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Application of atomic force microscopy in food sciences and detection of food toxin

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ABSTRACT

In recent years, atomic force microscopy (AFM) has shown to be a versatile tool for determining surface morphology, interaction forces and roughness measurement. The AFM technique is a 3D topographical modality with a high atomic resolution, especially in the vertical orientation. AFM is a type of scanning probe microscope that uses a near-field technique that involves a sharp tip interacting with the atoms on the sample surface. This adaptable method can be used to get high resolution sample images and examine local interactions in air or liquid environments. Qualitative macromolecule, quantitative structure analysis and polymer imaging, molecular interaction, surface topography, and Nano food characterization are all examples of AFM applications in food science and technology research. The current challenge in applying AFM to food research is a lack of adequate methods for various food systems. A better grasp of AFM technology and the development of related methodology for complex food systems would lead to a deeper understanding of food qualities at the macromolecular level and expand their applicability.

1. Introduction

Microscopes are increasingly being employed in food science and technology study research. This research has provided us with a multitude of fresh information about food microstructure and physical attributes. Transmission/ scanning electron microscopy (SEM/TEM) on fruits and vegetables (Xiao & Gao, 2012), light microscopy (LM) on ice cream (Pieniazek & Messina, 2016), and confocal laser scanning microscopy (CLSM) on fruit surface layers (Pieniazek & Messina, 2016) are just a few examples. These microscopes provide useful information about food structure at the macromolecular level, but they have several limitations that prevent them from being widely employed. In order to comprehend the functions of cell surfaces, electron microscopy has long been acknowledged as a critical tool in microbiology for elucidating cell surface ultrastructure(Dufrêne, 2014).

AFM (atomic force microscopy) has been widely employed to explore microbial surfaces at great resolution in recent years(Leeuwenhoek et al., 2019). The first images taken using a scanning probe microscope (SPM) were published in 1982 by Gerd Binnig and Heinrich Rohrer(Schuhmann, 2016).SEM and transmission electron microscopy can also provide high resolution, however their complicated sample preparation (e.g., chemical fixing, dehydration, metal coating, and ultrathin slice) can significantly distort the sample. AFM requires little or no sample preparation because it measures through direct contact between the tip and the sample (Rizvi et al., 2021). By raster scanning a sharp, conductive tip over a conducting sample, their innovation, the scanning tunneling microscope (STM), was able to resolve atomic structure. The AFM is similar to the STM, only it uses a sharpened tip attached on the end of a flexible cantilever to electronically map a surface instead of a conducting probe. The technology produces molecular-resolution three-dimensional photographs of the surface ultrastructure in real time, under physiological environments, and with minimal sample preparation. Force measurements can be used to explore physical aspects of the material, such as molecular interactions, surface hydrophobicity, surface charges, and mechanical properties, making AFM more than just a surface imaging tool. These studies help in the understanding of the structure-function interactions on microbial surfaces. The AFM has evolved into a structural imaging approach for biological materials such as proteins, nucleic acids, membranes, and cells in their naturalistic

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environment (Engel & Müller, 2000). Measurement of mechanical forces at the molecular level, in addition to high-resolution imaging of proteins, nucleotides, membranes, and living cells, provides precise insights into the operation and structure of biomolecular systems (Allison et al., 2010). AFM is the most frequently used method for structurally mapping the mechanical properties and responses of biological systems, with resolution ranging from millimetre to sub-nanometer and sensitivity ranging from micronewton to piconewton(Dufrêne et al., 2017).

2. Instrumentation

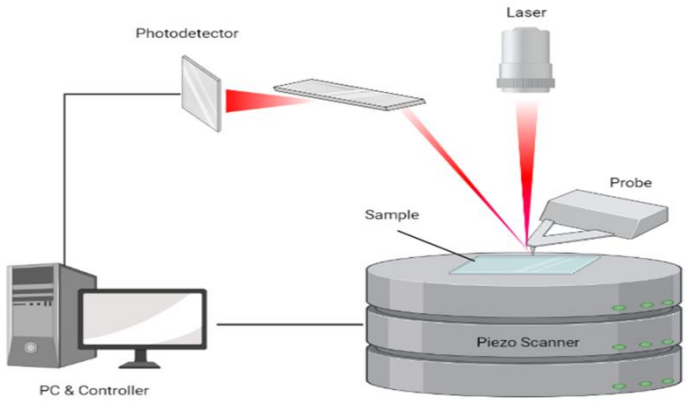
2.1 Contact mode: A microcantilever with a very sharp tip is brought into contact with the sample's surface in contact mode, which is also known as a static mode. It exerts significantly more force to the sample, which can occasionally result in poor pictures and sample distortion due to the tip (Bhushan & Marti, 2017). The micro-cantilever deflects due to the repulsive force acting on the tip. A beam deflection method is used to measure the deflection of a cantilever, in which a laser beam is reflected at the back side of the cantilever and the deflection is detected by a photodetector.

