Application of Atomic Force Microscopy as a Nanotechnology Tool in Food Science

HONGSHUN YANG, YIFEN WANG, SHAOJUAN LAI, HONGJIE AN, YUNFEI LI, AND FUSHENG CHEN

ABSTRACT: Atomic force microscopy (AFM) provides a method for detecting nanoscale structural information. First, this review explains the fundamentals of AFM, including principle, manipulation, and analysis. Applications of AFM are then reported in food science and technology research, including qualitative macromolecule and polymer imaging, complicated or quantitative structure analysis, molecular interaction, molecular manipulation, surface topography, and nanofood characterization. The results suggested that AFM could bring insightful knowledge on food properties, and the AFM analysis could be used to illustrate some mechanisms of property changes during processing and storage. However, the current difficulty in applying AFM to food research is lacking appropriate methodology for different food systems. Better understanding of AFM technology and developing corresponding methodology for complicated food systems would lead to a more in-depth understanding of food properties at macromolecular levels and enlarge their applications. The AFM results could greatly improve the food processing and storage technologies.

Keywords: atomic force microscopy (AFM), food science, macromolecule, microstructure, nanotechnology

Introduction

With the development of modern instruments, more and more microscopes are used in food science and technology research. This research has brought us a lot of new knowledge on food microstructure and further improved the processing and storage technologies. Some representative reports are transmission/scanning electron microscopy (SEM/TEM) on fruit and vegetables (He and others 2004); light microscopy (LM) on ice cream (Caillet and others 2003), and confocal laser scanning microscopy (CLSM) on fruit surface layers (Veraverbeke and others 2003). These microscopes bring insightful information of food structure at macromolecular scale; however, there are some drawbacks that limit them from being widely used. For example, it is difficult to get high-resolution molecule structures from LM. Samples for SEM/TEM and CLSM imaging should be pretreated: staining and vacuum for SEM/TEM; staining, fluorescence, and some other special treatment for CLSM. Furthermore, some fluorochromes are sensitive to laser illumination and will bleach before acquiring a good image. This results in many blurred images, and even destroys the native status of the specimen (Veraverbeke and others 2003).

In 1986, without the use of any lens or photon, a completely new type of microscopy was proposed, which directly investigates the sample surface by means of mechanical scanning. Since the microscopy of this type operates using atomic forces, it is frequently referred to as atomic force microscopy (AFM). AFM is also known as scanning force microscopy. This new scanning probe microscopy is based on the concept of near-field microscopy, which overcomes the problem of the limited diffraction-related resolution inherent in conventional microscopies (Braga and Ricci 2004). Thus, the notions of conventional microscope design are not applicable in AFM. In fact, AFM collects data for images by "feeling" rather than "looking" (Morris and others 1999). There are 3 kinds of operation modes (contact, noncontact, and tapping mode) that can be applied to different materials. AFM has been applied extensively in biological science, material science, chemistry, and recently food science.

In comparison with common forms of microcopies used by researchers in food science, AFM offers a number of unique features (AFM/LFM instruction manual 1997; Braga and Ricci 2004):

1. High magnification with high resolution. AFM is one of the few techniques that can detect atomic-scale defects.
2. Minimal sample preparation, no dyes as in LM; no vacuum, critical point, or gold sputtering as in SEM/TEM; no fluorescence as in CLSM. The samples can keep their native status or near-native status.
3. The ability to obtain different views of the sample from a single data collection; 2D and 3D images can be acquired at the same time.
4. The samples can be imaged in air or in an aqueous environment continuously, thus it is possible to observe ongoing processes directly.
5. The possibility of manipulating macromolecules and investigating the interaction between macromolecules.

With these unique features, AFM is a powerful tool used in investigating the fine structure information of food materials. For example, irregularities in polymer structure that escape detection in whole-sample-based analyses can be identified when individual polymers are examined (Round and others 1997). Thus AFM imaging offers the potential to characterize the integral heterogeneous structures of food macromolecules (Yang and others 2006c). However, there are some limitations of the technique such as relatively small scan size, the low scanning speeds, and the difficulties in imaging food and biological samples that are too soft (Braga and Ricci 2004). Table 1 shows the advantages and disadvantages of several commonly used microscopies.

MS20060644 Submitted 11/21/2006, Accepted 2/27/2007. *Authors Yang and Chen are with College of Food Science and Technology, Henan Univ. of Technology, Zhengzhou, Henan 450052, China. Authors Yang and Wang are with Biosystems Engineering Dept., Auburn Univ., Auburn, AL 36849-5417, U.S.A. Author Lai is with the Dept. of Biochemistry, Molecular Biology and Biophysics, Univ. of Minnesota-Twin Cities, Minneapolis, MN 55455, U.S.A. Author An is with Teda Bio-X Center, College of Food Engineering and Biotechnology, Tianjin Univ. of Science & Technology, Tianjin, 300457, China. Author Li is with the Dept. of Food Science and Technology, College of Agriculture and Biology, Shanghai Jiao Tong Univ., Shanghai, 201101, China. Direct inquiries to author Li (E-mail: yfli@sjtu.edu.cn).*

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Table 1—Advantages and disadvantages of different microscopy techniques

	Microscopy				
Characteristics	**AFM**	**LM**	**SEM**	**TEM**	**CLSM**
Advantages	High resolution, nanoscale Minimal sample preparation, near native status 2D and 3D In air/liquid, in situ, continuous process Can be manipulated	Large scan area Fast scan speed Cheap	Nanoscale High resolution Fast scan speed	Nanoscale High resolution Fast scan speed	Study dynamic process Fast scan speed 2D and 3D In situ
Disadvantages	Small scan size Slower scan speed Difficult for soft material	Only 2D Need pretreatment Low resolution and magnification	Only 2D Need pretreatment Not native status	Only 2D Need pretreatment Not native status	Complicated operation Need pretreatment

This review introduces the underlying fundamentals of AFM and briefly discusses the latest application of AFM in food science, which provides food technologists and the research community a panorama of past and current researches and future trends of the application of the AFM technology in this area.

Fundamentals of Atomic Force Microscopy

Principle

AFM images are obtained by measuring changes in the magnitude of the interaction between the probe and the sample surface (commonly van der Waal's force) as the surface is scanned beneath the probe. Figure 1 shows the schematic image of the imaging process. A laser beam (from laser diode) is focused onto the end of the cantilever (preferably directly over the tip), and then reflected onto a position-positive photodiode detector after being reflected by a mirror. As the tip moves in response to the sample topography during scanning, the angle of the reflected laser beam changes according to Hooke's law. The change of laser beam causes the laser spot falling onto the photodiode to move, which produces changes in the intensity in each of its quadrants. The difference in laser intensity between the segments produces an electrical signal that quantifies the normal motion of the tip (feedback). When the sample is scanned, the topography of the sample surface causes the cantilever to deflect as the force between the tip and sample changes. The map of the surface topography from the measured cantilever deflection is generated by computer and is shown in a monitor and the control part is shown in another monitor (Morris and others 1999).

