

FT-IR Imaging Data from Sectioned Tomato Seed



Sample: Tomato seed

Industry: Agriculture/Research

Technique: Transmission infrared imaging

Applicable Instrument: Spotlight™ FT-IR Imaging System

Introduction

The following data was obtained from a sectioned tomato seed and analyzed using the PerkinElmer® Spotlight™ FT-IR Imaging System. The section was placed onto a Barium Fluoride window and the infrared data was obtained in transmission mode.

The visible image of the sectioned seed is shown in Figure 1. The red box indicates the area where IR data was collected. The data was collected at a spatial (pixel) resolution of 25 microns and a spectroscopic resolution of 8 cm^{-1} . If required, a smaller pixel size of 6.25 microns could have been used. The whole analysis took less than 15 minutes.

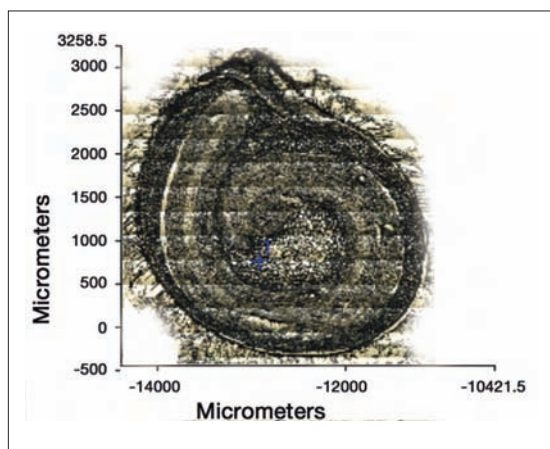


Figure 1.

The corresponding infrared image from the chosen area is shown in Figure 2. This image shows the integrated total IR absorbance from each of the pixel areas. A red-colored region is an area of high total IR absorbance, whereas, dark blue indicates an area of weaker total IR absorbance. This variation in intensity throughout the image can arise due to varying chemical composition or simply a variation in sample thickness.

Since a spectrum from each pixel area is stored in the image file, these can be subsequently retrieved and displayed. Spectra from the areas marked 1-4 are shown in Figure 3.

The individual absorbance bands are characteristic of functional groups in the various molecules present

and these can be used to determine the relative distribution of these molecules throughout the imaged area.

Previous work has assigned the following absorbance bands to specific functional groups;

OH (in Carbohydrates/sugars)
3000-3600 cm

C=O (Lipid) 1740 cm

C=O (Amide I) 1650 cm

N-H (Amide II) 1550 cm

PO (Phosphate) 1250 cm

OH (in Carbohydrates/sugars)
1050 cm

By plotting the intensity of the above frequencies as a function of position, it is often possible to create distribution maps for the

corresponding molecules. Figure 4 shows the distribution of the absorbance at 1740 cm⁻¹ corresponding to the lipid distribution. Once again, a red area corresponds to a high concentration of lipid relative to a blue area.

Similarly, a distribution for carbohydrates/sugars can be generated by looking at the distribution of the OH absorbance (Figure 5).

Another way of displaying this information is to generate band/ratio images. Instead of displaying the distribution of a single component, these images show the relative distribution of two components, and can help overcome variations in sample thickness effects. Figure 6 shows the relative distribution of sugar to lipid (3300 cm to 1740 cm).

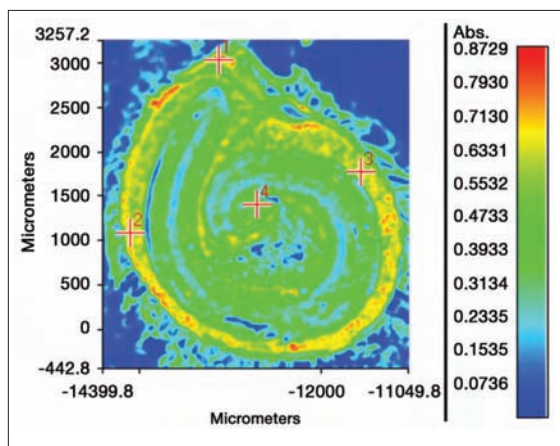


Figure 2.