2.2 Non-Contact mode: The cantilever is made to oscillate at a frequency slightly over its resonance frequency in noncontact mode, typically with amplitude of a few nanometers (10nm). The oscillation of the cantilever is affected by attraction forces present between the tip and the sample surface when the tip of the cantilever is brought close to, but not in contact with the sample surface. The main benefit of this technique is that it does not result in tip wear or sample damage. Its key disadvantage is that it has a lower spatial resolution (Jalili & Laxminarayana, 2004).

2.3 Tapping mode: In comparison to the non-contact mode, this mode was created to improve resolution. AFM works by contacting the surface intermittently with a tip attached to the end of an oscillating cantilever at the lowest point in the oscillation. When compared to the contact mode of operation, this mode greatly minimizes the forces exerted by the tip on the sample, reducing both sample and tip damage. When tapping mode is used instead of contact or noncontact modes, most samples have better lateral resolution. As a result, it is the most widely utilized mode in the domains of food and biological science. Food and biological samples, in general, are suited for tapping mode AFM since they are somewhat soft(Wang et al., 2019).

2.4 Principle

The AFM's working principle is based on the interaction of forces between a tip and a sample surface. The magnitude of this force alters the micro cantilever's deformation or motion state. The imaging technique is depicted schematically in Figure 1. After being reflected by a mirror, a laser beam (from a laser diode) is focused onto the end of the cantilever (ideally right over the tip) and then reflected onto a position-positive photodiode detector (Lekka, 2017).Sensors detect these changes and force distribution information is received when the sample is scanned, allowing the user to obtain surface structure information with nanoscale resolution. The angle of the reflected laser beam changes according to Hooke's law as the tip moves in response to the sample topography during scanning. The AFM scanner may travel in three directions: X, Y, and Z. The laser spot falling on the photodiode moves as the laser beam varies, causing changes in intensity in each of its quadrants. As the force between the tip and the sample changes during scanning, the topography of the sample surface causes the cantilever to deflect. A computer generates a map of the surface topography based on the measured cantilever deflection, which is displayed on one monitor, while the control portion is displayed on another(Sun, 2018).



3. Applications in Food Science

3.1. *Carbohydrates*

AFM can be used to characterize the microstructure of polysaccharides in a variety of ways. Scientists have looked into the starch granule's unique structural arrangement and tried to figure out how it relates to its physicochemical qualities. Relatively comprehensive structural models of starch have been created using standard physical and chemical approaches as well as microscopy (Liu et al., 2008) as well as developed observations on the interior structure of starch granules, with AFM playing a key role. After embedding in resin and slicing, the interior morphology of banana starch granules was investigated using AFM. Due to differences in molecular composition and organization, viscoelastic characteristics vary from granule to granule. The intrinsic structural changes between banana starch granules at various stages of ripening suggest that enzymatic hydrolysis of banana starch may begin near the surface (Peroni-Okita et al., 2015).Pectin is a family of galacturonic acid (GalA)-rich oligosaccharides and polysaccharides that make up the cell walls of higher plants. Water, sodium carbonate (Na_2CO_3), and a chelate agent such 1, 2-cyclohexanediaminetetra-acetic acid were used to extract different pectin fractions from cell wall components of fruits and vegetables (CDTA). AFM studied three pectin fractions isolated from fruits and vegetables: water-soluble (WSP), CDTA-soluble (CSP), and Na2CO3–soluble (DASP or SSP).The shape of papaya pectin molecules (Yang et al., 2017). The presence of various pectin chains, such as linear chains, short chains, branching points, aggregates, polymer, and so on, revealed the heterogeneity and complexity of pectin structures. In order to investigate the molecular mechanism, AFM has also been employed to analyze the alteration of pectin structure. Pectin chain widths and lengths reduced during storage, according to various AFM experiments.

