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Article title:

Imaging the Material Properties of Polished Bone Specimens using Reflection-Based
Infrared Microspectroscopy

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Fourier Transform InfraRed Microspectroscopy (FTIRM) is a spectroscopy technique most frequently used to measure the transmittance through a material. When the measured quantity is expressed as a transmittance spectrum $T(\omega)$, the sample's absorbance spectrum $A(\omega)$ can be calculated by:

$$A(\omega) = -\log_{10} T(\omega). \quad (1)$$

For optically thin samples, the absorbance spectrum can be further related to the molar absorptivity ε , the thickness of the sample b , and the concentration c by using the Beer-Lambert law:

$$A = \varepsilon bc. \quad (2)$$

Retrieval of Reflected Phase by Kramers-Kronig Analysis

The measured specular reflectance $R(\omega)$ from the front plane of a single interface at near normal incidence is related to the modulus of the complex reflectivity squared $r(\omega)^2$, which can also be expressed as the product of the complex reflectivity $r(\omega)$ and its complex conjugate $\overline{r(\omega)}$:

$$R(\omega) = |r(\omega)|^2 = r(\omega) \overline{r(\omega)}. \quad (3)$$

At normal incidence, Fresnel's equations relate the complex reflectivity $r(\omega)$ and the complex refractive index $N(\omega) = \eta(\omega) + i\kappa(\omega)$, where $\eta(\omega)$ is the refractive index and $\kappa(\omega)$ is the extinction coefficient, as:

$$r(\omega) = \frac{N(\omega) - 1}{N(\omega) + 1} = \frac{\eta(\omega) - 1 + i\kappa(\omega)}{\eta(\omega) + 1 + i\kappa(\omega)}. \quad (4)$$

Further, the reflective amplitude $r(\omega)$ can be written as the complex function:

$$r(\omega) = \sqrt{R(\omega)} \cdot e^{i\theta(\omega)}, \quad (5)$$

where the first term is the real part, the second term is the imaginary part, and $\theta(\omega)$ denotes the phase shift of the reflected light. Taking the logarithm of Eq.5 one obtains:

$$\ln r(\omega) = 0.5 \ln R(\omega) + i\theta(\omega). \quad (6)$$

Further, we may use the principle of relativistic causality and employ the Kramers-Kronig relationship to solve for the imaginary part using the real part of the complex function using:

$$\theta(\omega) - 1 = \frac{2}{\pi} P \int_0^\infty \frac{\ln|r(\omega')|}{\omega'^2 - \omega^2} d\omega', \quad (7)$$

where P denotes the Cauchy principal value that is needed for when the integral approaches the singular value of ω' . Thus, a measurement of the reflectance from a surface provides $|r(\omega)|$ from which the phase shift $\theta(\omega)$ can be determined. The complex refractive index can then be calculated using Eq.4. This is a well-established method for determining the optical response functions of bulk materials.¹

Calculation of Absorption Coefficient Spectrum

With the reflectance measured experimentally and the phase determined using a Kramers-Kronig relationship, we may rewrite Eq.5 as a trigonometric function and equate this to Eq.4 to give:

$$\sqrt{R(\omega)} \cdot (\cos\theta + i\sin\theta) = \frac{\eta(\omega) - 1 + i\kappa(\omega)}{\eta(\omega) + 1 + i\kappa(\omega)}. \quad (8)$$

From Eq.8, the values of $\eta(\omega)$ and $\kappa(\omega)$ can be solved for and written as:

$$\eta = \frac{1 - R(\omega)}{1 + R(\omega) - 2\sqrt{R(\omega)} \cdot \cos\theta(\omega)}, \text{ and} \quad (9)$$

$$\kappa(\omega) = \frac{-2\sqrt{R(\omega)} \cdot \sin\theta(\omega)}{1 + R(\omega) - 2\sqrt{R(\omega)} \cdot \cos\theta(\omega)}. \quad (10)$$

Although the refractive index $\eta(\omega)$ and the extinction coefficient $\kappa(\omega)$ can thus be derived from the measured $R(\omega)$ using a Kramers-Kronig relationship, we can further use $\kappa(\omega)$ to calculate the absorption coefficient spectrum α for a thickness d by considering Eq.2 and that $\kappa(\omega) = \alpha / \sqrt{4\pi}$:

$$(\mathcal{E}) = \alpha = 4\pi\sqrt{d}\kappa. \quad (11)$$

Although direct comparisons between reflection-derived absorption coefficient spectra and traditional transmission-derived absorption spectra are made, more accurate comparisons should take into consideration the reflection losses from the surface of the thin sections in the transmission geometry by:

$$T(\omega) = \frac{(1 - R(\omega))^2 e^{-\alpha(\omega)t}}{1 - R(\omega)^2 e^{-2\alpha(\omega)t}}, \quad (12)$$

where t is the sample thickness. In this equation, the numerator describes the transmission after accounting for the first reflective bounce, and the denominator is the sum of additional reflective bounces.

Spectral Extrapolation

The Kramers-Kronig relationship for solving the phase (Eq.7) contains a semi-infinite integral and thus requires knowledge of the entire bone reflectance spectrum. Given that spectrometers have a limited detection range, a standard Kramers-Kronig relationship cannot be satisfied. Several alternatives to the original Kramers-Kronig relationships have been suggested, such as singly subtractive² and multiply subtractive³ relationships, both of which rely on anchor points that can be used to control the extrapolation of data beyond the finite data set. Yet another alternative is the more general iterative maximum entropy model that does not rely on near-normal incidence light.⁴ Rather than extrapolating, the proposed solution in this work relies on *a priori* knowledge of the absorption features of the sample at an energy range beyond spectral features of interest.

REFERENCES

- (1) Lucarini, V.; Saarinen, J. J.; Peiponen, K.-E.; Vartiainen, E. M. *Kramers-Kronig Relations in Optical Materials Research*; Springer: Berlin, 2005.
- (2) Ahrenkiel, R. K. *J Opt Soc Am* **1971**, *61*, 1651-1655.
- (3) Palmer, K. F.; Williams, M. Z.; Budde, B. A. *Appl Opt* **1998**, *37*, 2660-2673.
- (4) Vartiainen, E. M.; Peiponen, K.-E.; Asakura, T. *Appl Spectrosc* **1996**, *50*, 1283-1289.