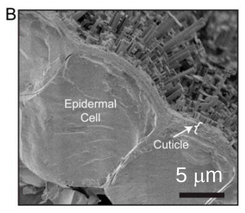
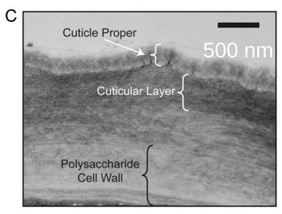
Introduction

Cuticle: lipid membrane on the plant surface

The multifunctional interface between the plant and the environment.
Critical for the development and survival of plants.
Barrier to protect plants against (1) Dehydration, (2) UV radiation,
(3) Atmospheric oxidants (OH, O_3) (4) Pathogen and insect attacks

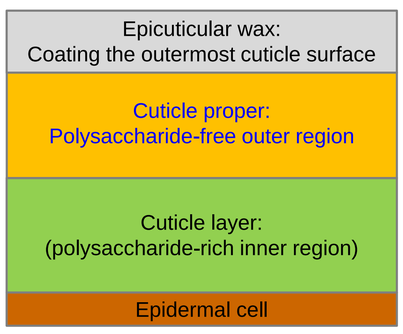


SEM of Arabidopsis leaf.



TEM of Arabidopsis stem.

Current cuticle model: A surface sensitive (nm-scale) spectroscopy is necessary.



The thickness of cuticle: 0.1 to 10 μm or more
(depending on the species)

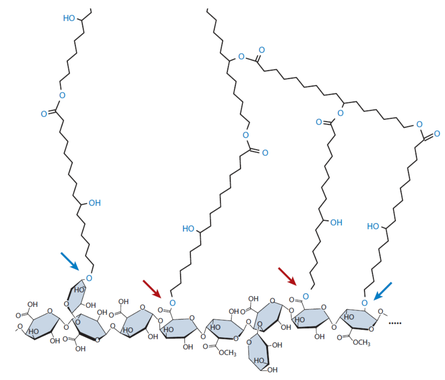
(1) Wax: Organic solvent soluble.
Aliphatic hydrocarbons (e.g., alkanes, alcohols)
Carbon chain lengths of C_{20}–C_{40}

(2) Cutin: Organic solvent insoluble polymer (polyester).
Chain lengths of C_{16} and C_{18} cross-linked by ester bonds.

(3) Polysaccharides: Pectins, and hemicelluloses.

Very little is known about the molecular arrangement (conformation, crystallinity, and orientation).
The key for the physicochemical properties.

"In situ observation" was technically difficult.
(i) Sample pretreatment, and (ii) Sample damage



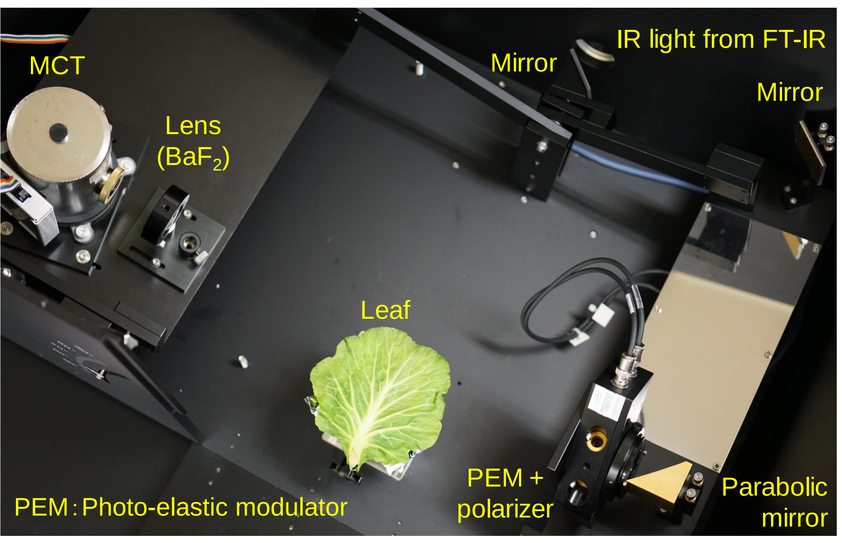
Yeats and Rose, (2013) Plant Physiol. 163: 5–20.