3.2. *Proteins*

Proteins are macromolecules that consist of amino acids and have a stable 3D conformation. They are essential substances for the structure and function of many biological units, ranging from subcellular structures (native membrane, etc.) to living cells, all the way up to tissues and organisms. Proteins in human body are mainly from in vivo synthesis and human diets. The structures of food proteins may be changed during food processing process (i.e. thermal processing and non-thermal processing) or a storage process (i.e. low temperature preservation), which may affect their in vivo bioavailability, metabolism, and nutrition abilities. In addition, in order to improve food processability and nutrition of food proteins, protein modification methods are applied during ingredient preparation and food processing. Therefore, it is a great need to study the structure characteristics of food proteins and their behaviors during different ingredient preparation, processing or preservation processes. Therefore, it is a great need to study the structure characteristics of food proteins and their behaviors during different ingredient preparation, processing or preservation processes (Shi et al., 2019).

Caseins are phosphoproteins that are divided into four subgroups: αS1, αS1, β, andκ. They are widely used in the food industry as a key component of cheese and milk, an emulsifier in meat products, a thickening and texture stabilizer in baked goods, and a thickener and texture stabilizer in dairy products. The interaction of casein with other compounds was studied using AFM Nano imaging(Chichti et al., 2013). Applied AFM to study the interaction of casein and modified starch in a simulated yoghurt system at various pH levels. Pure casein micelles collected and aggregated when the pH decreased. In any pH, the surface of starch granules was smooth. Many casein particles appeared on the surface of the mixed casein-starch region. It was discovered that introducing modified starch reduced the aggregate degree of casein.

The impact of processing and preservation conditions on meat proteins have been investigated using AFM Nano imaging. The effects of ultrasound, CaCl2, and sodium tripolyphosphate on the ultrastructure of the milk goat longissimus muscle fiber were studied using AFM by (J. Gao et al., 2016).The sarcomere length was dependent on the ultrasound bath, CaCl2 concentration, and trispolyphosphate concentration, according to AFM imaging studies. The ultrasonic bath was the preferred tenderization procedure among these options. This research indicated that AFM was an effective tool for analyzing the influence of processing procedures on dietary proteins, and that the ultrasound bath method was the optimal method for treating muscle fibers.

The effects of processing and preservation on wheat proteins were investigated using AFM Nano imaging. The proposed an AFM-based approach for progressively abrading the surface of wheat starch or gluten films. AFM was used to examine the surface topography of the films before and after abrasion. This research confirmed that gluten has mechanical qualities similar to soft materials, whereas starch has a higher hardness, based on the depth of the abrasion zones(Chichti et al., 2013).

3.3. Fats

Nanoemulsions offer a wide range of applications in food, including embedding, protecting, dispersing, and dissolving, as well as providing a larger surface area for target reactions. AFM can provide structural information on nanoemulsions, which can aid in the prediction of their functional properties.To investigate the physicochemical attributes and storage performance of a nanoemulsion system,

fish oil was used as the oil phase. AFM was used to obtain microstructural information on this nanoemulsion at the nanometer level, which helped to confirm prior research findings (Barrera et al., 2013). Pepper extracts contain antibacterial and antioxidant effects, but they are hydrophobic, making them difficult to fully disperse in a food system. The use of a nanoemulsion was suggested as a way to boost the availability of pepper extract. AFM revealed the uneven form of this nanoemulsion, which revealed probable response processes (Galvão et al., 2018).

4. Application of AFM in the detection of food spoilage caused by micro-organism

Antibiotics have a well-defined mechanism of action on bacteria in the majority of cases (L. Gao et al., 2021). The impacts on bacterial surfaces at the nanoscale, on the other hand, are poorly understood, and the AFM technique is particularly well adapted for this purpose, as it allows imaging and investigating Nanomechanical properties on single living cells. -lactams (Formosa et al., 2012), aminoglycosides and their derivate compounds, and fluoroquinolones (Montero et al., 2006)are some of the antibiotics studied using AFM. The morphology of P. aeruginosa after treatment with ticarcillin (-lactams) and tobramycin (aminoglycoside)(Formosa et al., 2012). Ticarcillin produces cell elongation, whereas tobramycin changes the cell surface. Antibiotics, on the other hand, alter the nanomechanical properties of cell walls, such as elasticity and spring constant.

