SAMPE Technical Conference Proceedings: Navigating the Global Landscape for the New Composites. Charleston, SC. 2012 October 22-25 Society for the Advancement of Material and Process Engineering. CD ROM; 9 pp.

NANOSCALE INFRARED SPECTROSCOPY OF BIOPOLYMERIC MATERIALS

Curtis Marcott[1], Michael Lo[2], Kevin Kjoller[2], Craig Prater[2], Roshan Shetty[2], Joseph Jakes[3], and Isao Noda[4]

[1]Light Light Solutions, LLC
P.O. Box 81486
Athens, GA 30608

[2]Anasys Instruments, Inc,
121 Gray Ave., Suite 100
Santa Barbara, CA 93101

[3]Forest Products Laboratory
One Gifford Pinchot Drive
Madison, WI 53726

[4]The Procter & Gamble Company
8611 Beckett Road
West Chester, OH 45069

ABSTRACT

Atomic Force Microscopy (AFM) and infrared (IR) spectroscopy have been combined in a single instrument capable of producing 100 nm spatial resolution IR spectra and images. This new capability enables the spectroscopic characterization of biomaterial domains at levels not previously possible. A tunable IR laser source generating pulses on the order of 10 ns was used for excitation of cast sample films or thin cross sections deposited on IR transparent ZnSe prisms. Short duration thermal waves, due to infrared absorption, were studied by monitoring the resulting excitation of the contact resonance modes of the AFM cantilever. Differences in the IR spectra as a function of spatial position provide insight into microdomain formation and lead to increased understanding of how nanomaterial additives affect the molecular structure and properties of biomaterials. Cellulosic materials and additives, as well as poly(hydroxyalkanoate) copolymers, are discussed.

1. INTRODUCTION

Atomic force microscopy (AFM) has been a widely used technique in basic nanoscale research, including the study of biopolymeric materials. One shortcoming of AFM, however, is its limited ability to provide definitive chemical identification of specific regions in a sample. The ability to chemically identify individual discrete domains is especially important in the study of heterogeneous materials like polymeric biomaterials. The manner in which different chemical and biological components mix and chemically interact plays an important role in their ultimate properties.

Infrared (IR) spectroscopy is an extremely useful technique for chemically characterizing domains in biopolymeric materials. Unfortunately, discrete domains of interest may often be too small to be spatially resolved by IR spectroscopy. Traditional IR microspectroscopy has a spatial resolution limit of roughly several micrometers. This is due to fundamental diffraction limitations associated with the wavelength of light used to make measurements in the diagnostically important mid-IR spectral range (2.5-25 μm). Although several AFM probe-based techniques have been used to exceed the diffraction limit of conventional IR measurements [1-2], in general, these near-field approaches are single or narrow band and do not produce information-rich spectra that can be used to definitively identify different chemical species. Other IR techniques are based on measuring local temperature increases originating from spectral absorption through the use of AFM cantilevers integrated with conventional Fourier transform infrared (FT-IR) spectrometers [3-4]. These approaches allow broader spectral coverage than near-field approaches, but the spatial resolution is typically limited due to thermal diffusion on the order of many micrometers.

1.1 AFM-IR technique

Instrumentation that combines atomic force microscopy and infrared spectroscopy (AFM-IR) has been developed which enables chemical characterization of biopolymeric materials at spatial resolutions on the order of 100 nm, well below the diffraction limit. The instrument takes advantage of the photothermal induced resonance (PTIR) effect and uses an AFM probe to measure local thermal expansion that occurs as a result of incident IR light being absorbed by a sample. The PTIR technique was originally developed by Dazzi et al. [5-8] using the CLIO free electron source facility at Université Paris-Sud. The AFM-IR instrument used in the current studies is based on a lab-scale tunable IR laser source [9-10].

