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Biological Applications of FT-IR Imaging – Large Samples at High Spatial Resolution

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FT-IR Imaging using a 2-D Focal Plane Array (FPA) has established itself as a mainstream technique. With its unique ability to provide spectral (chemical) and spatial (positional) information, application areas are growing substantially, especially in the study of biological samples. When coupled with spatial resolution enhancing techniques, such as ATR imaging (1), chemical information from features as small as a few microns can be achieved. FPA based FT-IR imaging provides significant time savings compared to linear array systems, since several thousands of spectra are collected simultaneously in “rapid-scan” mode, compared to 16 or 28 spectra with linear array systems. FPA imaging can be performed in high-spatial resolution over large areas (mm^2) in minutes, without resorting to low-spatial resolution modes ($\sim 25 \mu\text{m}$) used in linear array based systems.

Experimental Conditions

Wheat stem cross-sections were microtomed to 5–10 μm and placed on an infrared transparent ZnSe substrate. FT-IR images were collected in transmission mode using a Varian “Stingray” FT-IR imaging system, consisting of a Varian 7000 FT-IR spectrometer coupled to a Varian 600 FT-IR microscope with a mid-IR 64x64 MCT FPA detector (4,096 pixels). Images were collected in “rapid-scan” mode over a $2.5 \times 2.5 \text{ mm}$ area at 5.5 μm nominal spatial resolution (high spatial resolution mode), at 8 cm^{-1} spectral resolution and with 16 scans co-added. Total collection time was 21 min (compared to >16 h for a linear array system under equivalent conditions). ATR images were collected using a “slide-on” Ge ATR at 8 cm^{-1} resolution with a nominal spatial resolution of 1.1 μm and 64 scans. Total collection time was $\sim 1 \text{ min}$.

Results

Images of the wheat stem cross-section showed distinct chemical clustering (protein, carbohydrate, and lipid) that correlated very well with the known morphology and chemistry of the samples (Figure 1a). Using ATR imaging in the center areas, which were much less dense than the outer ring, proved very successful in highlighting difficult to observe features (Figure 1b). Not only was ATR imaging able to resolve cell walls of $\sim 5 \mu\text{m}$ thickness using $\sim 10 \mu\text{m}$ of IR light ($\sim 1000 \text{ cm}^{-1}$), but it was also able to provide stronger absorbances and better quality spectra free of artifacts due to scattering or diffraction compared to the transmission mode of measurement.

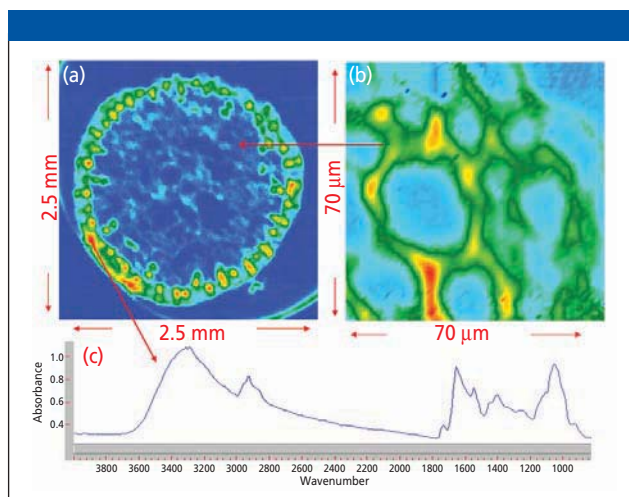


Figure 1: (a) Image of a wheat stem cross-section. (b) ATR image of the inner cells. Both images were created at 1045 cm^{-1} (carbohydrate). (c) Representative spectrum from image in 1a.

Conclusions

FT-IR imaging using a 2-D FPA provides an excellent means to visualize the chemical composition of wheat stem cells. Furthermore, ATR imaging demonstrates that features of only a few microns can be clearly imaged using relatively longer wavelengths of light.

References

- (1) E.M. Burka, R. Curbelo, Imaging ATR spectrometer. US patent 6,141,100.

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