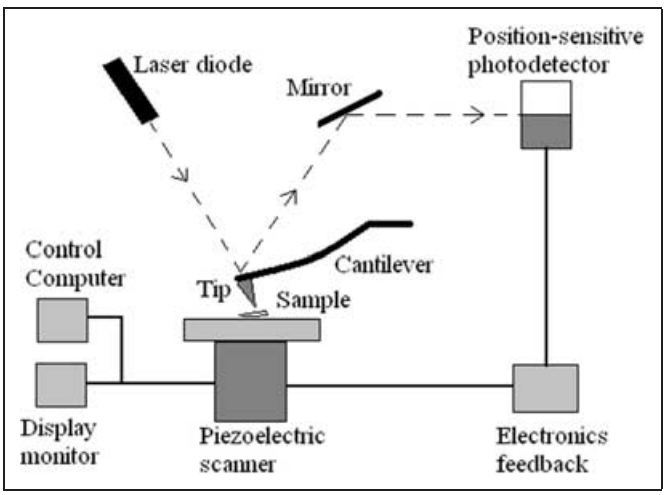

Figure 1—Schematic image of the AFM imaging process (based on Braga and Ricci 2004)

The AFM can be operated in 2 principal modes: with feedback control and without feedback control. When it is with feedback control, the positioning piezo can respond to any changes in force that are detected, and alter the tip-sample separation to restore the force to a predetermined value; thus a topographical image or height mode image will be obtained. When it is without feedback control, the AFM is operated in constant height or deflection mode. This is particularly useful for imaging very flat samples at high resolution. This is also called error signal mode. In this mode, slow variations in topography can be removed and the edges of features in the images can be highlighted (http://spm.phy.bris.ac.uk/techniques/AFM/).

The resolutions of AFM images include lateral (X, Y) resolution and vertical (Z) resolution. For lateral resolution, tip shape and pixelization are 2 crucial factors for most rough surface samples. The radius of curvature of the end of the tip will determine the highest lateral resolution obtainable with a specific tip, and, if the sample surface is atomically flat periodic lattice, it would be possible to achieve atomic resolution. Pixelization affects resolution in that we cannot get features smaller than the pixel size of the image. Regardless of the pixel size, the feedback loop is sampling the topography many times at each pixel. The data displayed at each pixel are the average of the sampling iterations by the feedback loop over the pixel area; for vertical resolution, scanner, pixelization, and noise are 3 related factors. The main factor is the noise on the power supply that controls the piezo-electric scanners. The resolution is also determined by the resolution of the vertical scanner movement that is < 0.1 nm. The number of data points in the vertical direction limits the size of the smallest resolvable height change. The Z-limit parameter may be used to increase the sampling resolution in the vertical dimension to image subangstrom height changes with large Z range scanners (AFM/LFM instruction manual 1997).

Operation

There are 3 primary imaging modes in AFM operation: contact mode, noncontact mode, and tapping mode.

In contact mode, AFM is operated using a tip attached to the end of a cantilever that essentially maintains contact between the tip and the sample surface at all times. It applies a far greater force to the sample and sometimes this will lead to poor images and distortion of soft samples by the tip because of capillary forces when imaging in air (Power and others 1998). In noncontact mode, the cantilever is oscillated at a frequency that is slightly above the cantilever's resonance frequency, typically with an amplitude of a few nanometers (<10 nm), in order to obtain an AC signal from the cantilever. In tapping mode, AFM is operated using a tip attached to the end of an oscillating cantilever that intermittently contacts the surface at the lowest point in the oscillation. This mode significantly reduces the forces exerted by the tip on the sample compared to the contact

mode of operation, and reduces both sample and tip damage. There is higher lateral resolution on most samples when applying tapping mode rather than contact and noncontact modes. Thus, it is the most commonly used mode in food and biological science fields. This tapping mode was used in most of the AFM literatures we discussed in this review as well. Recently, one of the developments in tapping mode is phase imaging. Phase imaging is obtained by measuring the phase difference between the oscillations of the cantilever driving piezo and the detected oscillations. Then image contrast is derived from image properties such as stiffness and viscoelasticity. However, one disadvantage to tapping mode is its slightly slower scan speed than contact mode AFM. Generally speaking, tapping mode AFM is suitable for food and biological samples because these samples are relatively soft. However, to select an operation mode, the important thing is to optimize the method used. Even though there are many similarities in imaging starch and protein, it is impossible that one imaging condition can be used for all starch and protein because when imaging different macromolecules, we should change the specific method according to their concrete properties, such as aggregation, elasticity, and then select the appropriate tip and imaging conditions.

Recently, force spectroscopy became one of the hottest issues in imaging mode. In this mode, a macromolecule sample is deposited onto solid substrate first, then the AFM tip is brought into contact with the substrate by moving the piezotube. The macromolecule chains can absorb onto the AFM tip to form a bridge structure between the tip and the substrate, then macromolecule chain bridge would be stretched when the tip and the substrate separate, which results in the deflection of cantilever. Meanwhile, deflection of the cantilever and the displacement of the piezotube are recorded. Then, the deflection is converted into force signal; thus the relationship between the force and the extension is obtained. This imaging mode has achieved a great performance on individual molecules and colloidal interactions (Liu and others 2005).

The sample can be imaged in air or fluid. After determining the imaging mode (contact, noncontact, or tapping) and sample status (in air or fluid), the manipulation can be conducted. For instance, AFM imaging food macromolecules in air is commonly conducted in a glove box at a low relative humidity that is controlled by silica gel. The food macromolecule solution is adjusted to room temperature and then disrupted by vortexing. A small volume (dozens of microliters) of votexed solution is deposited onto a surface of freshly cleaved mica sheet by pipette. Molecular combing or fluid fixation may be applied to manipulate the molecule to get more information (Yang and others 2006a). Fluid fixation is a manipulation that uses velocity gradient of the convective fluid flow produced by the evaporation of the droplet. The purpose is to yield well-elongated and aligned macromolecules, and then AFM imaging can investigate more delicate structural information. Then, the mica surface is air dried or dried in a dust-free enclosure (with free air convection or forced nitrogen gas convection). The solution also can be diluted to get desirable images.

After sample preparation, AFM software can be used for imaging in a process divided into 2 stages: real-time operation and offline analysis. Real-time operation is for obtaining the images and offline analysis is for analyzing the images from real-time operation.

The function of real-time operation is to run the actual microscope, change the size and location of the sample being scanned, control gains, and so on. Scanner settings, scan area, vertical range, and scan rate can be set according to the object properties. Other commonly used parameters in real-time operation are setpoint and integral gain (AFM/LFM instruction manual 1997). The scanner is adjusted to select and capture smaller images within the region accessible for scanning. The integrity of the AFM tip is verified by imaging a reference standard object before imaging sample.

