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Research

Nano-Fourier Transform Infrared Imaging Based on Atomic Force Microscopy for Milk Analysis

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Abstract

Mid-infrared (MIR) spectroscopy is a widely used tool for chemical group identification and molecular structure analysis in chemical, pharmacy, agricultural and food related-samples. However, the diffraction limit prevents the commonly used MIR spectroscopy from mapping on nano-scale particle studies. In this paper, we introduce an imaging of milk with 50 nm lateral resolution and sensitivity to individual protein and fat particle by nano-Fourier transform infrared spectroscopy (nano-FTIR) based on atomic force microscopy (AFM). Nano-FTIR provides similar infrared spectra to traditional spectroscopy. Moreover, the concentration distribution images of the protein and fat in milk of nano-scale are displayed. Particularly, samples with size up to 100 nm can be displayed clearly. Nano-FTIR imaging can be a powerful analytical technique for the research and development of food products.

Keywords: Nano-FTIR; Imaging; Milk; Particle; Spectroscopy

Introduction

Infrared spectroscopy is a widely used analytical technique for chemical identification in food [1], pharmaceutical [2], agricultural [3] and environmental [4] samples. To cope with the problems that infrared spectroscopy cannot provide the spatial information of the sample surface, infrared hyper spectral imaging have been introduced. Imaging indicates the particulate sizes of distribution within final products and have been applied in process control, like real-time monitoring at critical processing points [5]. However, because of the diffraction limit of the light, the conventional infrared technique cannot be applied for nano-scale imaging.

The quality of data obtained has been improved significantly when the atomic force microscopy (AFM) and infrared (IR) spectroscopy have been incorporated into a novel technique [6]. Nano-Fourier transform infrared spectroscopy (nano-FTIR) is based on recording the infrared light scattered at a scanning probe tip, so the diffraction-limited resolution of FTIR is break through and infrared images with nano-scale spatial resolution can be obtained. Acting as an antenna, a metalized AFM tip concentrates the incident field at the tip-apex for local probing of molecular vibrations of the analyzed sample [7].

The AFM–FTIR spectra generated from this technique contain the same information with respect to molecular structure as conventional IR spectroscopy measurements, allowing significant leverage of existing expertise in IR spectroscopy [8]. The AFM–IR technique can be used to acquire IR absorption spectra and absorption images with spatial resolution on the 50 to 100 nm scale, versus the scale of many micrometers or more for conventional IR spectroscopy. AFM-IR has been applied for analysis of polymer materials with organic matter, semiconductor materials, and nano-wires [9].

Precisely analytical methods are essential to ensure product quality, safety, authenticity and compliance with labeling. For example, milk is an emulsion or colloid of butterfat globules within a water-based fluid that contains dissolved carbohydrates and protein aggregates with minerals, which provides the most appropriate nutritional and immunological support for mammal infants. The impact of processing, like high pressure thermal (HPT) processing, ultrasonic or high pressure homogenization on the products quality should be studied [10]. To observe the processed milk sample, microscope equipped with a camera and ocular micrometer is usually applied to determine the diameters of fat globules and to evaluate the homogenization efficiency of milk samples [11]. Traditional microscope can only provide very limited spatial information and they are also affected by diffraction limit of the light.

In the present work, nano-FTIR spectroscopy imaging has been used to investigate the individual ingredients present with in milk. Nano-FTIR provide not only similar infrared spectra to traditional spectroscopy, but also the spatial information (concentration distribution imaging of components in milk) in nano-scale. This technique provides a powerful analytical tool, not just for milk, but for the food industry as a whole.

Experimental

Materials

Liquid milk sample was spread on the quartz microscope slide and

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dried with natural air under room temperature.

Instrumentation

A nano-FTIR system (Anasys Inc.) was used for all spectral acquisition with independent spectral graphs and IR imaging for the investigation. The instrument provides contact mode, tapping mode, force curve mode, and force modulation mode. The contact mode was used for AFM morphology analysis. The tapping mode was used for spectra collection. The AFM-IR spectra were collected over a range of1000–1800 cm^{-1}, with a spectral resolution of 4 cm^{-1}andan accumulation of 128 scans for each data point. Analysis Studio software (Anasys Inc.) was used for data collection and analysis.