In the AFM-IR technique, the radiation from a tunable IR laser source illuminates the sample from underneath through a high refractive index IR transparent prism in a configuration similar to attenuated total reflection (ATR) [11-12]. In contrast to that technique, where a high-sensitivity mercury-cadmium-telluride (MCT) single-element or focal-plane array detector senses the internally reflected IR beam, this approach uses an AFM cantilever to detect the rapid thermal expansion of the sample, caused by absorption of the nanosecond-long pulses of IR radiation at a given wavelength. Wavelengths where the sample absorbs light will cause greater heating and thus larger expansions. This sample expansion is picked up by the AFM tip and transmitted to the AFM cantilever. The induced resonance amplitude in the cantilever is proportional to the amount of IR radiation absorbed by the sample. The resulting spectrum is obtained by measuring the cantilever ringdown amplitudes while tuning the IR source over a range of wave numbers. A schematic diagram of the experiment, including a visualization of the raw cantilever ringdown signals and how they are processed into IR spectra, is shown in Figure 1. The AFM cantilever ringdown amplitude plotted as a function of laser excitation wavelength produces the IR spectrum [9,10].

More details regarding the AFM-IR experiment can be found elsewhere [5-10]. AFM-IR spectra are virtually identical to those obtained by conventional FT-IR spectroscopy, which means they can be digitally searched against readily available IR spectral databases.

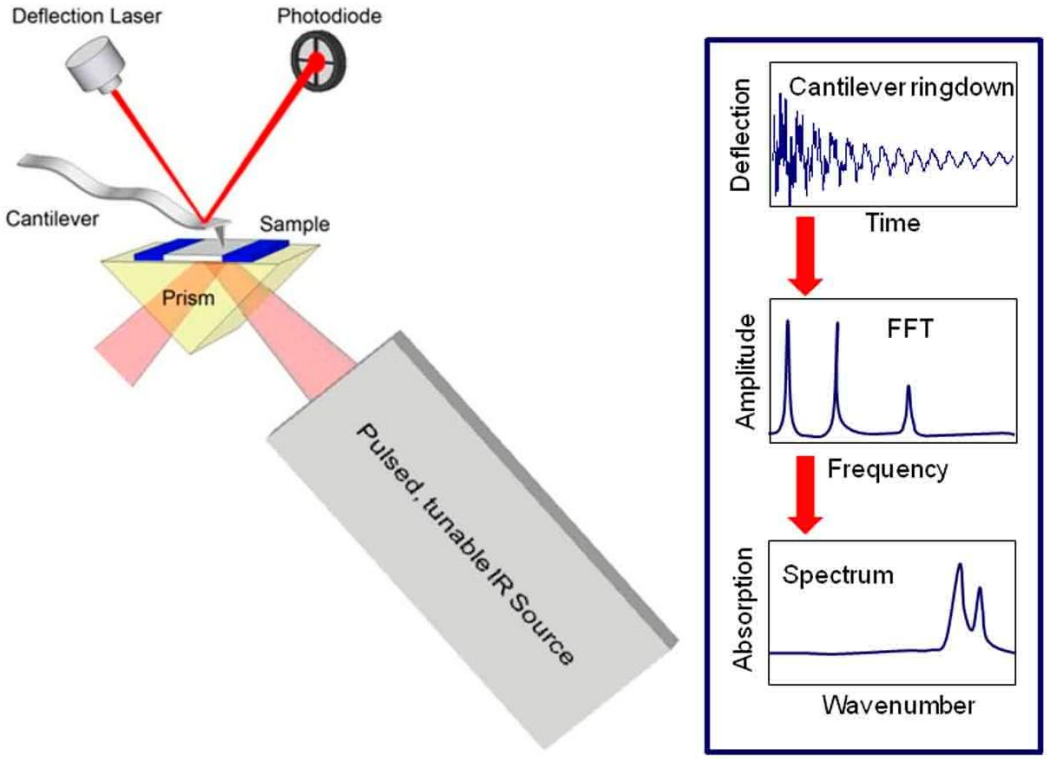

Figure 1. Schematic of the AFM-IR experiment.

1.2 Cellulose

Cellulose is one of the most abundant organic materials in nature. It is the major chemical constituent in plants, including wood. It is of interest to examine the structure of cellulose in wood using IR spectroscopy in order to better understand its inherent morphological structure at the molecular level [13]. This study shows how IR microspectroscopy with 100 nm spatial resolutions can also be used to examine how effective chemical treatments, such as acetylation, are at reaching the various morphological locations in wood.