Analysis

Images produced from scans are analyzed and/or modified using the software's offline functions. Special softwares were developed by companies for analyzing the AFM images. However, most of the functions in different softwares are similar and thus the results are comparable. The bright and dark areas in the images correspond to peaks and troughs in the objects. Commonly, different scales are used in the vertical and horizontal axes.

There are many offline operations for getting sample properties. For example, sectional profile analysis is used to measure the depth, height, and width of specimens. Roughness analysis is performed over an entire image or a selected part of the image to describe the surface status of the specimens.

There are many modification functions to improve the image quality. The correction of the images by offline analysis software can be used to reduce the noise of the samples and obtain high-quality images. The function of filters can be applied to AFM images to improve their quality. Flattening may be used to remove image artifacts due to vertical scanner drift, image bow, skips, and anything else that may have resulted in a vertical offset between scan lines. However, any modification operation may affect the measurement of interest, and it is better to modify the image as little as possible (AFM/LFM instruction manual 1997). Before modification operation, we should notice the influence of that operation on the parameters we want to determine. Do not use an operation that would greatly influence the parameters. For example, if we want roughness analysis results, it is better not to flatten the images. However, if we want to determine the chain width of macromolecules, the flattening operation has little influence on the results.

In addition to the parameters provided by software, researchers can utilize the molecule properties to obtain desired information. For example, the heights of molecular chains could be measured to judge whether molecular intersections were branched structures within molecules or overlapping molecules. A perpendicular line can be drawn across the molecular chains, and the vertical profile along that line is displayed on the screen. Several cursors can be placed on the line section to obtain the vertical measurements, which can be viewed as the heights of molecular chains. In general, the height of the chains is equal to the sum of 2 single chains when 2 chains cross over while it is equal to the height of a single chain at branch points (Adams and others 2003).

Recently, phase imaging and force spectroscopy were developed for investigating more information on the mechanical properties of samples. The principle of force spectroscopy is based on force-distance curve measurement. The curves are obtained directly from force-distance curves using the situations that the Z-piezo displacement has divided into 2 quantities, cantilever deflection and polymer extension length. During the force-distance curve movement, the cantilever is oscillated at its resonant frequency. Through measurement, frequency, amplitude, phase of the excitation signal, and the speed of Z-piezo scanner movement is kept at constant. Then variations in composition, friction, the chain stiffness, viscoelasticity, and other related mechanical properties can be obtained from these curves (Watabe and others 2006).

Applications in Food Science and Technology

Since the AFM was first invented in 1986, scientists and engineers have worked eagerly to apply this powerful instrument to almost all aspects, including food science and technology. Figure 2 shows the number of papers related to AFM technology applied in food

science field and its account for all the AFM papers in all the fields since 1993. In that year, AFM was first introduced as a powerful tool for monitoring changes of food proteins. It shows that the AFM papers related to food science field increased steadily until 2004. Meanwhile, a steady increase in AFM papers related to food science accounts for all the AFM papers in all fields accordingly. It should be noted that the numbers of these articles were searched under "atomic force microscopy AND food," and "atomic force microscope AND food" as topic (TS) in Institute for Scientific Information (ISI) search (there were several other related articles that might not be included in these results). Generally speaking, results suggest this field is promising and since it was created, more scientists have used AFM to study food science problems. One phenomenon is the relative ratio of AFM papers in food science decrease during 2005 to 2006, while the number of papers was almost the same as that during 2003 to 2004. One main reason is that new imaging modes were developed and they were applied in biological, material science and not much in food science.

Since tapping mode of AFM is the most applied mode in food science and height image is generally used to indicate the results, in this article, all AFM operations were in tapping mode and the images were showed with height mode if not specially indicated.

Commonly, the AFM application in food science can be divided into 6 main categories: (1) Qualitative imaging: AFM is used to qualitatively analyze the structure of food macromolecules. (2) Fine or quantitative structural analysis: Researchers try to find the characteristic parameters of AFM images that relate to food properties or its changes during processing or storage. (3) Molecular interaction: AFM is successfully applied to investigate molecular interaction. (4) Food macromolecular manipulation: AFM is applied to manipulation of food macromolecules. (5) Surface topology: Delicate physical properties of food surfaces are characterized by AFM. (6) Nanofood tools: AFM is used as a powerful tool for nanofood production and research. In the following sections, these 6 categories are discussed in detail and citations are listed for those with further interests in original papers.

Qualitative macromolecule and polymer imaging

The basic function of AFM is to provide the images of examined objects. AFM can be easily applied to describe a single macromolecular or polymer's qualitative information. Theoretically almost all food macromolecules can be imaged by AFM. In practical researches, almost all reports are focused on polysaccharides and proteins.

AFM has been used to image individual macromolecules and their intermolecular association for a long time (Morris and others 1997). Gunning and others (1996a) extended methods for AFM imaging of individual polysaccharides in order to image networks and gels formed by the bacterial polysaccharide gellan gum. Attempts have been made to image the surface of bulk aqueous gellan gels under butanol. Imaging in alcohols has the advantage of greatly reducing shear forces for keeping the samples intact. However, alcohols have the drawback that they may precipitate polysaccharides and alter the gel structure (Decho 1999). The AFM images of iota carrageenan showed that the network structure of refined iota carrageenan was homogeneous and composed of a continuous branched polymer network (Gunning and others 1998). Similar homogeneous structures also existed in polysaccharides from monoi. AFM images showed these consisted of linear chains with occasional long branches. The molecular weight distribution is wide with large variability in end-to-end lengths along single chains (Vardhanabhuti and Ikeda 2006). AFM could be used to illustrate different aggregation modes for different polysaccharides, which could provide auxiliary information that benefited in results from other instruments. The hyaluronan networks dispersed when diluted, which reveals individual molecules or small aggregates. However, at the same dilution the hylan retained the large aggregated structures (Gunning and others 1996b). Polysaccharide in motion also can be recorded by AFM (Gunning and others 2000). The 3 classically described states were postulated to be involved in polymer adsorption or desorption. Loops, trains, and tails were confirmed by direct observation of a water-soluble wheat pentosan. Adams and others (2003) illustrated two of the most common appearances for arabinoxylans that were linear and entangled "shoe-lace"-shaped structures. Furthermore, heterogeneity of individual molecules can be characterized by AFM.

