Nano TransMed 2023, 2(1): 69–73
ISSN 2790-7384 (print); 2790-6760 (online)
https://doi.org/10.26599/NTM.2023.9130017

RESEARCH ARTICLE

Profilometry and atomic force microscopy for surface characterization

Li Mei[1] and Guangzhao Guan[2]

[1] Department of Oral Sciences, Faculty of Dentistry, University of Otago, 310 Great King Street, Dunedin 9016, New Zealand
[2] Department of Oral Diagnostic and Surgical Sciences, Faculty of Dentistry, University of Otago, 310 Great King Street, Dunedin 9016, New Zealand

Received: 10 March 2023 / **Revised:** 20 March 2023 / **Accepted:** 20 March 2023

ABSTRACT

Aim: This study aims to evaluate and compare the profilometry and atomic force microscopy (AFM) for characterization of biomaterial surfaces. Method: The clinically commonly used titanium (Ti) was used as the specimen. Each of the specimen was prepared by different grits of sandpapers, including 2000, 1000, 800, 600, 400, 220, 180, and 100 grits. An unpolished Ti plate served as the control. Surface characterization of the Ti specimens was examined using profilometry and AFM. Results: Both profilometry and AFM were capable of producing two-dimensional (2D) and three-dimensional (3D) topography. The scanning speed of profilometry (12 ± 5 s/image) was faster than that of AFM (250 ± 50 s/image) ($p < 0.01$). The resolution of AFM was relatively higher than profilometry. AFM produced more precise value, especially at nano-scale. When the Ti surface roughness was less than 0.2 μm, the results of surface roughness measured by profilometry and AFM were similar (mean difference = 0.01 ± 0.03, $p = 0.81$). When the Ti surface roughness was more than 0.3 μm, the surface roughness measured by profilometry was slightly higher than that by AFM (mean difference = 0.43 ± 0.15, $p = 0.04$). Conclusion: Profilometry and AFM are both useful techniques for the characterization of biomaterial surfaces. Profilometry scanned faster than the AFM but produced less detailed surface topography. Both technologies provided similar measurement when the roughness was less than 0.2 μm. When the Ti surface roughness was more than 0.3 μm, the surface roughness measured by profilometry was slightly higher than that by AFM.

KEYWORDS

atomic force microscopy, profilometer, topography, roughness

1 Introduction

Titanium (Ti) and its major medical Ti alloy, are widely used in many medical fields as such as dentistry and orthopedics due to their excellent biocompatibility, mechanical properties, corrosion resistance, anti-microbial properties, and the neutral interference during modern imaging techniques, such as computed tomography or magnetic resonance imaging [1]. The Ti surface properties such as nanotopography and roughness affect osteoblast adhesion, proliferation, differentiation, and extracellular matrix formation [2]. The surface nanotopography and roughness could be measured using many techniques, including optical and scanning electron microscopy, contact and noncontact profilometry, and the atomic force microscopy (AFM) [3–5]. However, many roughness scales and the absence of a discernible pattern contribute to the complexity of measuring and researching surface roughness. The existence of voids and imperfections further complicates the correct measurement of surface roughness in ceramics [6]. There is no measurement capable of measuring all scales, thus it is essential to create methods for combining data from several devices. Optical profilometer and AFM measurements are ideally suited to cover an extensive variety of topographical scales [7].

Profilometers utilize a variety of optical concepts, including interferometry, focus detection, and light scattering to analyze the surface profile of an object. The data that generated by the profilometer could be used to create two-dimensional (2D) and three-dimensional (3D) surface profile graphs of the object [8]. These instruments offer a broad measuring range for amplitude and are consequently widely used to test the surface of materials. However, it may not provide a good visual description of the surface with 2D and 3D measurements [9]. AFM was first introduced by Gerd Binnig, Calvin Quate, and Christoph Gerber in 1986, as the first effective instrument for imaging, measuring, and documenting the structural character of a material in real time [10]. It has been widely used in a wide variety of scientific areas, including dentistry and medicine. AFM provides 3D scanning of the topography of the contact surfaces of various materials at the micro and nano levels,

---

Address correspondence to Li Mei, li.mei@otago.ac.nz; Guangzhao Guan, simon.guan@otago.ac.nz

Cite this article as Li M. et al. *Nano TransMed* **2023**, *2*, e9130017.

producing high-resolution 3D pictures of the data [11]. When compared with a traditional profilometer, the resolution of AFM has a higher level of accuracy due to its precise tip and lower loading force [12]. But the AFM's greatest measurement range is generally restricted to a surface that is 100 μm × 100 μm in size [13].

