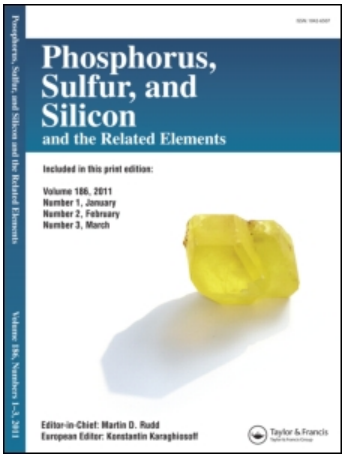

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Infrared Microspectroscopic Imaging of Biomineralized Tissues using a Mercury-Cadmium-Telluride Focal-Plane Array Detector

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A 64 × 64 Mercury-Cadmium-Telluride (MCT) focal-plane array detector attached to a Fourier transform infrared (FT-IR) microscope was used to spectroscopically image 5-μm sections of human bone tissue in the fingerprint region of the infrared spectrum. Infrared band contours in the 1200–900 cm^{-1} spectral region, due to phosphorous-containing mineral, change shape as a function of radial distance from centers of bone growth, called osteons. This technique is providing new insights into the biomineralization process.

Keywords: FT-IR; microspectroscopy; focal-plane array; MCT; imaging; bone

INTRODUCTION

An infrared (IR) focal-plane array (FPA) detector used in conjunction with a Fourier transform infrared (FT-IR) microscope has recently been shown to be a powerful technique for obtaining spectroscopic images with unprecedented image fidelity[1-10]. Use of a step-scanning FT-IR spectrometer with an array detector placed at an image focal plane of an IR microscope enables full infrared spectra (or spectroscopic images) to be collected across the entire unapertured field of view of the sample in a single measurement. The first such measurements were demonstrated using InSb FPAs, but they were sensitive only down to 1850 cm^{-1}.[1-4] It is now possible to use longer wavelength cut-off MCT FPAs to obtain spectroscopic images throughout the mid-infrared region of the spectrum (4000-900 cm^{-1}).

FT-IR microspectroscopy is a powerful technique for addressing site-to-site variation in mineral quality in histological sections.[10-14] The information obtained from analysis of thin sections of calcified tissue includes a measure of the amount of mineral and organic matrix present (from the phosphate and Amide I peaks, respectively). In addition, information about the crystallinity (i.e. crystal size, perfection, and maturation) of the apatitic mineral phase may be obtained.[13] In this work, a 64 x 64 MCT FPA positioned at the focal plane of a commercial step-scanning FT-IR microscope is used to collect spectroscopic images of human iliac crest bone tissue sections.

EXPERIMENTAL

Biopsied bone tissue from a human iliac crest was fixed in 70% ethanol, dehydrated through serial acetones, embedded in poly(methyl methacrylate) (PMMA), and a 5-μm-thick cross section cut. The section was then carefully sandwiched between two NaCl windows.

Spectroscopic images were recorded in transmittance with a UMA 300A FT-IR microscope with a 15x objective coupled to an FTS-60A step-scanning FT-IR spectrometer (Bio-Rad). A 50-mm ZnSe image formation lens was used to focus the entire field of view (0.5 mm x 0.5 mm) onto a 64 x 64 MCT FPA detector (Lockheed Martin Santa Barbara Focalplane). A cold 1800-cm^{-1} long-pass optical filter (Corion) was in place when the measurements were taken. The camera head contained a 14-bit analog-to-digital converter which was used to coadd 150 image frames during the 1 s the moving mirror of the interferometer was stopped at a particular optical retardation. Prior to acquiring data, the focal-plane array is flat fielded to compensate for variation in detector element response and non-uniform illumination. The camera integration time was 0.163 ms per frame. A total of 4096 asymmetric interferograms were collected (one for each detector pixel) at a spectral resolution of 16 cm^{-1} and an under-sampling ratio of eight (every eighth zero crossing of the HeNe reference laser). The entire data set took less than five minutes to collect. After Fourier transformation with a zero-filling factor of two and triangular apodization, sample and open-beam files were ratioed and converted to a single spectroscopic image file in absorbance units, which occupied about 4.1 Mbytes of disk space.

RESULTS AND DISCUSSION

The left image in Figure 1 (A) represents the area of the phosphate band region between 1200 and 900 cm^{-1}. Red indicates areas of highest absorbance, blue areas lowest absorbance, with green representing regions of intermediate absorbance. Some of the

smaller blue areas are osteons, or centers of bone growth, where the bone is thinner. The image on the right in Figure 1 (B) has been classified according to the relative absorbances at 1110 and 1018 cm^{-1}. Green represents areas of the image with little bone, where absorbances at both wavenumbers are low. Red areas represent regions with the highest mineral content, where the absorbances at both 1110 and 1018 cm^{-1} are high. Dark blue areas have higher absorbance at 1110 cm^{-1} than at 1018 cm^{-1}, while cyan-colored areas have higher absorbance at 1018 cm^{-1} than at 1110 cm^{-1}. The IR spectra shown in the bottom of Figure 1 (C) represent the average of all the similarly colored pixels in the top right image. Two different types of new (less mature) mineral

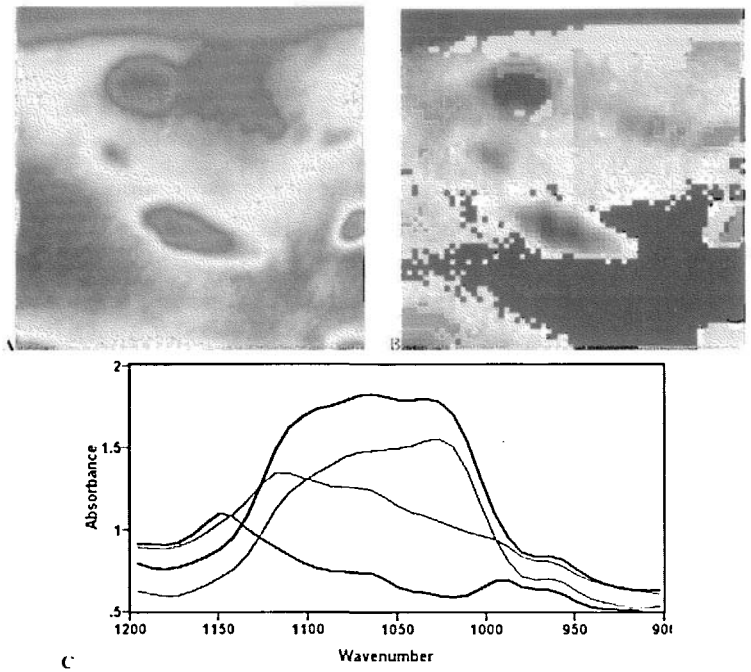

FIGURE 1 Spectroscopic image of a 0.5-mm x 0.5-mm area of a 5-μm-thick cross section of human iliac crest biopsy representing: A) the area under the phosphate band region; and B) classified according to the absorbances at 1110 and 1018 cm^{-1}. The IR spectra in C) represent averages of the corresponding colored areas in B).

are clearly seen in these spectroscopic images of bone. The blue spectrum (peak wavenumber of 1110 cm^{-1}) represents mineral with significant amounts of poorly crystalline apatites ($Ca_3(PO_4)_2$), while the cyan spectrum (peak wavenumber of 1018 cm^{-1}) represents mineral with more non-stochiometric apatites containing HPO_4^{2-} and/or CO_3^{2-}.[13,14] The ability to obtain thousands of IR spectra in a single imaging experiment facilitated the discovery of these two spatially segregated types of new mineral.

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