Results and Discussion

Spectra at Different Locations on the Surface of the Milk Sample

Consecutive height nano-images of milk sample at two different locations was first analyzed using AFM (see Figure 1 a) and b)). The bright and dark colors in the map display the height of the sample surface. The air bubbles produced during preparation in the milk sample were shown as dark area with diameter of 200nm-1000nm in the image of Figure 1 a). Figure 1 b) displays some bigger air bubbles compare the area in Figure 1 a). As milk is a homogeneous food product, the protein, fat and sugar particles in milk are difficult to be observed even though the spatial resolution of the AFM image is in 50 nm scale. The milk sample was soft, so that the image show some tiny scratch when the tip of the instrument moved on the surface of the sample.

In Figure 1 c) and d), infrared spectra of milk located on the red, green dots (see Figure 1 a) and the cyan, blue, violet, black dots (see figure 1 b) are displayed, and those spectra displayed different characteristic bands. The colors of the spectra are corresponding to the color of the dot indicating their locations. Most of the spectra have the characteristic bands on 1224 cm^{-1}, 1452 cm^{-1},1536 cm^{-1}, 1650 cm^{-1}, and 1740 cm^{-1}. Some dots shows special characteristic bands like 1100 cm^{-1} (green), 1148 cm^{-1}(green), 1164 cm^{-1}(red and violet), 1216cm^{-1} (blue).

The characteristic bands of milk samples obtained by AFM-FTIR were compared with the published papers about milk analysis using traditional infrared spectroscopy[10,12,13]. AFM-FTIR and IR, two different techniques, show similar characteristic