1.3 Poly(hydroxyalkanoates) (PHAs)

Polyhydroxyalkanoates (PHAs) are a class of polyesters that can be accumulated as energy-storing inclusion granules in the cells of certain microorganisms [14–17]. PHAs are of interest because they can be biologically synthesized from renewable resources and degraded by bacteria in the soil [15,18]. PHAs show promise as a new family of environmentally friendly polymeric materials because their physical properties bear a fairly close resemblance to some petroleum-based synthetic polymers and they possess an attractive combined set of end-use properties. One of the simplest and largely produced PHA polymers is poly(3-hydroxybutyrate) (PHB) [16,17]. Its ultimate tensile strength is comparable to that of isotactic polypropylene (iPP) [17]. Since PHB is biocompatible, its utilization for medical purposes is also being contemplated. However, PHB is stiff and brittle due to its high degree of crystallinity, and it can become thermally unstable during processing [19–22]. Consequently, efforts have been undertaken to copolymerize PHB with other comonomers to improve its mechanical properties [19–22]. The

Procter & Gamble Company (Cincinnati, OH) has introduced a new family of PHA copolymers comprised of 3-hydroxybutyrate and other medium-length side-chain 3-hydroxyalkanoates. Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (P(HB-*co*-HHx)), with a melting temperature (T_m) of 110 to 160 ºC and 35 to 45% crystallinity, is a typically available grade of such copolymers.

Recent IR studies of the melting and temperature-induced crystallization of PHA copolymers have focused on collecting average bulk spectra of the polymer as a function of temperature [23,24]. Although these studies have provided important information about the melting and crystallization processes, no information concerning the spatial distribution of the crystalline and noncrystalline microdomains in the final processed polymeric material was obtained. This study shows the spatial location of crystalline and noncrystalline microdomains on a sub-micrometer spatial scale [10].

2. EXPERIMENTATION

2.1 Wood samples

Toothpick samples (both untreated and acetylated) were embedded in epoxy. Cross sections approximately 500 nm thick were cut using a microtome and transferred to the surface of a zinc selenide (ZnSe) prism. Both transverse and longitudinal cross sections of the untreated and acetylated wood samples were prepared and examined using AFM-IR.

2.2 PHA samples

Films of P(HB-*co*-HHx) were solution cast as evaporated films from chloroform onto a ZnSe prism. The specific polymer studied was 7.6 mol % 3HHx co-monomer, which had a molecular weight of 624,000. The samples were then heated to 160 ºC for one hour and annealed at 90 ºC overnight to generate crystalline lamellae. The sample thickness was determined to be 450 nm by AFM. A nucleation site for polymer recrystallization was generated by bringing a self-heated AFM tip that had been heated to 350 ºC to a distance of 3 µm from a specific location on the sample for 90 s and then withdrawing it [10].

2.3 AFM-IR data collection conditions

The ZnSe prisms with the samples mounted/deposited on them were placed onto the sample stage of the nanoIR™ AFM-IR instrument (Anasys Instruments, Santa Barbara, CA). The sample was allowed to equilibrate thermally with the sampling system prior to commencement of the measurements. All AFM topographic images are obtained in contact mode with either an AppNano SICONA 450 µm (Santa Clara, CA), Mikromasch CSC37-ALBS (Tallin, Estonia), Team-Nanotec Improved Super Cone 450 µm (Villingen-Schwenningen, Germany) contact mode cantilevers, or ThermaLever™ selfheating cantilevers (Anasys Instruments, Santa Barbara, CA). The IR laser produced pulses of 10 ns at a repetition rate of 1 kHz; the power levels incident on the ZnSe prism were set to *ca.* 0.5 mW by the software and the focused laser spot size was about 40 µm. Local spectra were collected over a 1800-1200 cm^{-1} spectral range using a data point spacing of 4 cm^{-1}. The spectral resolution is determined by the laser line width and is estimated to be about 8 cm^{-1}. The spatial resolution of the IR spectra collected using AFM-IR

is limited by mechanical and thermal properties of the sample. For the ~500 nm thick samples used in this study, we estimate the spatial resolution of the IR spectra presented here to be about 100 nm.

3. RESULTS

3.1 Wood cellulose

Figure 2 shows contact mode AFM images (top) of thin cross sections cut in the transverse direction of a control wood sample and an acetylated wood sample. Corresponding AFM-IR spectra collected from the locations indicated in the S2 cell wall lamina (S2CWL) and compound corner middle lamella (CCML) regions of the wood structure are highlighted in red and blue, respectively. The band near 1500 cm^{-1} is due to lignin, which is clearly more prevalent in the CCML than it is in the S2CWL region of the wood. A carbonyl band at 1736 cm^{-1}, due to acetylated cellulose, shows a significant increase in intensity in both regions of the acetylated sample. A higher degree of acetylation is apparent in the higher lignin content CCML regions of the wood sample.

