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Application Note

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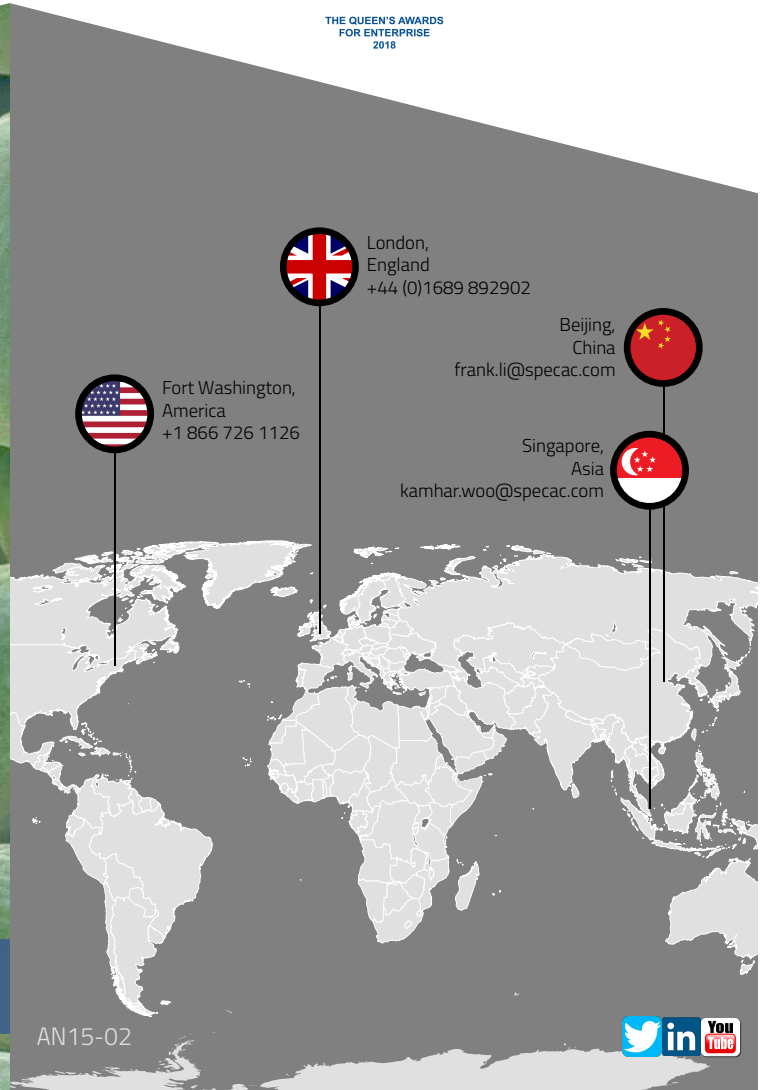
FTIR Analysis of Biological Yeast Cells using the Gateway ATR

THE QUEEN'S AWARDS FOR ENTERPRISE 2018



AN15-02

Inside: Learn how the Gateway ATR can yield spectroscopic information on low concentration biological samples

Introduction

Aqueous suspensions of biological materials represent a challenge for infrared spectroscopists. While there are many reports on spectroscopic studies on molecules of biological significance, there are far fewer examples of spectra obtained from whole organisms.

Water absorbs very strongly in the mid-IR, dwarfing contributions from other components in the sample. By controlling environmental factors, such as the temperature of the sample, it is possible to perform spectral subtractions to obtain information about the peaks that occur in the same region as the water absorptions.

The Gateway accessory is a 6-reflection ATR typically used for viscous liquids such as creams, pastes and gels. Such samples are readily applied to and cleaned from the accessible and removable crystal assembly on the ATR top plate.

In this application note we describe its use for measuring large biomolecules (yeast cells and protoplasts) in aqueous suspensions.

The Gateway™ is a multibounce ATR accessory

- 6 reflections
- ZnSe, Ge and Si crystals
- Range of top plates offering solid and liquid options plus flow and temperature control
- Provides sensitive measurements of liquids and reactants
- Ideal for oils, biofuels and liquid-lipid interactions
- Consistent, qualitative measurements of heterogenous solids, such as foodstuffs

Acknowledgement: The data presented here was collected by Dr J.E Newbery of Goldsmiths College, University of London, UK.