In recent years, a growing number of researchers have studied the polysaccharides from commonly used food materials. Starch is an important food component and widely used in food industry. Gunning and others (2003) applied a new process involving incubation of hot amylose solutions with iodine and the nonionic surfactant Tween-20 (molecular weight 1230; purchased as a 10% solution [Surfact-Amps 20] from Pierce Biotechnology Inc. [Rockford, Ill., U.S.A.]) to stabilize the amylose molecules. AFM images of the sample revealed a distribution of extended chain-like molecules, directly visualizing a small number of branched macromolecules for the 1st time. Rindlav-Westling and Gatenholm (2003) found that the outmost surfaces of all films were covered with small protrusions when they examined the surfaces of solution-cast films of starch, amylose, and amylopectin with AFM. Dimantov and others (2004) reported on the surface of high amylose corn starch (HACS)-pectin coatings characterized by AFM, showing increased roughness with increased HACS content. Pectin is another polysaccharide that is closely related with food properties. Round and others (1997) imaged pectin polysaccharides extracted from unripe tomato plant cell walls. The obtained AFM images revealed for the 1st time a branched structure for tomato pectins that differs from that proposed for the neutral sugar side chains from enzymatic hydrolysis and sugar analysis. Further, AFM was used to investigate the nature of the long branches attached to the pectin (Round and others 2001). The detailed pectin polymer from the AFM images was also illustrated. Other detailed structures of polysaccharides, such as gum arabic and water-soluble soybean polysaccharide, were also investigated using AFM. The AFM results can be used to verify the theoretically calculated macromolecular structure (Li and Xie 2006), which will greatly extend the utilization of AFM.

Protein is another basic nutritional and structural component in food. AFM has been used to characterize the protein structure and is helpful for other chemical and physical analysis. AFM has provided

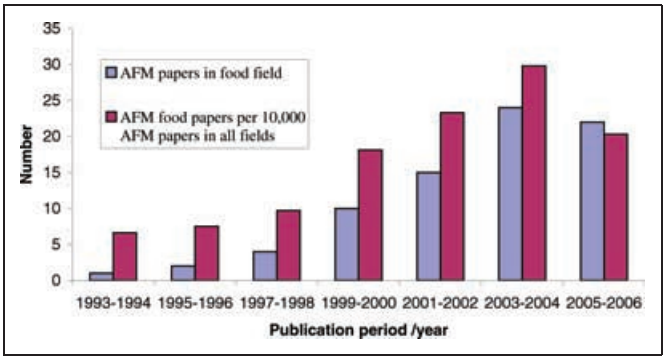

Figure 2—Publications about AFM in food research listed in ISI web of science and the amount that they account for per 10000 papers published in all the AFM fields

good-quality images of heat-induced whey protein aggregates that were maintained as intact as possible after their formation. Whey protein isolate aggregates exhibited similar microstructures to those observed for β-lactoglobulin aggregates (Ikeda and Morris 2002). At pH 7, aggregates were composed of ellipsoidal particles, regardless of the concentration of added NaCl. This result supported the previously proposed 2-step aggregation model at neutral pH. The study provided structural information essential for understanding the diverse physical properties of heat-induced whey protein gels. Tapping mode AFM results indicated that the fibrils formed have a periodic structure with a period of about 25 nm and a thickness of 1 or 2 protein monomers (Arnaudov and others 2003). The data implied multiple steps during the heat-induced formation of β-lactoglobulin fibrils at low pH and at low ionic strength. Fibril formation itself also involves at least 2 steps. Iwasaki and others (2005a) observed heat-induced morphological changes in myosin filaments using AFM, and found that the strands became rope-like at 70 °C, and individual filaments in a strand were not distinguishable.

As macromolecules, polysaccharides and proteins have some similar properties during AFM imaging. Ikeda and Morris (2002) found that when the tip was brought into contact with the protein sample in air, the hydration layers coalesced, resulting in strong capillary-induced adhesive forces. These forces can damage or displace molecules during scanning, the result being similar to that of polysaccharides. Furthermore, the AFM imaging methodologies on protein and polysaccharide macromolecules and polymers have several common imaging parameters and protocols. These include the selection of scan area, scan rate, resolution adjustment, and preparation of the samples (AFM/LFM instruction manual 1997). These shall give some useful guidance on AFM imaging of new polysaccharides or proteins.

There are some limitations for AFM molecular imaging. The main problem is caused by the crystallization of buffers and the distortions of deposited samples. This can be reduced using sublimable buffers. Another problem is that the tips are easily contaminated, which could result in limited tip lifetime as well as reduced image quality.

Complicated or quantitative structure analysis

It is a great challenge to investigate the spatial structure of the components in complicated food systems. With great efforts, AFM has been used to characterize the complicated food systems and the results can be speculated on illustrating the mechanism of physical and chemical processes during food processing and storage. Meanwhile, more quantitative parameters of the AFM images are being developed, which could provide useful information to investigate the food physical and/or chemical properties.

Many polysaccharides or proteins can form a gel system in certain conditions. AFM is an alternate instrument to characterize the gel structure information, which traditionally comes from chemical or rheological analysis. AFM imaging of a bulk gel of polysaccharide has succeeded only in limited cases because bulk gel surfaces easily deform on contact with the probe during imaging. However, it is still very effective for obtaining gel structure information.

Decho (1999) studied the polysaccharide structure of alginic acid that was examined as individual molecules and as dense gels using tapping mode AFM. The molecules exhibited frequent "kinks" or abrupt right-angle changes in orientation. This work demonstrated the efficiency of tapping mode AFM for examining the structures and gel conformations of a pliant polymeric matrix.

Some researchers studied the effects of gelation condition on the gel structure. Power and others (1998) used AFM to image hydroxypropylguar (HPG) gel structures formed under quiescent conditions as well as under varying levels of steady shear. AFM images showed an aligned, ordered structure in the high gel strength material with a random network structure in the gel formed under quiescent conditions. At moderate levels of shear (200/s) HPG molecules are straightened and aligned, while at high levels of shear (1000/s) the aligned structure collapses into clusters of molecules that would significantly lower gel strength because of reduced network crosslinking. Such information could not be obtained by traditional chemical or other physical analysis.

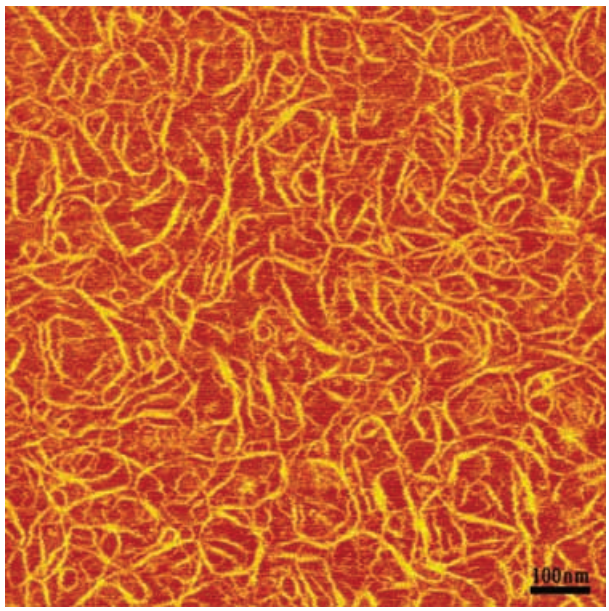

Figure 3—Phase-shift image of an acid gel made from orange albedo pectin, sucrose, and sodium citrate buffer solution. Reprinted with permission from *Biomacromolecules* 2004, 5, 334–41. Copyright 2004 American Chemical Society.