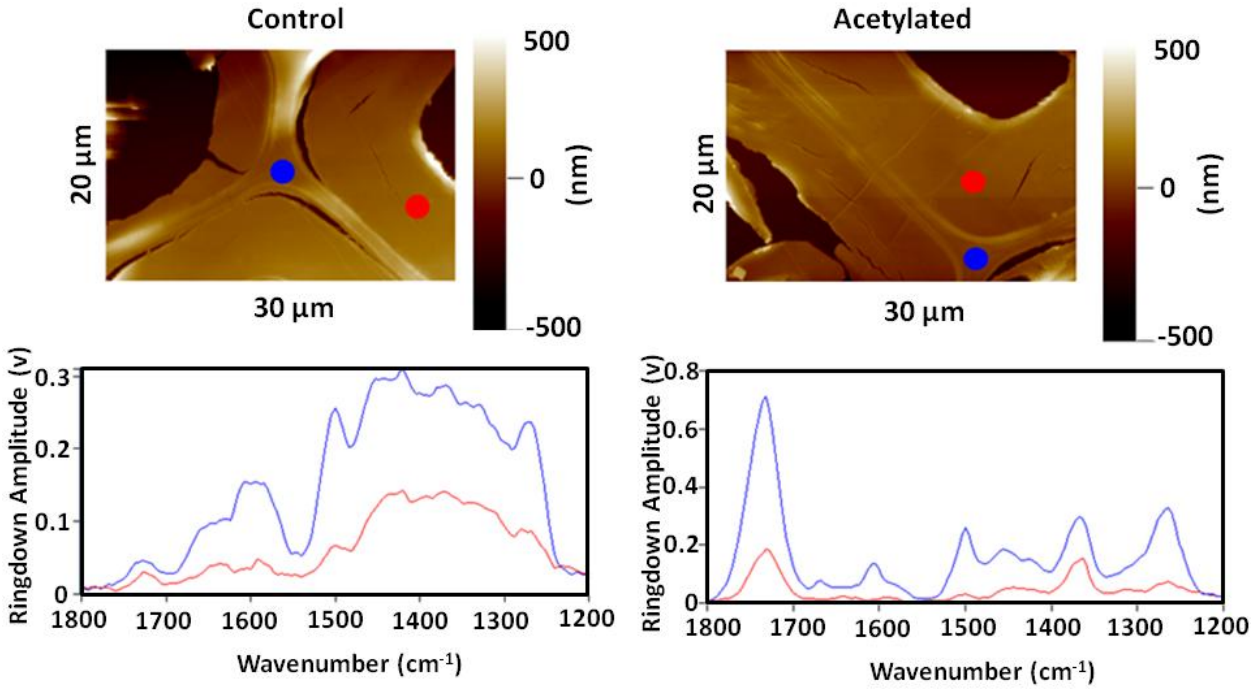

Figure 2. AFM images (top) of thin cross sections cut in the transverse direction of a control wood sample (left) and an acetylated wood sample (right). Corresponding AFM-IR spectra collected from the locations indicated in red and blue are highlighted using the corresponding colors in the spectra (bottom).

Figure 3 shows contact mode AFM images (top) of thin cross sections cut in the longitudinal (radial) direction of a control wood sample and an acetylated wood sample. Corresponding AFM-IR spectra collected from the locations indicated in the S2CWL and CCML regions of the wood structure are highlighted in red and blue, respectively. An additional spectrum indicated by

the green dot was also collected. The lignin band near 1500 cm^{-1} is clearly more prevalent in the blue and green locations than it is in the S2CWL (red) region of the wood. Once again, the 1736 cm^{-1} shows a significant increase in intensity in both regions of the acetylated sample, but is significantly stronger in the higher lignin content regions of the wood.