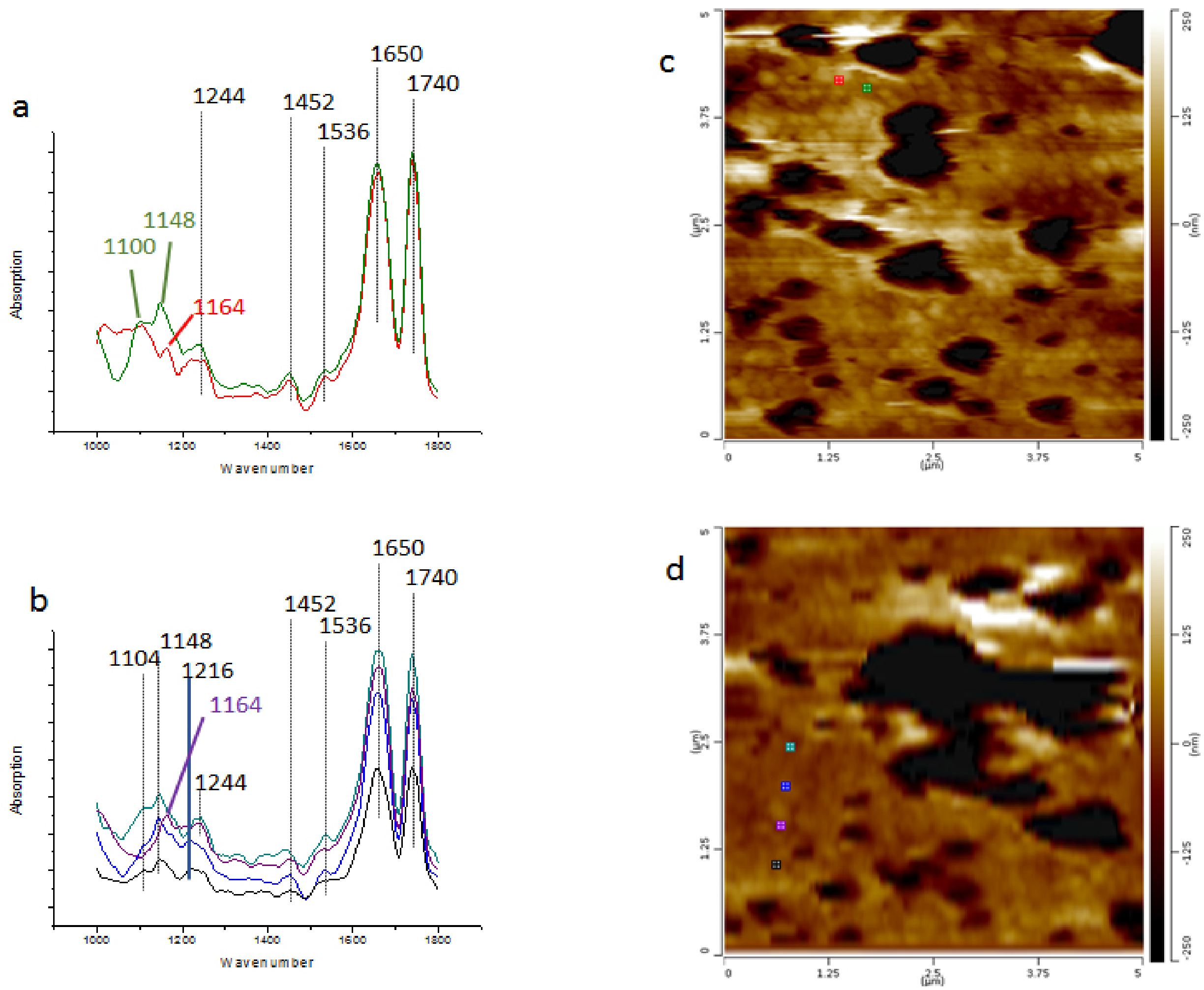

Figure 1. a) and b) Consecutive height nano-images of milk sample on two different locations. c) and d) Infrared spectra of milk samples on the color dot marked in the images. The color of the spectra are corresponding to the color of the dot indicating the location.

bands in spectra of milk. From the published report, IR peaks at 1740cm^{-1}, and 1164cm^{-1}all arise from the fat in the milk. 1452 cm^{-1} is corresponding to functional group like CH_2 and CH_3, and they may include most of the organic components in milk. the milk are difficult to be resolved directly from this image.

Figure 2 b shows corresponding concentration distribution of the components on this location. Figure 2 b1) is the component with