Experimental Methods

PM-IRRAS: Polarization-modulation infrared reflection absorption spectroscopy

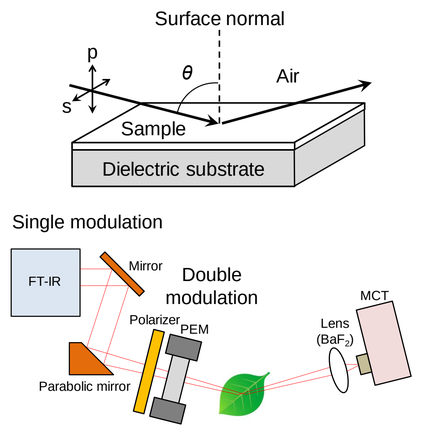
Double modulation FT-IR spectroscopy based on the difference in IR reflectance between the p- and s-polarizations

PM-IRRAS of a wild cabbage (Brassica oleracea L.)



(1) Rapidly switched measurements of R_p and R_s (e.g., 50 kHz) using a PEM
↓
(2) The electric signal is separated into the direct current form (I_{DC}), and the alternate current form (I_{AC}).
↓
(3) The ratio spectrum (S) is obtained. [The ratio of I_{AC} to I_{DC} (I_{AC}/I_{DC})]
↓

$$S = C\frac{J_2(\phi_0)(R_p - R_s)}{(R_p + R_s) + J_0(\phi_0)(R_p - R_s)} \quad \frac{I_{AC}}{I_{DC}}$$



IR spectra of thin sample films can be obtained on a metallic substrate and even on a dielectric substrate (e.g., silicon, liquid water, and a plant leaf).

Blaudez et al., (1996) Faraday Trans. 92: 525. Itoh et al., (2010) Appl. Spectrosc. 64: 1374.

In situ analysis of a living specimen is possible without sample pretreatment

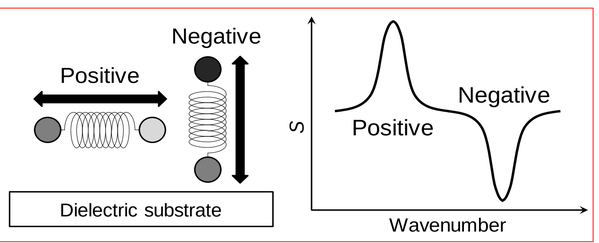
1. Background-free measurement
↔ Conventional IR measurements *need* background and sample measurements.
Impossible to measure a leaf surface (substrate) without the cuticle (sample).

2. Nondestructive analytical technique
↔ Structural analysis methods using electrons, ions, X-rays. or lasers.

3. Average molecular orientation can be determined
Similar surface selection rule to that of external reflection spectroscopy using a dielectric substrate (p-polarization).

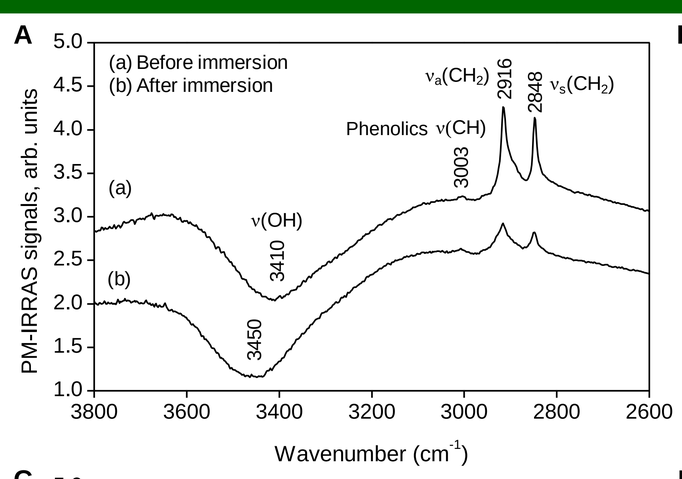
Surface-parallel vibration
→ Positive peak*