Antimicrobial peptides have been developed for a number of years, but their mechanism of action is still unknown, and more research is needed. AFM can be used to assess the nanoscale effects of such molecules, assisting in the knowledge of how these peptides interact with the bacterial cell wall.Because antimicrobial peptides are thought to have a detergent-like impact on bacterial membranes, numerous research have focused on peptide effects on phospholipidic layers that imitate these membranes(Grzeszczuk et al., 2020). However, as microorganisms become increasingly resistant to antibiotics, alternative means to killing bacteria must be devised.Colistin is the most well-known antimicrobial peptide; it is effective against gram-negative bacteria including *Acinetobacterbaumannii, Pseudomonas aeruginosa*, and*Klebsiellapneumoniae,* which are responsible for many nosocomial infections.The effects of colistin on gram-negative bacteria have been studied in a small number of AFM studies (Soon et al., 2009), with the major conclusions being that treated bacteria exhibit enhanced cell wall elasticity and spring constant, as well as a decrease in cell adhesive qualities. These findings support the theory that colistin acts as a detergent on the bacterial cell wall, removing the LPS from the surface. In reality, the results of these investigations include colistin-resistant bacteria, which exhibit cell wall structural changes from susceptible strains(Babu et al., 2016).

4.1. *Bacillus*

Bacillus is a Gram-positive bacterium that can be either aerobic or facultatively anaerobic. This genus is notable for its ability to form spores, a biologically inactive structure that allows it to withstand extreme climatic conditions. As a result, some common sterilization methods are unable to harm all Bacillus, resulting in food intoxications like Bacillus anthracis as well as Bacillus cereus, or the break - down of food materials such as Alicyclobacillus acidoterrestris and C. tyrobutyricum, making Bacillus a emphasis in the food manufacturing and research. Bacillus studies Because of its relatively high stiffness and significant degree of morphological uniformity, the cell outer surface (S-layer) is particularly well suited for AFM study amongst the different investigations on bacillus(Soni et al., 2016). The S-layer of bacillus, which is made up of individual proteins, glycoproteins (MW 40–200 kDa), or higher sophisticated lattices, influences adhesion and ocytosis, and is significant in food poisoning as well as infection defense(Sleytr et al., 2014).

4.2. *Lactic acid bacteria (LAB)*

Lactic acid bacteria (LAB) are Gram-positive bacteria possessing metabolic and physiological properties in common. *Lactobacillus brevis*, *Lactobacillus lindneri*, and *Lactobacillus paracollinoides* are lactic acid bacteria that can cause pathogenic deterioration of beer, meat, or milk via anaerobic lactate breakdown (Mokoena, 2017). To explore bacterial adhesion at the nanoscale scale, *PriscaSchaer-Zammaretti* utilized AFM to analyze the surface topography, elasticity, and molecular interactions of different types of lactic acid bacteria. The findings revealed that *L. helveticus* and *L. johnsonii* had a membrane with a uniform elasticity and no attachment events, which was most likely due to the S-layer that entirely occupies the bacterial membrane. Nonetheless, due to their polysaccharide-rich surfaces, certain *L. johnsonii* species have strong stickiness forces.One of the most important criteria for characterizing and evaluating food-borne pathogens and probiotics is the tendency of lactic acid bacteria to adhere to the intestinal epithelium. Other AFM-derived metrics (surface topography and elasticity) can also be used to discriminate between different bacterial pathogens for appropriate preventative therapies (Garcia-Gonzalez et al., 2018). The physical characteristics of aggregated Lactobacillus sakei L3 exopolysaccharide (EPS) polymers from Hubei meat using AFM.(Ihnatouski et al., 2020).

4.3. *Salmonella*

Salmonella is one of the main widespread food pathogens, causing gastrointestinal disorders around the world and livestock mostly via the faecal matter pathway after consuming infected foodstuff. Several Salmonella iso forms are host-specific, whereas many infect a broad spectrum of people and killing thousands of individuals each year. Salmonella cells as tiny as a single cell can be pathogenic, highlighting the need for more study into food safety procedures(Qi et al., 2019). Salmonella, like other microbes, can easily attach to solid substrates and create biofilms, which are the most common cause of food borne outbreaks(Bai et al., 2021). AFM was frequently utilized to observe biofilm development and assess bacterial adherence to the substrate. AFM was used to study whether the components, such as curli (amyloid fibres), cellulose, as well as the cell membrane protein BapA, influenced the shape of bacteria and the production of biofilms. Curli and cellulose, but not BapA, were identified to play a key role in the production of Salmonella biofilms. The findings showed that AFM is a useful method for monitoring the microstructure of bacteria beneath liquid biofilms (Bai et al., 2021).