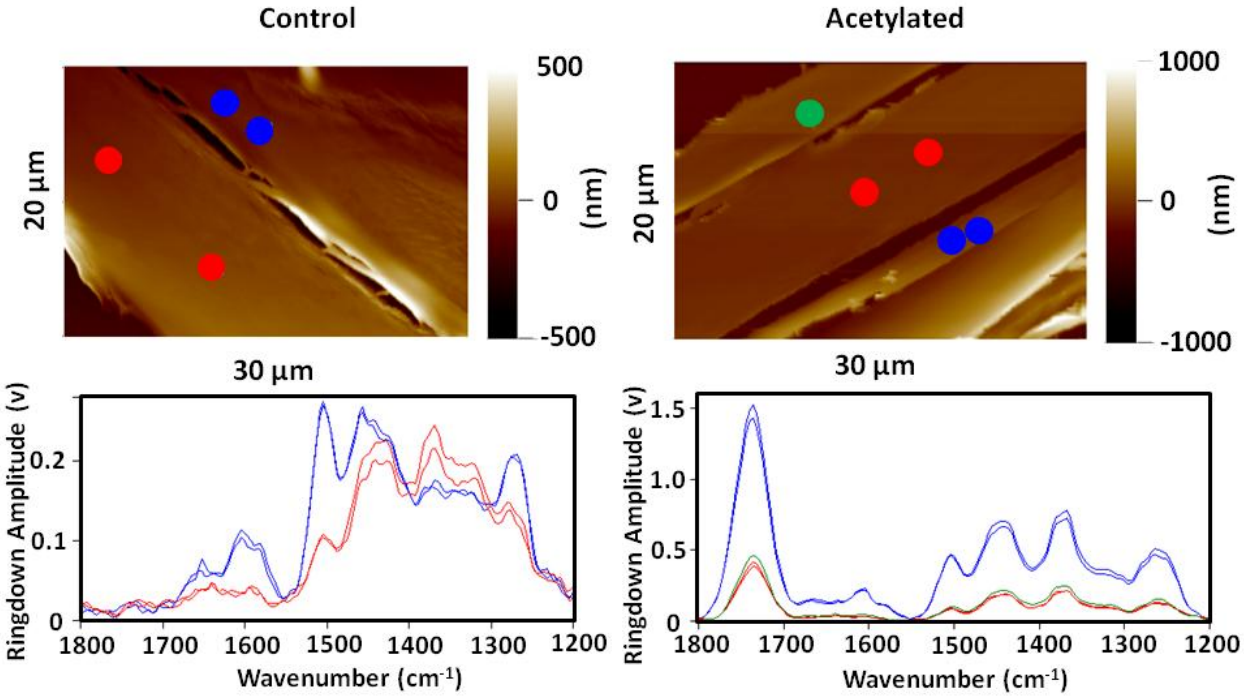

Figure 3. AFM images (top) of thin cross sections cut in the longitudinal (radial) direction of a control wood sample and an acetylated wood sample. Corresponding AFM-IR spectra collected from the locations indicated are shown (bottom).

It is also interesting to compare the IR spectra collected in the transverse cross sections with those collected in the radial sections. Clear differences in the relative intensities and band shapes are apparent for both the control and acetylated samples. This may be due to orientation effects that are detectable because the IR laser source is polarized.

3.2 Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (P(HB-*co*-HHx))

Figure 4 shows a contact mode AFM image and seven AFM-IR spectra collected on a sample film of P(HB-*co*-HHx) at the specific locations indicated. The bright spot in the top AFM image in Fig. 4 is a ‘‘high’’ area generated when an AFM tip that was heated to 350 °C for 90 s and then withdrawn was brought near that location. The AFM and AFM-IR measurements shown were recorded after the sample had cooled back to room temperature. It is clear that spectra collected near the site of the heated spot have much higher IR absorbance near 1276 cm^{-1}, where C-O-C skeletal backbone stretching vibrations occur, than spectra which were collected further away. The observed differences in the relative IR absorbance intensities and bandshapes in the vicinity of 1276 cm^{-1} band would be impossible to resolve at this spatial resolution using conventional FI-IR microspectroscopy.

