Imaging of Live Cells in Water Using an Agilent 620 FTIR Microscope and an Agilent Cary 670 FTIR System Equipped with Standard Thermal Source

Application Note

Life Science Research

Authors

Mustafa Kansiz and Alan Rein
Agilent Technologies, Inc.

Introduction

FTIR chemical imaging is rapidly becoming a key analytical technique for life science research, as it provides the unique capabilities of micro-scale spatial resolution as well as simultaneous chemical identification and quantification of biological macro molecular components, such as proteins, lipids, nucleic acids, and carbohydrates. In the past, the technical limitations of mid-infrared FTIR spectroscopy made the imaging of live cells in an aqueous environment difficult, and necessitated the use of an ultra-bright synchrotron IR source. As a result of optimized instrument design, the Agilent Cary 670 FTIR spectrometer combined with the Agilent Cary 620 FTIR imaging microscope (Figure 1) is capable of real-time measurement of live cells in an aqueous background, eliminating the access and cost penalties associated with securing experiment time using a synchrotron.

Figure 1. Agilent Cary 670 FTIR and Agilent 620 FTIR Imaging Microscope.

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This Agilent technology provides both the spatial and spectral (chemical) information in a time framework that enables the measurement of dynamic events. The result is FTIR imaging of live, viable cells in water over relatively large fields of view (FOVs) in minutes with an image pixel size of ~1 µm. This technology offers scientists a powerful and convenient method for studying cellular processes.

Methods and Measurement

Micrasteris hardyi algal cells (courtesy Phil Heraud, Monash University, Melbourne, Australia) were transferred from a growth medium using a pipette into a liquid sampling cell (courtesy of Lisa Vicarri, Elettra Synchrotron Light Laboratory, Trieste, Italy). The cell is comprised of two 1-mm thick CaF_2 windows separated by a 7-µm telflon spacer (Figure 2).

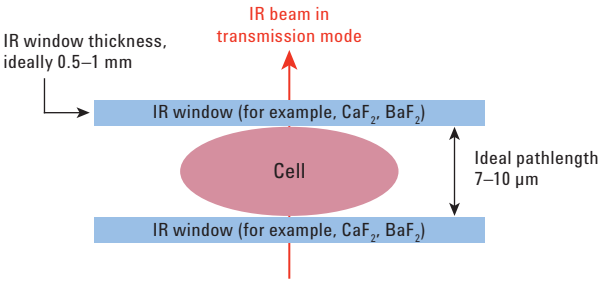

Figure 2 A schematic of the CaF_2 cell used in this work for live imaging of Micrasteris hardyi cells.

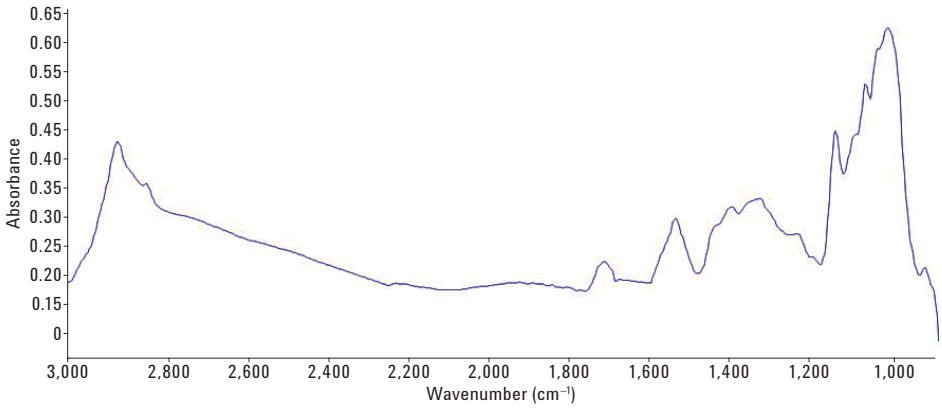

Figure 3. Mid-infrared spectrum of Micrasteris hardyi cells as measured in a Caf_2 cell. Infrared bands arising from the specific chemicals contained in the cellular substructures are clearly observed. Spectral bands arising from aqueous background are mathematically eliminated.

The Cary 670 FTIR spectrometer, interfaced with a Cary 620 FTIR imaging microscope, was used in all measurements. The optical configuration consisted of a pair of matched 15x, 0.62 NA objectives, with a 21-mm working distance and data collected in high mag transmission mode, yielding a 1.1-µm pixel size. The FPA used in this work contained 64 × 64 (4,096) elements. Spectral resolution was set to 8 cm^{-1}, with 256 scans co-added per tile. A 3 × 3 tile mosaic was collected to provide a total measurement area of 210 × 210 µm in approximately 1 hour. Agilent also offers a 128 × 128 FPA, in which case the system would collect an area of 280 × 280 µm in ~30 minutes (7 minutes per tile).