4.4. *Listeria*

Listeria monocytogenes is a saprophytic Gram-positive bacterium that grow and thrives in the soil as a transcriptional extracellular Gram-positive bacterium. It can, however, become a pathogen after being consumed by vulnerable humans or animals(Freitag et al., 2009). To understand L. monocytogenes attachment method to inert surfaces or its pathogenicity in food-borne pathogens, AFM quantification for bonding force determination between L. monocytogenes and silicon nitride sample surface was performed. The results reveal a logarithmic relationship between mean nanometer adherence (in nN) as well as the 50 percent fatal dosage (LD_{50}) of strain pathogenicity evaluated in mice. The nanometer adherence might be utilized as a benchmark to discriminate among virulent and avirulent L. monocytogenes strains, according to the study(Gordesli-Duatepe et al., 2020). Furthermore, AFM in flexible force configuration was utilized to examine food-borne pathogenic organisms' bacterial cells upon drying in the absence of food and even in the presence of minuscule amounts of food, such as carrot beverage, an aqueous phase of nori, milk, as well as soymilk, in order to evaluate the relationship between food byproducts and food-borne pathogen preservation. According to the findings smaller food particles, either protein- as well as carbohydrate-rich, exhibit a protective impact on surface bacteria, disabling sanitization mechanisms and increasing cross contamination(Dangaran et al., 2009).

5. Detection of food toxin by AFM

Recognition Because of its non-destructive nature and high resolution under biologically relevant conditions, AFM has shown to be a viable instrument for investigating diverse biological processes. It has been used to identify DNA, specific proteins, and proteins on live cell membranes with great effectiveness. employing an antibody-functionalized needle to identify the toxin ricin attached to a gold surface(Main et al., 2021). Detection also confirms by single molecules antibody-ricin interaction forming its bonding strength to be 64.89 ± 1.67 pN. The capacity to quantify interactions with single-molecule specificity is a major benefit of AFM-based approaches. You can quantify accurate binding structure with AFM since it provides you more flexibility over your experiments(B. Wang et al., 2012). False alarms can occur in any biosensor, even AFM-based ones, due to non-specific interactions on the detecting molecule, demanding further investigation and decreasing the price. 4D histograms connecting desolation forces, elongation length, loading speed, and repetition may be used to visualizethis phenomenon. Interaction peaks from DFS's force-distance graphs are displayed by bigger data points, making it easier to distinguish between specific and non-specific interactions. Salmonella typhimurium, a foodborne pathogen, has been used to show this strategy (Benedetti et al., 2011). Additionally, putting anti - fouling films to substrates before to modifying them with biomolecules has been investigated as a technique of minimizing non-specific protein adsorption and common bacterium adherence. In solid phase microextraction for gas chromatography, it has been shown to avoid fouling by food matrix(Zhang & Chiao, 2015).

6. Application of AFM in the study of nanoemulsion

The use of AFM to examine the droplet interface properties of nanoemulsions, including mechanical properties (e.g. elasticity, hardness, adhesion, surface charge densities), distribution and adsorption behaviours of surfactants, and surface interaction forces between droplets or between droplets and surfactants; all of which are crucial when designing nanoemulsion-based delivery systems for functional compounds in various fields, including the food industries (Ho et al., 2022). To prevent droplet agglomeration and coalescence, the majority of nanoemulsions must be diluted 100–1000 times into distilled water. The diluted solution is then deposited on the freshly cleaved mica substrate. In some cases, the deposited droplets are washed with distilled water before dehydration by either leaving it overnight in a dust-protected environment at room temperature or using a furnace/heater to accelerate the drying process (Salvia-Trujillo et al. 2013). The binding (adhesion)

