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Synchrotron infrared confocal microspectroscopic spatial resolution or a customized synchrotron/focal plane array system enhances chemical imaging of biological tissue or cells

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ABSTRACT

Spectroscopy and spatially resolved chemical imaging of biological materials using an infrared microscope is greatly enhanced with confocal image plane masking to 5–6 μm with a third generation microspectrometer and illumination with a synchrotron radiation source compared to globar illuminated and array detection or singly masked system. Steps toward this instrumental achievement are illustrated with spectra and images of biological tissue sections, including single cells, brain, aorta, and grain specimens. A recent, customized synchrotron infrared microspectrometer installation enables focal plane array detection to achieve both rapid and high definition chemical imaging. Localization of the ester carbonyl population in single modified starch granules was used to provide direct comparison of the two advanced imaging capabilities.

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1. Introduction

1.1. FT-IR spectroscopy through a microscope

For infrared microspectroscopy (IMS), obtaining excellent spatial resolution is essential while maintaining spectral resolution. To achieve this, a high signal-to-noise ratio (SNR) is required, with sufficient coadded scans obtained in two minutes or less and good microscopic focus. High fidelity chemical imaging has the same requirements. Image fidelity of selectively obtaining only the spectrum of a particular microscopic feature surrounded by adjacent material of a different composition presents an optical challenge, primarily due to diffraction effects. Performance has advanced from an initial globar source confocal IMS through synchrotron use as a source, focal plane array (FPA) microspectroscopy, and small step mapping, to the present custom built, newly available combined multiple synchrotron beam source illuminated commercial IMS with FPA detection that is now in use at the Wisconsin Synchrotron Radiation Center (SRC).

The first infrared research microscope IR-PLAN^{TM} (Spectra Tech, Stamford, CT) supplied with a globar source featured a Schwarzschild front surface objective and condenser with confocal remote image plane masking and a dedicated liquid nitrogen cooled 250 μm mercury–cadmium–telluride (MCT) detector to fit the microscopic beam. Fig. 1 shows spectra that we obtained via IMS, targeting small deposits of plaque in diseased human brain tissue [1,2]. Our first reported series of spectra [3] was obtained from targeted microscopic apertured areas on microtomed frozen wheat sections and the imaging [4] of a normal mouse brain (cerebrum) tissue section, as a contour map that required interpolation between evenly separated raster scanned data points. With a microprocessor controlled motorized stage and baseline correction software, the first single wheat aleurone cell images [5], Fig. 2, were achieved from mapping with a step size equal to the 6 μm × 7 μm confocal masking with 256 scans coadded and moderate spectral smoothing.

1.2. Enhanced spatial resolution of synchrotron infrared microspectroscopy

A few months after the first synchrotron IMS experiment at the National Synchrotron Light Source (NSLS) in 1993, excellent spectra were obtained from frozen wheat sections with 6 μm × 6 μm confocal masking from 64 scans in a few minutes with no smoothing. Fig. 3 shows aleurone cells imaged with enhanced spatial resolution on experimental infrared beamline U2b at Brookhaven [5]. From 1993–2010, globar data from KSU complemented by annual synchrotron data from NSLS (including 15 months in continuous residence) resulted in further exploitation of synchrotron IMS on a

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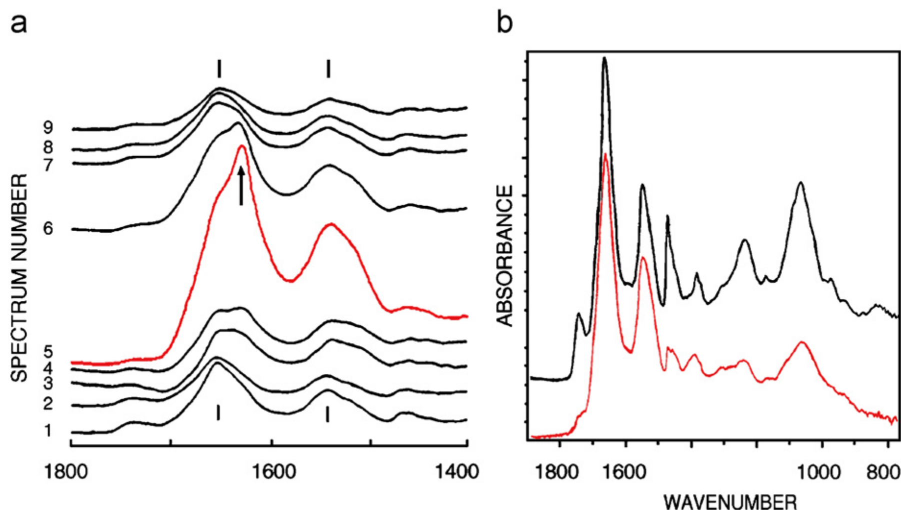