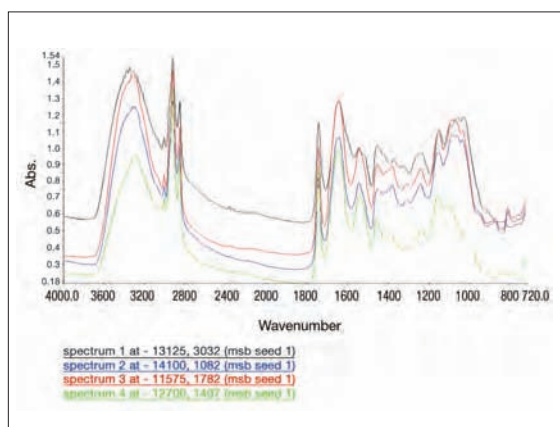


Figure 3.

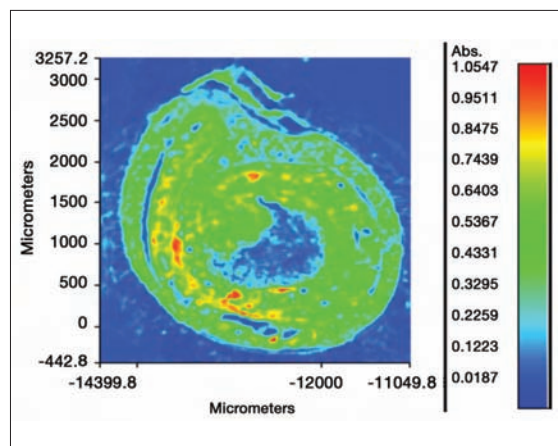


Figure 4.

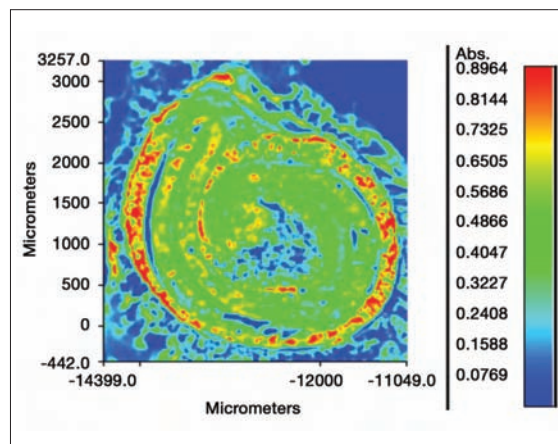


Figure 5.

Conclusion

In conclusion, data indicating the distribution of molecules within thin sections of seeds is rapidly obtained using the IR imaging technique and this data is of such a quality that distribution images are easily generated.

The thin section was kindly supplied by Royal Botanic Gardens, Wakehurst, UK.

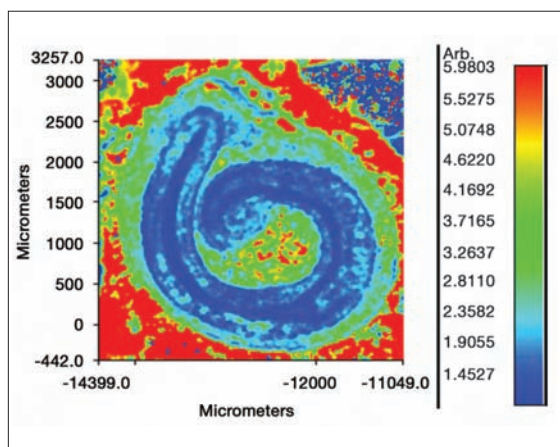


Figure 6.

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