

Investigating Bone Chemistry using Reflection-Based Infrared Imaging

Beamline:

U2B, U10B

Technique:

Infrared Microspectroscopy

Researchers:

A. S. Acerbo (Stony Brook Univ.)
L. M. Miller (BNL), D. R. Sumner (Rush Medical Center), C. Rubin (Stony Brook Univ.), D. Judex (Stony Brook Univ.), M. E. Faillace (Mount Sinai), A. Nazarian (Harvard Medical).

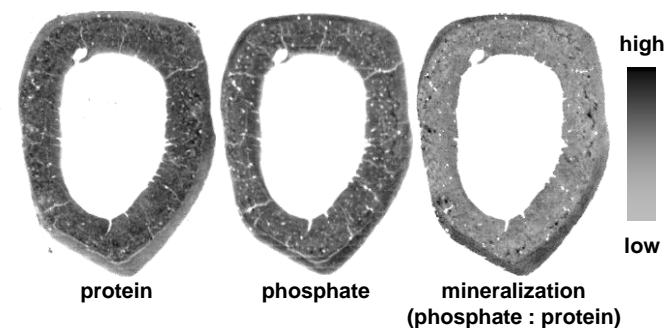
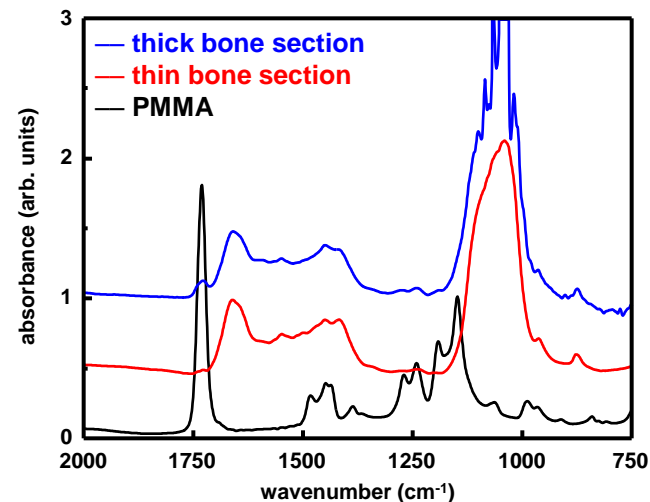
Publications:

A. S. Acerbo, G. L. Carr, S. Judex, L. M. Miller. *Analytical Chemistry* **84** (8), 3607-3613, 2012.

A. Bhatia, M. Albazzaz, A. A. Espinoza Orías, N. Inoue, L. M. Miller, A. S. Acerbo, A. George, D. R. Sumner. *Journal of the Mechanical Behavior of Biomedical Materials* **5** (1), 1-8, 2012.

Motivation: Fourier Transform Infrared Microspectroscopy (FTIRM) has become the most widely used technique for determining the spatially resolved chemical makeup of bone. To date, FTIRM has required thin sections of bone (typically $<5\ \mu\text{m}$) in order to provide sufficient transmission of infrared (IR) light through the tissue. Specimens that are too thick will cause insufficient illumination of the detector and prevent an accurate measurement, making quantitation impossible. Moreover, thin sections require bones to be embedded in an infiltrating plastic resin that can alter the chemical composition and cause spectral overlap with absorption features of bone. Here, we developed reflection-based FTIRM as an alternative to the widely adopted transmission-based FTIRM, which reduces specimen preparation time and broadens the range of specimens that can be imaged.

Results: We have shown theoretically that spectra produced by reflection-based FTIRM can be transformed into absorbance like spectra using a Kramers-Kronig transform. These absorbance-like spectra can be analyzed with standard methods used for transmission-based FTIRM. Furthermore, no additional hardware is required beyond what is already available for transmission FTIRM. Specimen preparation consists of polishing the specimen and orienting its surface perpendicular to the normal of the incident IR beam to ensure that spectral data are collected with a high SNR. We have shown empirically that reflection-based FTIRM can be used to determine the mineralization, crystallinity, carbonate substitution, and collagen cross-linking parameters that are equivalent to the transmission-based approach. We anticipate that reflection-based FTIRM will more readily allow for pixel-to-pixel correlations within a single specimen between bone's material, mechanical, and morphological properties on the micrometer (and perhaps nano) scale by combining FTIRM with techniques such as nanoindentation, Raman microscopy, qBSE, and 2-D surface slices from μCT



(Top) (Red) Typical IR bone spectrum showing the characteristic protein and mineral components. (Blue) IR spectrum from a thick bone section, illustrating effects of detector saturation at the ν_1, ν_3 phosphate peak. (Black) IR spectrum of the embedding medium PMMA, showing peaks that overlap with characteristic bone features. Spectra offset for clarity.

(Bottom) High spatial resolution IR map of single polished bone specimen, showing amount of protein (left), phosphate (middle), and tissue mineralization (right). Circumferential lamellae appear with a different chemical makeup compared to intracortical and periosteal bone.