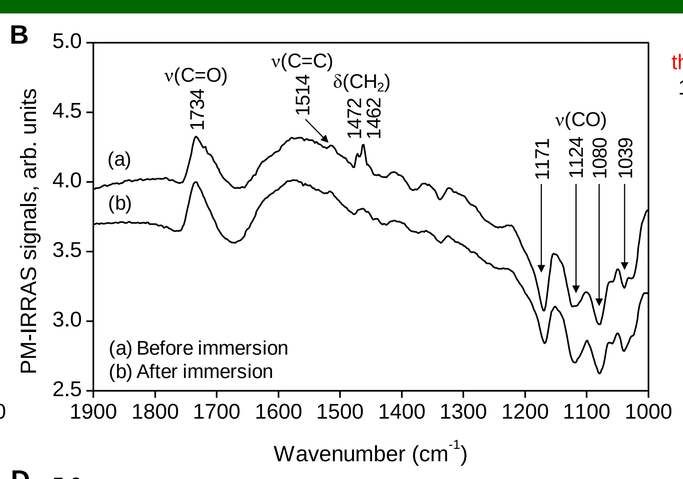
Surface-normal vibration
→ Negative peak*



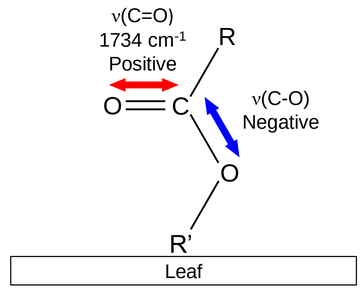
*When the incidence angle (76°) is larger than Brewster angle of the air/leaf interface (55 °)

Results and Discussion

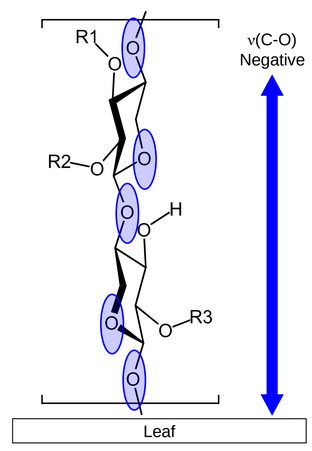




The positive ν(C=O) band at 1734 cm^{-1}:
the non hydrogen-bonding character of cutin.
1713 cm^{-1} or lower when hydrogen bonded.

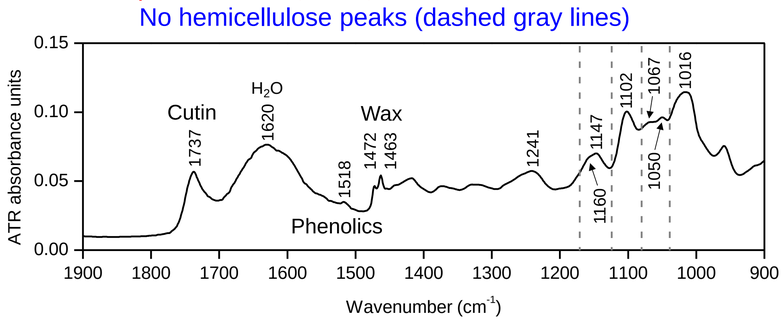


The intense negative ν(CO) bands:
1171, 1124 cm^{-1}: Glycosidic linkage of xylan
1080, 1039 cm^{-1}: Ring vibration of xyloglucan



Probing an inner region by ATR-IR spectroscopy

Pectin peaks at 1241, 1147, 1102, 1067, 1050, 1016 cm^{-1}
No hemicellulose peaks (dashed gray lines)