AFM also can be used to image the time-influenced process. AFM images revealed that pores in high methoxyl sugar acid pectin gels were fluid and flattened out when measured as a function of time (Fishman and others 2004). Figure 3 shows the general features for 3 types of gels (commercial citrus pectin, orange albedo pectin, and lime albedo pectin). One micrometer-square area contained a complex network of curvilinear strands and junctions. The dimensions of strands and pores could be obtained by quantitative analysis. These images revealed for the 1st time the structure of adsorbed sugar on pectin in the hydrated native gels and how the pectin framework was organized within these gels in the different times. Furthermore, images indicated relatively small differences in the organizations of native commercial citrus pectin, orange albedo pectin, and lime albedo pectin gels at optimal pH as determined in this study.

Another AFM application is to investigate the gelation mechanisms of polysaccharides. Association and formation of κ-carrageenan gel networks can be detected by AFM even in the absence of factors that promote side-by-side aggregation (Ikeda and others 2001). The results supported and improved the 2-stage model of κ-carrageenan gelation. Regardless of the concentration of added NaCl, AFM images of heat-induced gel precursors revealed that aggregates were composed of ellipsoidal primary particles. This confirms that aggregation occurs in 2 steps: the formation of primary aggregates and the subsequent aggregation of the primary aggregates (Ikeda 2003). Similar results were reported for curdlan gels; AFM images of molecular assemblies of curdlan provide the 1st direct evidence for their structural heterogeneity, suggesting that

heat-induced gelation of this polysaccharide involves 2 steps: The 1st step is to release single molecular chains from microfibrils; however, some single chains are not completely dissociated. The 2nd step is that these partially dissociated single chains crosslink the parent microfibrils through the formation of triple-stranded helices, by hydrophobic association between multiple single helices, and by association between a single helix and microfibril (Ikeda and Shishido 2005). Also effects of cations on the network formation of the microbial polysaccharide gellan can be illustrated through AFM (Ikeda and others 2004a). AFM images of cation-induced bulk gels of gellan revealed straight network strands similar to the case of the previously visualized acidset bulk gels under butanol (Gunning and others 1996a), but at somewhat lower network strand densities. Discrete aggregates rather than uniform or continuous coverage of polymers and polymer aggregates were observed for heat-induced gelation of many polysaccharides (Ikeda and others 2004a; Ikeda and Shishido 2005). These AFM images bring new knowledge on gel mechanisms.

Phase imaging is a promising method to study mixed systems. It was used to probe the mechanical properties of phase-separated lipid monolayers made of a mixture of the surface-active lipopeptide surfactin and dipalmitoylphosphatidylcholine. The data emphasized the complex nature of the contrast mechanisms of AFM on mixed systems (Deleu and others 2001). The phase imaging for protein-DNA oligonucleotide complexes also showed that it is a promising method (Kim and others 2004).

For quantitative aspects, AFM was successfully applied to investigate the effects of polysaccharide concentration on gelation process. Ikeda and others (2004b) found that gellan and xyloglucan did not form a gel at concentrations below 0.5% w/w and 0.75% w/w, respectively, while a mixture of 0.05% w/w gellan and 0.7% w/w xyloglucan formed a single-phase gel, demonstrating synergistic interactions between gellan and xyloglucan. AFM images reveal the formation of a network in a mixture at a concentration much lower than the gelation threshold of gellan in an individual system. Recently, quantitative features of food macromolecular polymers from different processing and storage stages were considered. The characteristics of pectin chain widths of yellow peach during storage were studied by AFM (Yang and others 2005a, 2006b, 2006c). Figure 4 shows a typical AFM image of chelate-soluble pectin from yellow peach. The quantitative data of pectin chain widths from AFM images suggest the possible degradation mode of fruit pectin during storage. The frequency of small pectin chain widths increased with time in controlled and regular atmosphere storage groups, but was greater in the regular atmosphere group. This suggested controlled atmosphere could inhibit the degradation of pectin molecules. Interestingly, almost all widths of pectin chains were composed of 4 basic units. For different pectins there was little difference in the values. Parallel linkages or intertwists between the basic units were proposed as fundamental structural conformations for pectin molecules. These experiments could be easily extended to investigate cellulose and other polysaccharides. The degradation mode of structural polysaccharides from quantitative AFM analysis could be correlated with other physicochemical analysis and has been used to illustrate the roles of these structural polysaccharides.

Force spectroscopy has supplied an alternative way to illustrate more quantitative information of macromolecules. Researchers could quantitatively estimate elongation-dependent changes of stiffness and viscosity of a single chain using a phenomenological model (Watabe and others 2006). Force spectroscopy is the bridge connecting nanostructure with physiochemical properties of macromolecules.