Results and Discussion

The infrared spectrum of the *Micrasteris* cell (Figure 3) displays assignable bands associated with chemistry in various cellular components.

The label-free, FTIR imaging of this living cell displays a rich chemical profile (Figure 4). Clear lipid inclusion domains are evident when false color images are created using the lipid ester carbonyl band at 1,713 cm^{-1}. These lipid inclusions in the IR image correlate well with the darker spots observed in the visible image,

Relying on the inherent chemical specificity and simultaneous spatial information afforded by FTIR imaging, simply highlighting spectral bands associated with certain macromolecular groups, such as proteins and carbohydrates, enables visualization of the cellular constituents (Figures 4B, 4C, and 4D) containing those groups. Also, highlighting all the protein, lipid, and C-H containing components can provide a composite chemical image (Figure 4E). Note that although pixel size does not necessarily equate to the achievable spatial resolution, 1.1-mm pixels ensure operation at the diffraction limit (which for the mid-infrared is in the region of 4–10 µm), a claim that is supported by the ~8-µm thick cell appendages that are readily visualized.

We also found subtle changes in the carbohydrate spectral region (~1,180–1,000 cm^{-1}) differentiating ghost cells (where internal components were emptied) from live cells (Figure 5).

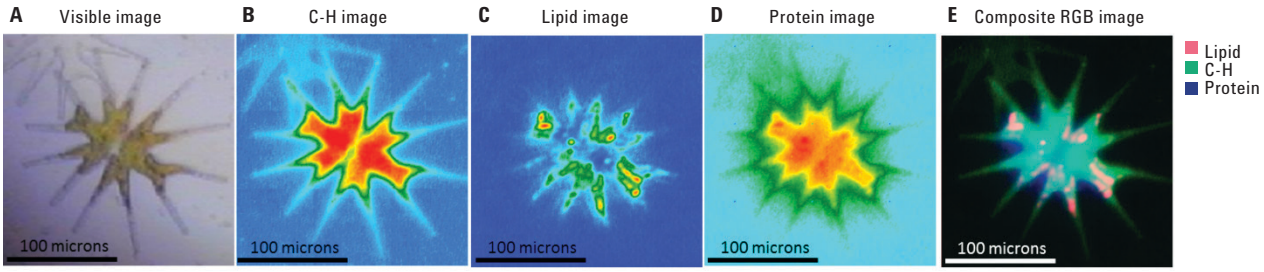

Figure 4. A) Photomicrograph of Micrasteris cells. High-resolution FTIR chemical images of Micrasteris showing (B) the integrated area under the C-H absorbance with a band maximum at 2,928 cm^{-1}, (C) the integrated area under the lipid band at 1,713 cm^{-1}, and (D) the integrated area under the protein band centered at 1,640 cm^{-1}. Red and blue colors represent strong and weak absorption of the infrared beam, respectively. The complete image comprises a 3 × 3 mosaic collected using a 64 × 64 FPA detector, which generates 4,096 spectra, each with a pixel size on the sample plane of 1.1 mm.

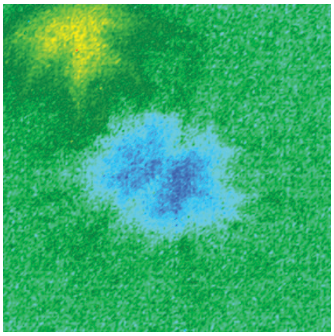

Figure 5. Chemical image showing a carbohydrate band intensity centered at 1,109 cm^{-1}, displaying a ghost cell in the upper left corner.

Conclusions

We report here, for the first time, chemical imaging of live cells in aqueous media using a benchtop FTIR system equipped with a thermal source (nonsynchrotron). Measuring the IR spectrum in an aqueous environment eliminates cell dehydration and preserves cellular chemistry, resulting in more accurate and relevant deduction of cellular processes.

The combination of the high optical throughput Agilent Cary 670 FTIR spectrometer and the optimally matched Agilent Cary 620 imaging FTIR microscope, with its exclusive high magnification/high NA and large working distance (21 mm), enables the use of the liquid transmission cell, and makes these measurements possible. This state-of-the-art technology allows scientists to image cellular processes such as cell division or cellular response to external stresses such as salinity, temperature, or various chemicals and drugs. With the Agilent FTIR technology, the cost and limited availability of a synchrotron source is eliminated, thereby making chemical imaging of live cells in native media using mid-infrared spectroscopy far more practical and accessible.

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