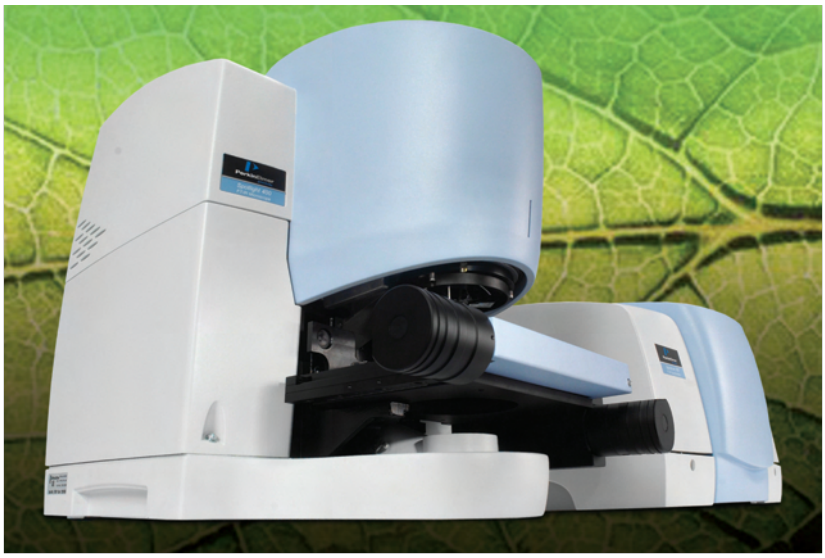
FT-IR Imaging Study on Stoma of *Limonium*



Sample: Leaf cells

Industry: Agriculture/Research

Technique: Transmission infrared imaging

Applicable Instrument: Spotlight™ FT-IR Imaging System

Introduction

There are many stomata (thousands per square centimeter) in the lower epidermis of plant leaves. Two crescent-shaped cells called guard cells, which are rich in chloroplasts, flank the stoma. Starch is produced in daytime by photosynthesis within the guard cells, not in adjacent epidermal cells. The guard cells regulate the opening and closing of the stomata in order to exchange gases between the leaf and the surrounding atmosphere. In some plant species, additional cells may be differentiated from the ordinary epidermal cells. These cells are called accessory cells.

Recently FT-IR imaging technology has been developed.[1] By this method, molecular information for an area of a sample is obtained to see the distribution of concerned molecules or functional groups. These chemical 'pictures', called IR images, provide information that is highly complementary to images obtained from techniques such as Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM) and visible light microscopy.

In this paper, the FT-IR images of the lower epidermis of a leaf are discussed.

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Experimental

Leaf lower epidermis of Statice (*Limonium sinuatum,* Family: *Plumbaginaceae*) was placed on a BaF_2 plate as an imaging sample. Image data (transmission mode) were collected between 4000 and 720 cm^{-1} at 4 cm^{-1} resolution with 4 scans per pixel. The area of the sample was imaged over 225 µm x 262.5 µm. Using 6.25 µm pixel resolution, acquisition of an image consisting of 1512 spectra took less than 10 minutes.

Results and discussion

The visible image of the leaf lower epidermis of Statice is shown in Figure 1. IR imaging was performed in the whole area shown in Figure 1. Figure 2 shows typical C-O stretching bands of cellulose and starch, respectively. The cellulose band across 1165 and 1155 cm^{-1} was ratioed against the starch band across 1023 and 1013 cm^{-1}, as shown in Figure 3(a). The bands used to obtain the band ratio image are indicated in Figure 3(b). Starch-rich areas are clearly differentiated from others, indicating that starch is located not only in the guard cells but also in the accessory cells.

1. E.N. Lewis, I.W. Levin (1995) Appl. Spectrosc. 49, 672.

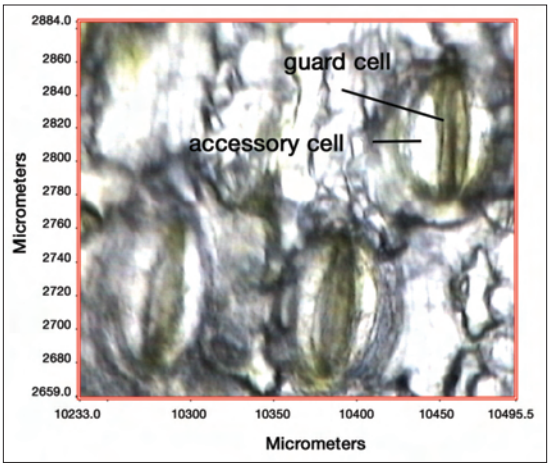

Figure 1. Visible image of the leaf lower epidermis of Statice.

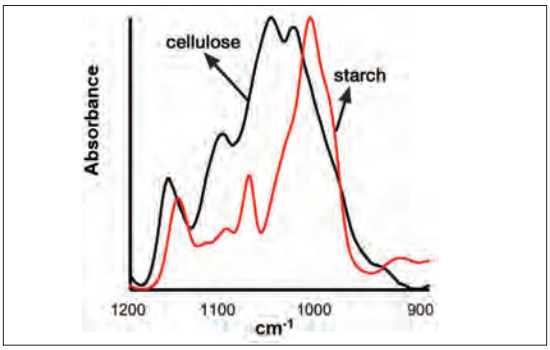

Figure 2. FT-IR spectra of cellulose (black) and starch (red).

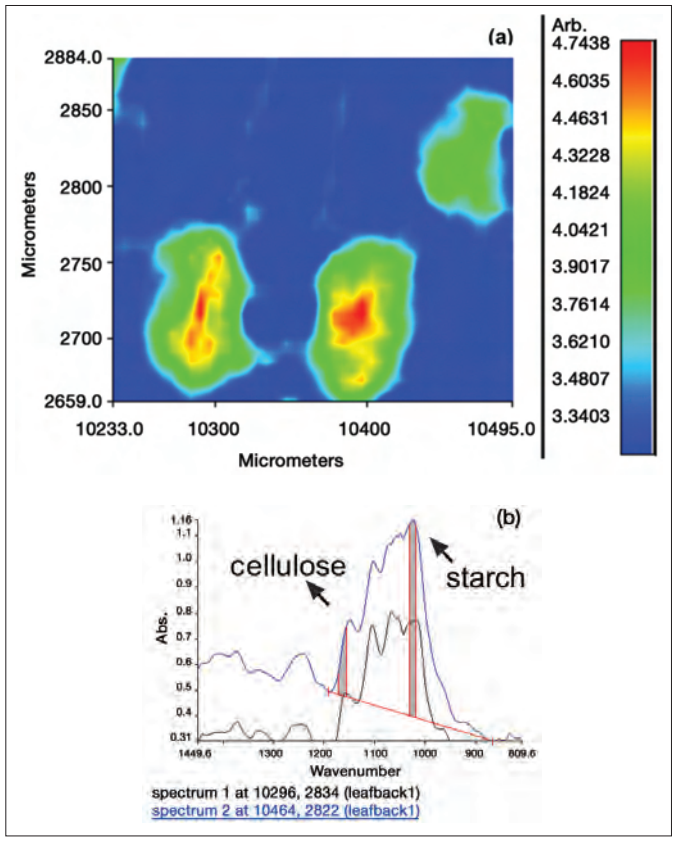

Figure 3. Band ratio image (1023-1013 cm^{-1}/1165-1155 cm^{-1}) of cellulose (a), and the cellulose and starch bands used for the upper image (b).

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