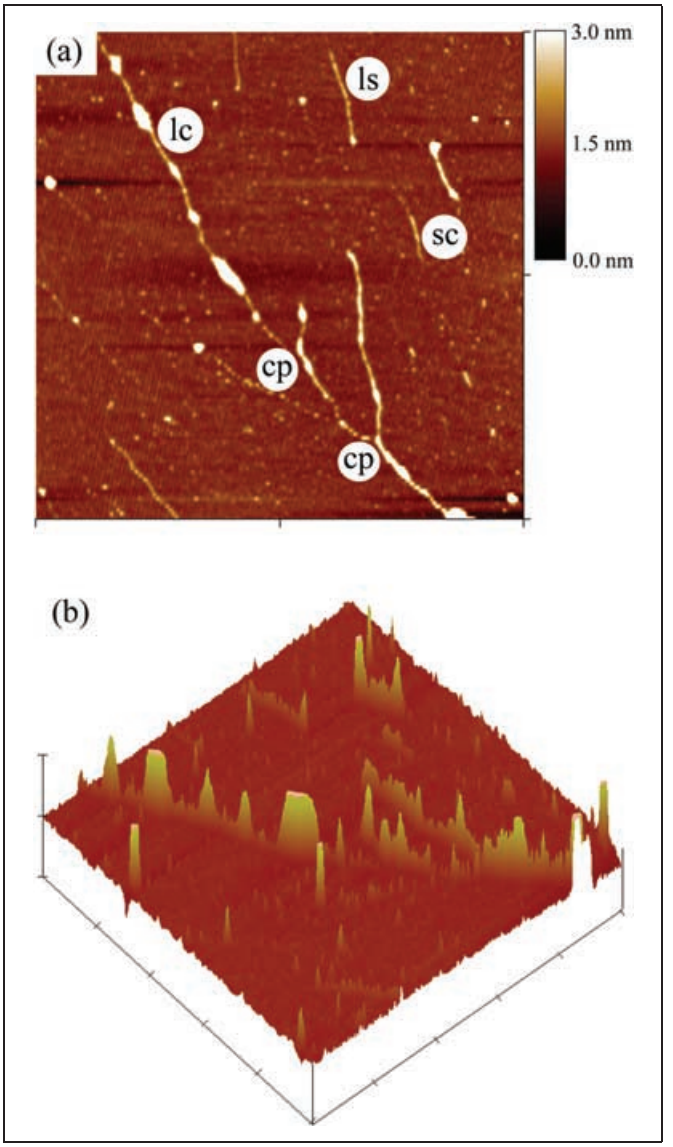

Figure 4— AFM images of chelate-soluble pectin from yellow peach (from Yang and others 2006c): (a) plane image; (b) 3-dimensional image. cp = cleavage points; lc = long chains; ls = linear single fractions; sc = short chains. Reprinted from *Postharvest Biol Technol*, 39, Yang H, Lai S, An H, and others, Atomic force microscopy study of the ultrastructural changes of chelate-soluble pectin in peaches under controlled atmosphere storage, 79, Copyright (2006), with permission from Elsevier.

Recent tendency of AFM imaging is to illustrate the structure information as well as some functional properties of food macromolecules at the same time. During AFM imaging, Iwasaki and others (2005b) reported the different elasticities of the strands of thermal and hydrostatic pressure-induced filamentous myosin gels using the force-indentation relationship. Development of powerful enhancements such as phase imaging, force modulation mode, and some others allow AFM for quantitative determination of elasticity, which is the material's relative measure to deform reversibly under applied loading. Elasticity can be measured from the loading and unloading force curves.

Molecular interaction

Molecular interaction was thought to have a great influence on food physical and chemical properties, and researchers have

tried many up-to-date instruments to investigate the processes and mechanisms. AFM testified as a promising tool. Gad and others (1997) successfully used functionalized AFM tips to sense specific forces of interaction between ligand and receptor, allowing the positions of polysaccharides on a living microbial cell surface to be mapped. The distribution of mannan on the cell surface was mapped by carrying out the force measurement in the force volume mode of AFM.

The fundamental of measuring molecular interactions can be explained from the view of forces. The interaction force between tip and sample can be measured by moving the AFM tip perpendicular to the surface, then the force-distance curves can be obtained; thus it is possible to detect surface interaction forces or single molecular bonds. This method of detecting forces is straightforward: the functionalized force probe with a biomolecule is brought into contact with or very near a surface that is covered with the countermolecule and then molecular interaction is formed. Upon retraction of the tip from the surface, the molecular bond or interaction is broken and the adhesion force determined at that point denotes the molecular interaction forces (Willemsen and others 2000).

In the food industry, the role of emulsifiers in the stabilization of foams and emulsions is of widespread technological importance. Emulsifiers can generally be classified into 2 main groups, large molecules such as proteins and small molecules such as surfactants (Woodward and others 2004). There has been substantial interest in understanding the interactions between proteins and surfactants and between different proteins (Mackie and others 2001). AFM has also proven to be a powerful tool for interpreting the rheology of food polymers at the molecular level. The adsorption of mixed β-casein/β-lactoglobulin films to the air/water interface and the subsequent displacement by the nonionic surfactant Tween 20 was observed (Mackie and others 2001). AFM was also used to trace the displacement of the mixed film by surfactant. The displacement of a globular protein from the air/water interface by sodium dodecyl sulfate (SDS) was observed using a combination of the Langmuir-Blodgett method and AFM (Mackie and others 2000). Gunning and others (2004b) studied the effects of surfactant types on surfactant-protein interactions at the air-water interface. Morris (2004) reported molecular interactions between air-water and oil-water interfaces by AFM. Figure 5 shows displacement of β-lactoglobulin from an air–water interface by surfactant. These images directly confirm the existence of protein networks at the interface. Besides qualitative description of the process, quantification of the AFM images was possible by calculating the areas occupied by either protein or surfactant. The results reveal the fundamental nature of the displacement process. Roesch and others (2004) observed mixes of κ-carrageenan and β-lactoglobulin by AFM, which demonstrated that AFM imaging allowed direct visualization of mixed biopolymer systems such as β-lactoglobulin and κ-carrageenan. The interaction between food components and food additives can be investigated by AFM also. Tay and others (2005) monitored the aggregation process of the 11S, 7S, and 2S proteins coagulated by glucono-δ-lactono by using AFM. The functional and structural properties of 2S soy protein in relation to other molecular protein fractions were illustrated (Tay and others 2006).

Modification of the surfaces of mica or silica extends the AFM applications on molecular interaction analysis. AFM images illustrated the mucin-chitosan complex layer formation on negatively charged surfaces of silica and mica (Dedinaite and others 2005). The mucin adsorption layer is negatively charged. While chitosan is a positively charged polyelectrolyte, researchers have shown how chitosan associated with the preadsorbed mucin to form mucin-chitosan complexes that resisted desorption by SDS even at SDS concentrations as high as 1 critical micelle concentration.

AFM also has great application potential in molecular interactions in microbiology. Spores of the filamentous fungus *Aspergillus oryzae* show a great biotechnological ability to produce highly active proteins. To date, little is known about the molecular mechanisms of spore aggregation. Under aqueous conditions, AFM imaging and force measurements are helpful to characterize the surface morphology and macromolecular interactions of *Aspergillus oryzae* spores in relation to their aggregation behavior (Van der Aa and others 2002). This study emphasizes the potential of AFM imaging and force measurements for investigating the molecular basis of microbial adhesion and aggregation processes.

Utilizing the interaction between enzyme and substrate, AFM has successfully illustrated the mechanisms as well as structural information of the enzyme and substrate. The complex structures of water-soluble wheat arabinoxylans and its different binding mode to different inactivated enzymes were illustrated by AFM (Adams and others 2004, 2005). It would bring in more useful information if combined with the real time investigation, which has been