Fig. 1. (a) Alzheimer plaque (gray matter) and (b) multiple sclerosis plaque (white matter) showing both beta-amyloid (middle spectrum) at 1630 cm^{-1} and lipid reduction (bottom spectrum) at 1740 and 1409 cm^{-1}. (Adapted from Refs. [1,2] with permission from *Applied Spectroscopy Reviews* and *Biophysical Journal*).

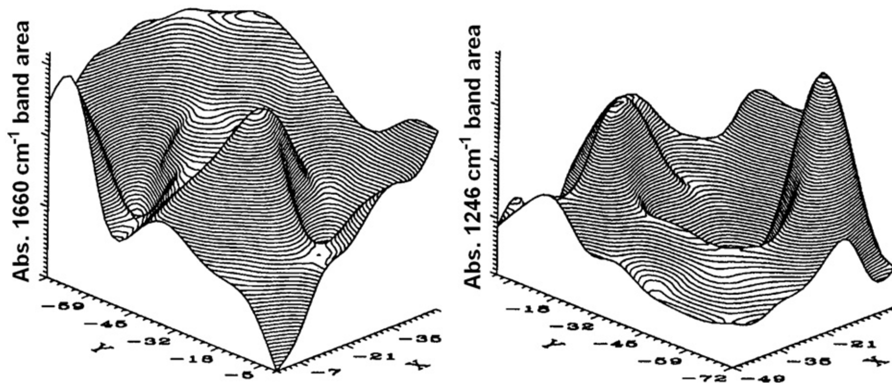

Fig. 2. First single cell map of wheat aleurone (protein) and cell wall at contrasting wavelengths. (From Ref. [5] with permission from *Cereal Foods World*).

variety of biological materials with several research partners [6]. Meanwhile, Jamin et al. [7,8], also working at NSLS beamline U2b, mapped live cells with 3 μm confocal masking that produced revealing images from diffraction truncated spectra. Use of a 50 μm cooled MCT detector and 32 times infinity corrected matched objective and condenser was a significant later optical refinement.

With a third generation IMS Continμum™ (Spectra Tech/Nicolet, Shelton, CT), we subsequently used 1 μm steps with 5 μm × 5 μm confocal masking at NSLS beamline U10b to provide detailed CH_2 stretch image contrast (Fig. 4) of a 10 μm diameter organic domain in silicate organic copolymer [9]. Dumas contrasted confocal synchrotron IMS with globar FPA images at BNL [10] of the same transverse section of human hair (Fig. 5) [11]. With a typical FPA, designed for fast data acquisition, image fidelity was sacrificed by diffraction effects without the benefit of confocal operation and the brightness of a synchrotron source. The optical dilemma was how to take advantage of array detection with a synchrotron source.

1.3. Custom imaging system at a synchrotron dedicated bending magnet

A theoretical, potential image fidelity enhancement was proposed by Carr et al. [12] and subsequently implemented at the Wisconsin SRC using a synchrotron source combined with a FPA microspectrometer in the optical configuration, as shown in Fig. 6. Graphics presented by Hirschmugl, in a SRI 2010 plenary lecture [13], compared two images of the same prostate gland tissue from different instruments [14], showing enhanced image fidelity of the SRC system. The combined 320 mrad horizontal × 25 mrad vertical synchrotron beam provided brightness. A high magnification objective was designed to achieve a favorable point spread function (PSF) on the *x*, *y* axes. Carr [15], also suggested array readout PSF deconvolution.

2. Experimental

2.1. Comparison of three instruments on single modified starch granules

2.1.1. Sample preparation

Waxy maize starch was reacted with octenyl succinic anhydride in a slurry under carefully controlled conditions as described elsewhere [16]. Under a microscope, granules were flattened to allow transmission with a small 7 mm stainless steel roller on the surface of either a 2 mm thick BaF_2 window or an IR reflective (Low-e) glass.

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