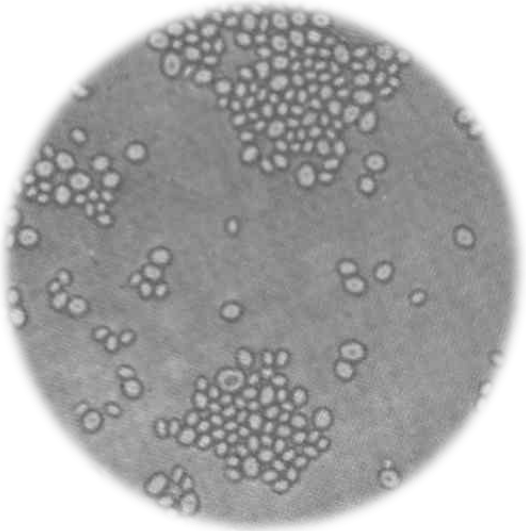


Figure 1: Microscopic image of Torulopsis glabrata

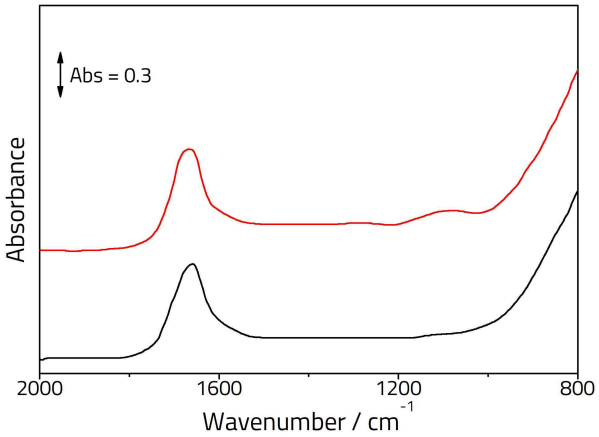


Figure 2: IR spectra of yeast cells in Water (red) and distilled Water (black).

Experimental

A suspension of Torulopsis (T.) glabrata cells was loaded onto the ZnSe crystal. A commercially available spectrometer was used at a resolution of 4 cm^{-1} and 1000 scans to maximise signal-to-noise.

T. Galabrata protoplasts were obtained by treating a solution of the yeast with a snail enzyme to break down the cell wall. The extracted protoplasts were then analyzed in the same manner as the whole cells.

Results and Discussion

Figure 1 shows a micrograph of T. Glabrata cells deposited on a glass slide. Usually such low spatial coverage would give a poor spectrum, however, multibounce ATR increases the signal intensity such that the spectrum of the cells can be obtained.

The spectra shown in Figure 2 have been acquired from an aqueous suspension of the yeast (upper trace) and from distilled water (lower trace). Apart from a slight disturbance in the vicinity of 1000 cm^{-1} these two spectra seem almost identical. However, spectral information can be obtained either by subtraction of the water peaks or by the use of a water background.

The lower trace shown in Figure 3 is the result after the digital subtraction of the water spectrum from that of the yeast suspension. It will be evident that as well as absorbances at 1050 cm^{-1} there are significant features at 1630, 1550, 1410 and 1240 cm^{-1}. The main band at 1050 cm^{-1} has a complex composition and represents absorptions mainly from the carbohydrates of the yeast cell wall. The peaks at 1630 and 1550 cm^{-1} have been shown to be indicative of the presence of amides and probably come from proteins either attached to or embedded into the cell wall.

The upper trace in Figure 3 represents the spectrum obtained from production of protoplasts, is an essential step in the formation of new genetic hybrids. The protoplasts and the whole cells have very similar infrared spectra; The differences between the two lies in the detailed band structure and in the relative peak intensities. In the protoplast spectrum the peaks due to the carbohydrate features (1050 cm^{-1}) are significantly reduced in intensity compared with the peaks from the proteinaceous material. The change is entirely as expected from the structures of the yeast cell and the yeast protoplast as much of the carbohydrate present is located in the cell well.

Conclusions

The Gateway ATR provides a quick and efficient method of obtaining in-situ spectra of biological samples. This is very convenient without the disadvantage of issues associated with sedimentation in conventional transmission cells and ATR cells.

Studies such as these are capable of yielding information of value in the taxonomy of yeast hybrids produced for biotechnological reasons and can also be of use in improving methods for the production of protoplasts.

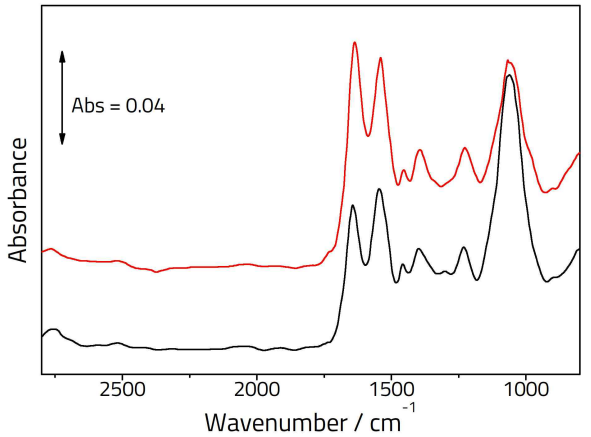
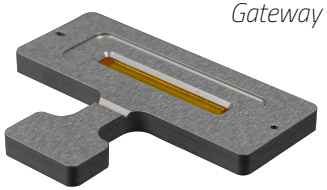


Figure 3: IR spectra of T.Glabrata protoplasts (red) and whole cells (black).

Gateway Top Plates



Trough Plate

Heated Flow-thru Plate

Flow-thru Plate

Have a look at our Product catalogue for more options of Gateway Top Plates