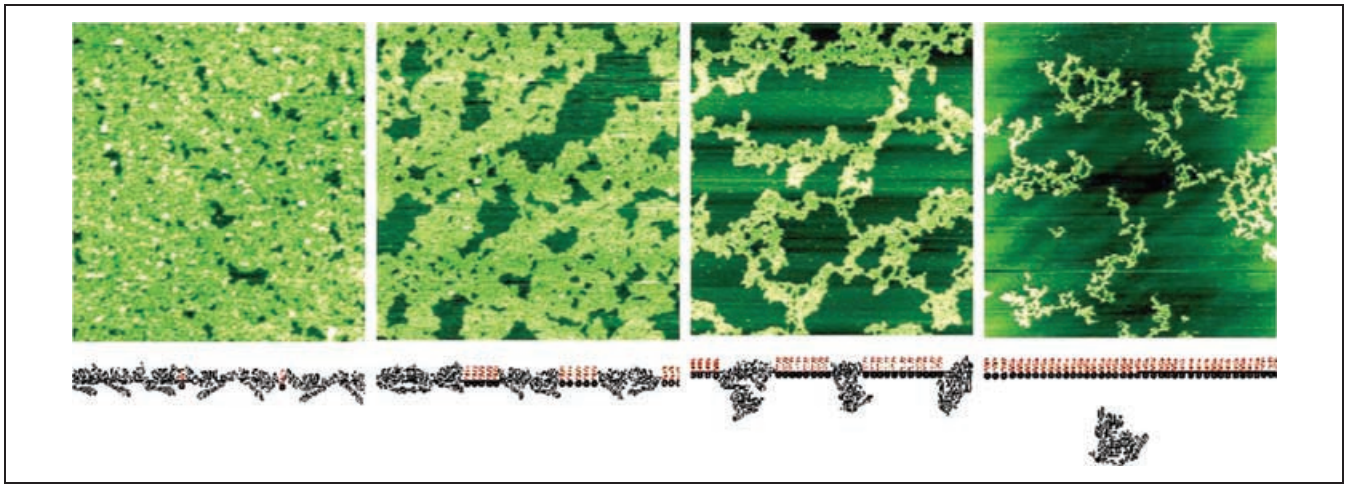

Figure 5—AFM images showing the displacement of a spread β-lactoglobulin protein film from an air-water interface by the progressive addition of the surfactant Tween 20. A schematic model of the displacement process is illustrated below the images. Reprinted from *Trends Food Sci Tech*, 15, Morris VJ, Probing molecular interactions in foods, 293, Copyright (2004), with permission from Elsevier.

successfully applied in watching molecular processes for dynamics of polymer adsorption and desorption (Gunning and others 2004a).

Since the force spectroscopy experiments were conducted at a solid–liquid interface in a liquid cell, it could be used to study the interaction between small molecules and macromolecules by changing buffers in the liquid cell. The results could indicate whether interactions exist between the macromolecules and small molecules when comparing the single-chain elasticity before and after the addition of small molecules (Liu and others 2005).

Recently, AFM was successfully applied in molecular interaction in real food systems. Woodward and others (2004) reported the displacement of a commercial whey protein system and the action was compared to that of β-lactoglobulin. The AFM results showed that whey protein isolate was more resistant to displacement from the air-water interface than native β-lactoglobulin. However, in highly complex real food systems, there are many interactions and many components that will interrupt the desired objects of investigation. Thus, it is difficult to propose a widely useful protocol for applying the AFM techniques to real food systems.

Food molecular manipulation

Many food macromolecules have a tangled structure. Aggregation of molecules or entanglement of a single linear molecule will result in potentially observable molecular characteristics being obscured. Manipulation of molecules offers us an opportunity to observe the reactions between food macromolecules directly (Yang and others 2006a).

Nakao and others (2002) developed substrates for straightening and fixing DNA using polymer-coated glass substrates for DNA, stretching and fixation, and analyzing the precise gene location on DNA by fluorescence microscopy and AFM. Observed images demonstrated that DNA was sufficiently fixed and stretched on modified coated surfaces. This specific interaction is considered to be attributed to π-π interaction (π-stacking) between aromatic amines in polymers and base pairs in DNA molecules. In addition, Nakao and others (2005) used AFM and other instruments to fabricate and align π-conjugated polymer-functionalized DNA nanowires. They developed a simple method to functionalize DNA with π-conjugated polymer, forming highly aligned and integrated arrays of π-conjugated polymer nanowires of a few nanometers diameter. Furthermore, a functionalized AFM tip was developed for nanodissection of DNA (An and others 2005). Even though these researchers did not use food molecules directly, they definitely offer references for food macromolecular manipulation.

Manipulating and stretching single food molecules are useful in determining the chain lengths and further illustrating interactions between macromolecules. Recently, Yang and others (2006a) have manipulated and stretched single pectin molecules with modified molecular combing and fluid fixation techniques. AFM images of the effects of manipulation on the chelate-soluble pectin from peaches are shown in Figure 6. Pectin molecules on mica surfaces, which were imaged in air with AFM, can be aligned and stretched through applying modified molecular combing and fluid fixation techniques. Compared to fluid fixation, modified molecular combing showed more effective and powerful for molecular manipulation. Chelate-soluble pectin can be straightened into lines in 1 direction by molecular combing while sodium carbonate-soluble pectin showed "V" structures. The authors also gave an interpretation of the different results for different pectins.

Surface topography

AFM is a powerful instrument for qualitatively determining roughness and other surface characteristic parameters. Topographical and frictional investigations using AFM allow for measuring surface characteristics, including roughness, homogeneity, surface morphology, and fractal analysis. In biological samples the surfaces characteristics also include other interactions, including electrostatic interaction, steric interaction, and specific adhesion forces at molecular contact; all these forces are usually called colloidal forces, which can also be analyzed by AFM. MaMaster and others (1999) reported AFM imaging on pure and mixed films of α- and ω-gliadins by tapping mode. Wang and others (2003a) reported the topography of zein layers after the solvent evaporated by AFM, and the result showed that the surface features of zein deposits depended on the adsorbing surface. Wang and others (2003b) applied AFM to compare the roughness results of modified stainless steel with the unmodified surfaces. The results showed that the modified surfaces were less rough, which could reduce bacterial contamination on surfaces encountered in food processing environments.

Today, emphasis is being put on the development of sensors for nondestructive assortment in real-time mode (Butz and others 2005). Hershko and Nussinovitch (1998) applied AFM to investigate the fine structural analysis of the onion skin and to compare the surface roughness of alginate-coated onion skin and the control. The results showed that the smoother the alginate-coated samples, the lower the roughness value calculated by the AFM. There were similar phenomena for garlic (Hershko and others 1998). Yang and others (2005b) tried to utilize the roughness analysis of fruits and vegetables to characterize their surface status. It was found that the roughness value increased with the storage time. However, the specimen areas were too small compared to the whole sample. The sample areas that Hershko and Nussinovitch (1998) determined were no larger than 250 μm^2, and the vasculature part should be avoided carefully. Furthermore, AFM imaging in air could result in staining of the tip and affect the next imaging, which would definitely affect the roughness results (Yang and others 2005b). If the image areas can be greatly enlarged and stain of the tip eliminated, the parameters obtained from AFM would be more reliable and could be used to investigate the appearance quality of produce in real time.