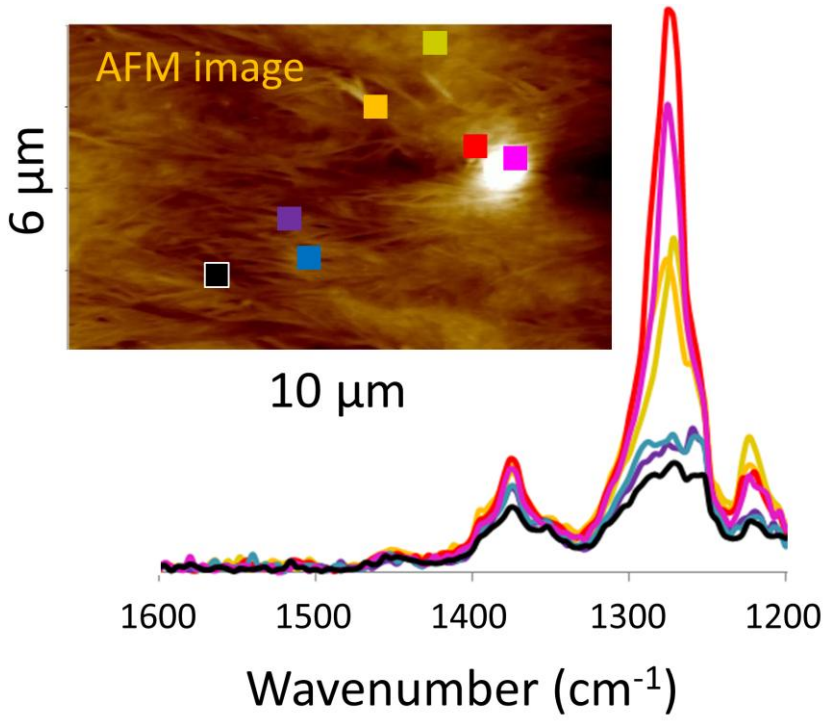

Figure 4. Contact mode AFM height image (left) and IR spectra at selected positions (right) of P(HB-co-HHx) (7.6 mol % HHx).

4. CONCLUSIONS

A new capability that uses an AFM to detect light absorption from a pulsed tunable IR laser source enabled the spectroscopic characterization of domains of wood cross sections and cast films of a poly(hydroxyalkanote) polymer. The functional group identification power of IR spectroscopy enabled the differentiation of specific microdomains at spatial resolutions on the order of 100 nm, well beyond with is possible with conventional FT-IR microspectroscopy. The understanding provided by the AFM-IR technique should prove valuable in helping guide formulation and processing conditions that produce morphologies with the most desirable material properties.

5. REFERENCES

1. Martin, Y., Zenhausern, F., and Wickramasinghe, H.K. "Scattering Spectroscopy of Molecules at Nanometer Resolution." *Applied Physics Letters* 68(18) (1996): 2475-2477.
2. Knoll, B. and Keilmann, F. "Near-Field Probing of Vibrational Absorption for Chemical Microscopy." *Nature* 399(6732) (1999): 134-137.
3. Anderson, M.S. "Locally Enhanced Raman Spectroscopy with an Atomic Force Microscope." *Applied Physics Letters* 76(21) (2000): 3130-3132.
4. Hammiche, A., Bozec, L., German, M.J., Chalmers, J.M., Everall, N.J., Poulter, G., Reading, M., Grandy, D.B., Martin, F.L., and Pollock, H.M. "Mid-Infrared Microspectroscopy of

Difficult Samples Using Near-Field Photothermal Microspectroscopy." *Spectroscopy* 19(2) (2004): 20-42.