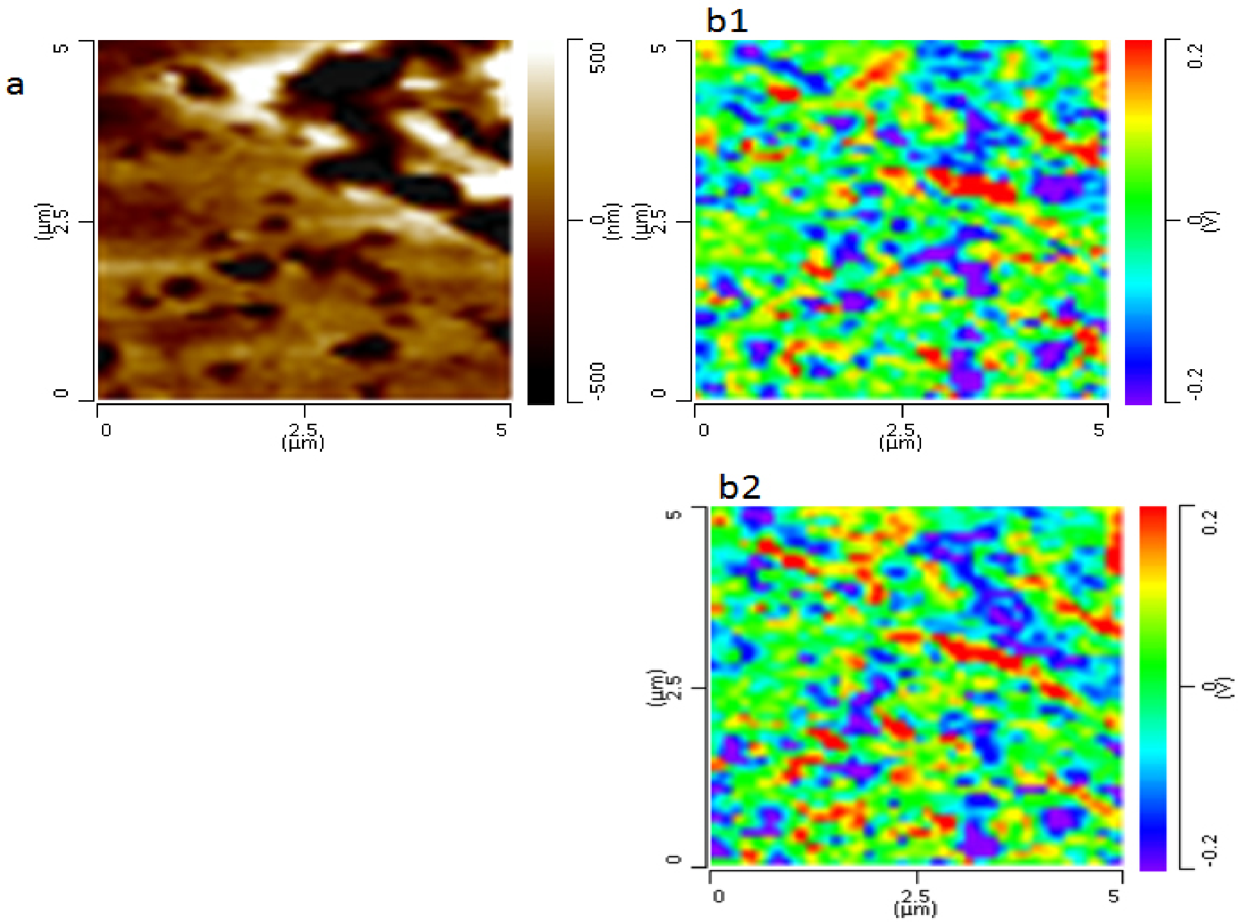

Figure 2. a) Consecutive height nano images of milk sample on the third location, and b) corresponding concentration distribution of the components on this location. b1) corresponds to the component with characteristic band at 1650 cm^{-1} and b2) corresponds tothe component with characteristic band at 1740 cm^{-1}. In figure 2b, red color means the component has relatively higher concentration.

The bands around1100 cm^{-1}, 1224 cm^{-1}stand for the vibrations of amide I bands of protein.1650 cm^{-1} and 1536cm^{-1}can be assigned to amide II. 1216cm^{-1} of spectra on blue dot stands for the C–O vibration in sugar. In this way, the spectra of different locations in the image of milk sample can provide useful chemical information corresponding components in the milk.

Chemical Concentration Distribution of Components in the Milk

Nano-FTIR imaging can provide not only the spatial distribution information about the constituents, but also the size information. Milk in those locations displayed very different components, which can be assigned as proteins, fat or sugars.

Figure 2 a shows consecutive height nano-images of milk sample on the third location, which displays almost similar characteristics topography from the locations chose in above session. Some bulbuls can be found in milk and the particle of the components in

characteristic band at 1650 cm^{-1},which is the characteristic band of amide I, and can be used for displaying the distribution of protein particle. The red color areas show high concentration of amide I and represent the shape of the particle of the proteins in the milk sample. The Nano-FTIR is based on photo thermal induced resonance, the intensity of the signal is independent scattering and proportional to the absorption energy. Therefore, even though water band has strong influence at centered around 1640 cm^{-1} normally in traditional infrared spectroscopy, it does not affect the analysis of protein in milk using AFM nano-FTIR in band at 1650 cm^{-1}. Figure 2 b2) is the component with characteristic band at 1740 cm^{-1}, which displays the fat particles in the milk sample.

Both Figure 2 b1) and b2) show relatively low concentration in the location where there are bulbuls in Figure 2a, but they are not totally similar to the distribution in Figure 2a. Figure 2b gives much more details of the particle size information of protein and fat in the milk sample. From the Figure 2b, the distribution of fat and protein show complement to each other in some areas and overlaps in some other areas. The size of the particles of the two components is in a large range from 100nm to 1000nm. The long stripes normally have consistent width and they may be consisted by several partials crowding together.

Nano-FTIR enables reliable probing and imaging of components structure in milk with nano-scale resolution, and close to single particle of those components sensitivity. Our results indicate that infrared spectra of sample on very accurate location can be obtained, which demonstrates extraordinary sensitivity of nano-FTIR to ultra-small amounts of in food material.

In summary, nano-FTIR is a technique based on AFM, the light tip have to move on the surface of the samples, and the surface of the sample must reach certain hardness to hold the tip, so that this method cannot be applied on liquid food samples analysis, so far. The speed of the equipment is not as fast as low spatial resolution IR imaging instruments. Since the spectra obtained by nano-FTIR spectroscopy are similar to low spatial resolution IR spectroscopy, some useful characteristic bands can be obtained using traditional IR spectroscopy beforehand, and only certain range of wavelength interesting are suggested to be used for imaging. Then the concentration image of corresponding component can be displayed in nano-scale level within a short analysis time.

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