AFM can be applied to characterize the delicate food surface at the micrometer scale. The topography of edible films including some tiny features was characterized by AFM (Lent and others 1998). Morton and others (2003) reported that shallow depressions in the caramel surface of 1 to 10 μm dia that had a higher adhesion to the AFM tip, a lower stiffness, and different thermal character than the surrounding sample. AFM results brought fine information on food surface structures and amend our previous knowledge. The surface structure of commercial milk chocolate is examined using AFM, the results showed that following many temperature cycles there was crystal growth around some pores. The pores themselves did not change much, which suggested that they were not directly involved in bloom mediation or liquefied fat transport in milk chocolate; without these AFM results, this was thought to be directly involved in these processes (Rousseau 2006).

Phase imaging AFM has been utilized to characterize the complex surfaces, map the surface friction and adhesion, and identify surface contamination. Phase imaging has supplied detailed smooth and rough domains of maize starch films, which cannot be obtained by height imaging (Thiré and others 2003).

Nanofood characterization

Although the practical nanofood has not been marketed until now, scientists have taken the "bottom up" approach, which allows nanostructures to be built from individual atoms or molecules that are capable of self-assembling, to build nanofood. With the development of AFM, this process can be simulated and optimized by

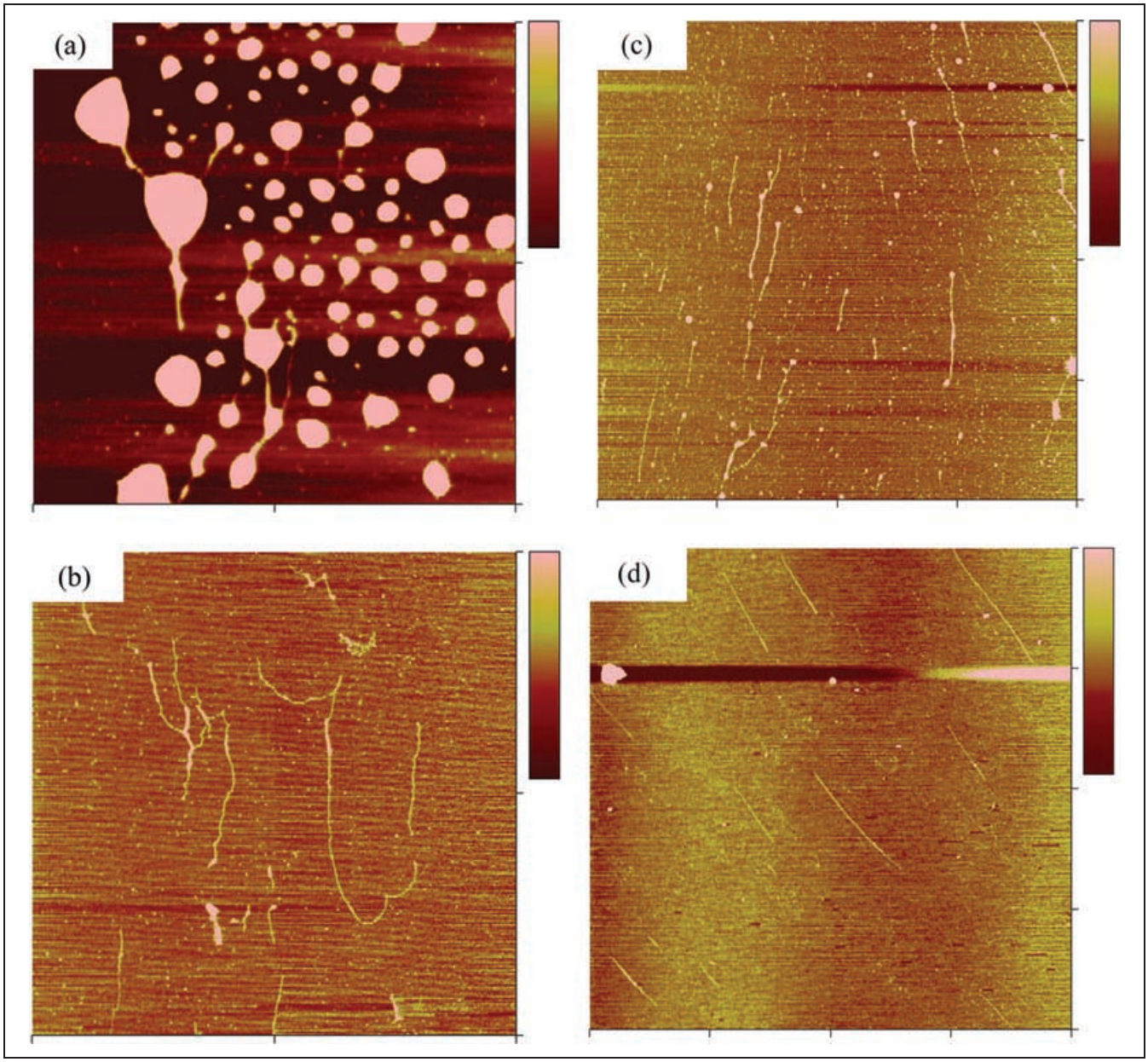

Figure 6— AFM images of effects of manipulation on the chelate-soluble pectin from peaches. Height bar: 2 nm (from Yang and others 2006a, with kind permission of Springer Science and Business Media). (a) Typical image without manipulation, image size: 5.0 × 5.0 μm. (b) Untypical image without manipulation, image size: 5.0 × 5.0 μm. (c) Image after fluid fixation manipulation, image size: 10.0 × 10.0 μm. (d) Image after modified molecular combing manipulation, image size: 10.0 × 10.0 μm.

AFM and useful information would be applied to direct the future nanofood production.

Conclusions and Research Needs

AFM has been applied to investigate fine food molecule structure and molecular interaction on nanoscale. It has been successfully applied on qualitative and quantitative analysis of macromolecule structure, molecular interaction, and molecular manipulation. AFM has brought in much original knowledge on food properties and could be used to direct food processing and storage. By means of AFM, researchers have succeeded in modifying our previous understanding of the pectin molecular structures (Round and others 1997), proposed the degradation mode of pectin in fruits through the statistical results of pectin chain widths (Yang and others 2005a, 2006b, 2006c), and obtained direct process images of the molecular interactions between protein and surfactant (Morris 2004); this information cannot be obtained by other techniques. However, AFM is still a relatively new technique for food scientists. There is a need to develop standard methodologies for applying this technology to different food systems (Morris 2004).

Unfortunately, not all the macromolecules have a good attachment to the mica surface. Therefore, it will be essential to modify the mica or the macromolecule in order to investigate or manipulate successfully.

For some health-related phytochemicals, AFM will offer an alternative way to understanding their interactions and thus lead to a sophisticated, holistic approach to disease prevention and treatment (Lila and Raskin 2005).

Presently, methods used to detect quality of food have their strengths, weaknesses, and special applications. Future researches

on food quality and functionality in terms of genetics, metabolic processes, product composition, molecular structure, and physical status need new technologies and techniques to better measure quality attributes (Butz and others 2005). AFM is a promising technology and would provide a great opportunity to combine other techniques and measure the overall quality of food.

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