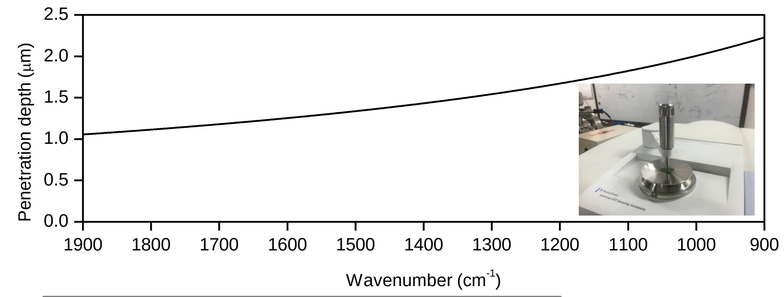
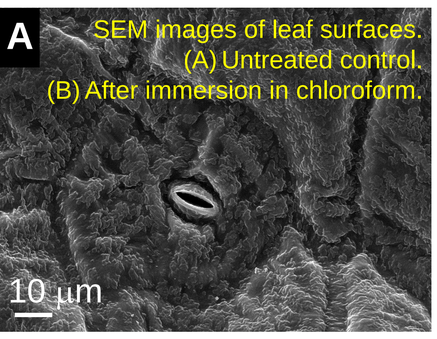
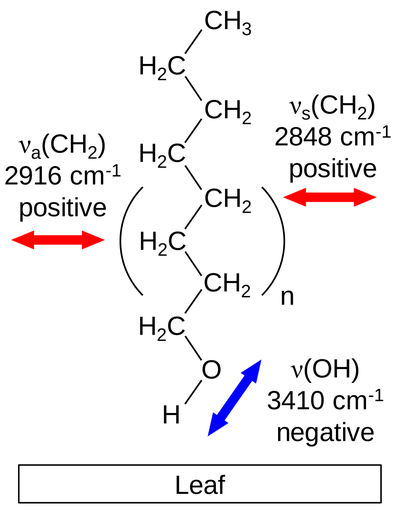


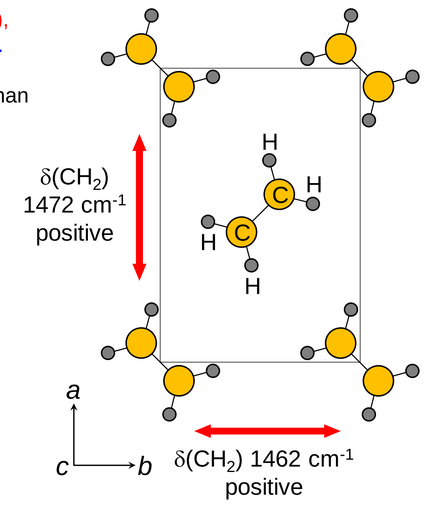
Figure: Ratio spectra of a wild cabbage leaf at (A) 3800–2600 cm^{-1} and (B) 1900–1000 cm^{-1}.
(a) before solvent treatment and (b) after immersion in chloroform. (C) and (D) Magnification of spectra in (A) and (B).

The presence of waxes, cutin, and hemicelluloses in the outer cuticle region.
↔ The cuticle model (the cuticle proper as the outer region free from polysaccharides)?



The thickness of the cuticular wax (50-375 nm),
The cuticle thickness of wild cabbage: 3-6 μm.
↓
PM-IRRAS probes the outer cuticle region less than about 100-500 nm surface region.





Orthorhombic or Monoclinic: 1472, 1462 cm^{-1}
Triclinic: 1473 cm^{-1}
Hexagonal: 1468 cm^{-1}
Amorphous: a broad peak at 1467 cm^{-1}

Conclusions

(1) PM-IRRAS is an easy-to-use approach for studying the plant cuticle
No need for sample pretreatment or background measurements

(2) The positive $\nu_a(CH_2)$, $\nu_s(CH_2)$ and $\delta(CH_2)$ bands:
The all-trans zigzag alkyl chains of the epicuticular wax.
Packed in the orthorhombic subcell.
Oriented perpendicular to the leaf surface.

(3) Polysaccharides are widely distributed across the leaf cuticle.
Hemicelluloses in the outer cuticle region (less than about 100-500 nm).
Pectins in the inner 2 μm region and are more abundant than hemicelluloses.

Hama et al., JPC B, 121, 11124 (2017) Hama et al., Plant Cell Physiol., in press.