5. Dazzi, A., Prazeres, R., Glotin, F., and Ortega, J.-M. "Local Infrared Microspectroscopy with Subwavelength Spatial Resolution with an Atomic Force Microscope Tip used as a Photothermal Sensor." *Optics Letters* 30(18) (2005): 2388-2390.
6. Dazzi, A., Prazeres, R., Glotin, F., and Ortega, J.-M. "Analysis of Nano-Chemical Mapping Performed by an AFM-Based (AFMIR) Acousto-Optic Technique." *Ultramicroscopy* (2007) 107(12): 1194-1200.
7. Dazzi, A., Prazeres, R., Glotin, F., Ortega, J.-M., Alsawaftah, M., and De Frutos, M. "Chemical Mapping of the Distribution of Viruses into Infected Bacteria with a Photothermal Method." *Ultramicroscopy* 108(7) (2008): 635-641.
8. Dazzi, A. "Sub-100-Nanometer Infrared Spectroscopy and Imaging Based on a Near-Field Photothermal Technique (PTIR)." *Biomedical Vibrational Spectroscopy*, Eds: Kneipp, J. and Lasch, P. Hoboken, NJ: Wiley, 2008. 291-312.
9. Prater, C., Kjoller, K., Cook, D., Shetty, R., Meyers, G., Reinhardt, C., Felts, J., King, W., Vodopyanov, K., and Dazzi, A. "Nanoscale Infrared Spectroscopy of Materials by Atomic Force Microscopy." *Microscopy and Analysis* 24(3) (2010): 5-8.
10. Marcott, C., Lo, M., Kjoller, K, Prater, C., and Noda, I. "Spatial Differentiation of Sub-Micrometer Domains in a Poly(hydroxyalkanoate) Copolymer Using Instrumentation that Combines Atomic Force Microscopy (AFM) and Infrared (IR) Spectroscopy." *Applied Spectroscopy* 65(10) (2011): 1145-1150.
11. Kazarian, K.G., Chan, K.L.A. "Micro- and Macro-Attenuated Total Reflection Fourier Transform Infrared Spectroscopic Imaging. *Applied Spectroscopy* 64(5) (2010): 135A-152A.
12. Gulley-Stahl, H.J., Bledsoe, S.B., Evan, A.P., and Sommer, A.J. "The Advantages of an Attenuated Total Internal Reflection Infrared Microspectroscopic Imaging Approach for Kidney Biopsy Analysis." *Applied Spectroscopy* 64(1) (2010):15-22.
13. Mohebby, B. "Application of ATR Infrared Spectroscopy in Wood Acetylation." *J. Agric. Sci. Technol.* 10 (2008): 253-259.
14. Williams, S.F. and Martin, D.P. "Application of PHAs in Medicine and Pharmacy." *Biopolymers*, Eds. Doi, Y. and Steinbuchel, A. Vol. 4, Weinhiem: Wiley-VCH, 2002. 91–128.
15. Lara, L.M. and Gjalt, W.H. "Metabolic Engineering of Poly (3-Hydroxyalkanoates): From DNA to Plastics. *Microbiol. Mol. Biol. Rev.* 63(1) (1991): 21-53.
16. Anderson, A.J. and Dawes, E.A. "Occurrence, Metabolism, Metabolic Role, and Industrial Uses of Bacterial Polyhydroxyalkanoates." *Microbiol. Rev.* 54(4) (1990): 450-472.
17. Jendrossek, D. "Extracellular Polyhydroxyalkanoate Depolymerases: The Key Enzymes of PHA Degradation." *Biopolymers*, Eds. Doi, Y. and Steinbuchel, A. Vol. 3B, Weinhiem: Wiley-VCH, 2002. 41–84.
18. Lee, S.Y. "Bacterial Polyhydroxyalkanoates." *Biotechnol. Bioeng.* 49 (1996): 1-14.

19. Satkowski, M.M., Melik, D.H., Autran, J.-P., Green, P.R, Noda, I., and Schechtman, L.A. *Biopolymers*, Eds. Doi, Y. and Steinbuchel, A. Vol. 3B, Weinhiem: Wiley-VCH, 2002. 231–264.

20. Noda, I., Green, P.R., Satkowski, M.M., and Schechtman, L.A. "Preparation and Properties of a Novel Class of Polyhydroxyalkanoate Copolymers." *Biomacromolecules* 6(2) (2005): 580-586.

21. Noda, I., Satkowski, M.M., Dowrey, A.E., and Marcott, C. "Polymer Alloys of Nodax Copolymers and Poly(Lactic Acid)." *Macromol. Biosci.* 4(3) (2004): 269-275.

22. Poliakoff, M. and Noda, I. "Plastic Bags, Sugar Cane, and Advanced Vibrational Spectroscopy: Taking Green Chemistry to the Third World." *Green Chemistry* 6 (2004): G37-G38.

23. Padermshoke, A., Sato, H., Katsumoto, Y., Ekgasit, S., Noda, I., and Ozaki, Y. "Thermally Induced Phase Transition of Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) Investigated by Two-Dimensional Infrared Correlation Spectroscopy." *Vibrational Spectroscopy* 36 (2004): 241-249.

24. Sato, H., Murakami, R., Mori, K., Ando, Y., Takahashi, I., Noda, I., and Ozaki, Y. "Specific Crystal Structure of Poly(3-hydroxybutyrate) Thin Films Studied by Infrared Reflection–Absorption Spectroscopy." *Vibrational Spectroscopy* 51 (2009): 132-135.