of droplets on the substrate surface is usually accomplished via electrostatic attraction (e.g. adsorption) between the charges on the sample and those on the mica surface. The most common AFM operating mode used to characterize the droplets in nanoemulsions was the tapping or noncontact mode to avoid damaging on the droplet surface, and the imaging was performed under dry conditions. The size and shape of the droplet were then determined using the photos that had been taken. In order to characterise nanoemulsions, AFM has been employed to confirm the properties of nanoemulsions in addition to other analytical techniques. Nevertheless, the results are not always comparable, indicating challenge in the use of AFM to study nanoemulsions. Thymol nanoemulsions with sodium caseinate (NaCas), lecithin, and propylene glycol were reported to have highly compatible droplet size and morphology measurements between AFM and other analytical techniques, such as dynamic light scattering, confocal laser-scanning microscopy, and TEM (Xue and Zhong 2014); fish-oil nanoemulsions with whey protein isolate (WPI) alone or in combination with Tween 80 and Span 80 (Nejadmansouri et al. 2016a; Nejadmansouri et al. 2016b); For nanoemulsions of numerous functional components with various emulsifiers, such as essential oils with Tween 80, similarity in droplet size evaluated by AFM and dynamic light scattering was also found (Javanshir et al. 2020): cholesterol with castor oil, medium-chain triglycerides, and scallop gonad protein isolates (Han et al., 2020);

7. Conclusion

AFM is a potential technology that might be used to combine other techniques, and it is frequently used in basic research on basic food components as well as complex food systems. AFM has been shown to be a useful method for food morphology and rheology research, providing unique insights into nanoscale structures and functional behaviors. Standard techniques for applying this technology to various food systems must be developed. Scientists have used AFM to characterize the microstructural and mechanical properties of food items in a variety of domains. The functional properties and stability of dispersed systems such as nanoemulsions are highly dependent on the surface properties of dispersed droplets and interaction forces between them. These properties allowed us to manipulate and effectively design nanometric delivery systems for many bioactive compounds, nutraceuticals, polyunsaturated fatty acids, prebiotics, probiotics, and pharmaceuticals in a wide range of fields, such as the chemical, pharmaceutical, food and nutrition, and cosmetic industries. For these purposes, AFM technology could emerge as one of the most meaningful techniques for imaging the surface topography and determining the surface forces of droplets in nanoemulsions. The current review will demonstrate that AFM applications to food systems are numerous, with the potential to reveal more about the architecture of foodstuff at the nanoscale.

8. Abbreviations:

AFM: Atomic force microscopy; **CLSM**: Confocal laser scanning microscopy;**LM**: Light microscopy;**SEM**: Scanning electron microscopy; **SPM**: Scanning probe;**STM**: Scanning tunnelling microscope;**TEM:** Transmission electron microscopy.

9. Disclosure statement

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11. Conflicts of Interest The authors declare no conflicts of interest.

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Table 1. Parts of Atomic Force Microscope

Parts of AFM	Function	Application	References
Cantilevers and tips	Detect the sample surface and undergo deflection	Microlithographic processes are used to make AFM tips, which are usually curved resembling a pyramid. The higher the resolution of the final image, the finer the tip.	(Kim et al., 2009)
Laser	Concentrating on the cantilever's rear	Experimental data from various investigations confirms that a beam of light provides sufficient radiation pressure to move a microstructure.	(Shimoni, 2008)
Laser deflector	A laser beam deflection mechanism is used in Atomic Force Microscopes, in which a laser is deflected from the rear of the reflecting AFM switch and over a position-sensitive detector. AFM tips and cantilevers are commonly made of Si or Si3N4 microfabrication. The typical tip radius ranges from a few to tens of nanometers.	Evaluate height variation of the laser demonstrated on the photo-diode, across to the laser centering position on the cantilever. The level of cantilever movement, in instance, may vary depending on where the laser beam focuses on the cantilever.	(Jensen, 2013)
Feedback Loop	Controlling the force and tip location via laser deflection	The AFM tip is reflected by a laser from the back of a cantilever. The laser location on the photodetector is employed in the feedback loop to monitor the surface for scanning and monitoring as the tip interacts with it.	(Bourauel et al., 1998)
Piezo Scanner	Piezo scanners can be configured to rotate in x, y, and z by extending and compressing in different directions.	Scanners are composed of piezoelectric substance, which expanded and shrinks in response to a voltage supplied. The polarity of the supplied voltage determines whether they lengthen or shrink. The material expands in one dimension and shrinks in the other when exposed to a positive voltage. When a voltage is negative, the opposite happens.	(Wang et al., 2019b)