Roughness parameters are used to define various surface morphological qualities. Areal roughness parameters are defined in the International Organization for Standardization (ISO) 25178 (www.iso.org) series. As the surface characteristics should be described as using more than one surface measurement parameter, multiple parameters such as height, spatial, hybrid, functions and related parameters, and parameters related to segmentation could be used, in order to give reliable information on the profile shape [6].

The aim of this study was to evaluate and compare the roughness and topography by profilometer and AFM to characterize the surface quality in Ti with different grit of sandpapers.

2 Methods

2.1 Preparation of specimens

The clinical commonly used Ti plate (Synthes Maxillofacial plate) was cut into nine identical plates. Each of the plates was prepared by different grits of sandpapers (2000, 1000, 800, 600, 400, 220, 180, and 100 grits). An untouched Ti plate was used as the control. All specimens were cleaned with an ultrasound cleaner and naturally dried before the profilometry and AFM measurements and analyses.

Surface characterization was performed using a profilometer (Polytec TopMap Micro.View, Baden-Württemberg, Germany) and an AFM (BioScope ResolveTM, Bruker Billerica, Massachusetts, USA). Three different regions were evaluated in each specimen [6]. With the ScanAsyst mode, the AFM adjusted most of the parameters such as set-point, drive frequency, scan rate, feedback gains, and other important scanning parameters automatically to obtain a given sample [14]. The specimen surface was scanned using a triangular DNP-10(B) tip (frequency 23 KHz, spring constant 0.12 N/m, tip radius 20 nm). Three areas were randomly selected from each specimen for measurement, and 2D and 3D AFM images were then taken at 50 μm × 50 μm planes, at 256 × 256 resolutions, and at a scan rate of 0.5 Hz in ScanAsyst mode [15].

Surface characterization was made using areal ISO 25178 (www.iso.org) (Eq. (1)). Sa is the average roughness and defined as the average height of all measured points in the measurement. It is the extension of Ra (arithmetical mean height of a line) to a surface. It is commonly used for surface roughness evaluation.

$$\mathrm{Sa} = \frac{1}{A}\iint_A \left|Z(X,Y)\right| \mathrm{d}x\mathrm{d}y \tag{1}$$

2.2 Statistical analysis

Data obtained from profilometry and AFM measurements were recorded and subjected to statistical analysis on SPSS 28 software (SPSS Inc, Chicago, IL, USA), Origin 2022b (Academic) for Windows (OriginLab Corporation, Northampton, MA, USA), Topography Measurement System 4.2.1.1 (Polytec GmbH, Waldbronn, Baden-Württemberg, Germany) and Mountains 9.3 (Digital Surf, Besançon, France). The results were expressed as percentages and numbers. The surface roughness parameters were expressed as the mean ± standard deviation (SD) if normally distributed, or as the median and interquartile range (IQR) if they had a skewed distribution. The measurements of profilometry and AFM were compared using One-Way ANOVA and Tukey's post hoc test at $\alpha = 0.05$.

3 Results

Scanning speed of the profilometer (12 ± 5 s/image) was faster than that of the AFM (250 ± 50 s/image) ($p < 0.01$). Both profilometry and AFM produced good quality 2D and 3D images. The peak and valley could be seen and measured in both 2D and 3D images (Figs. 1 and 2). AFM provided images with near-atomic resolution and more parameters of surface topography compared with the profilometry (Table 1). The topography of AFM revealed a non-uniform with distinct peaks and deep valleys surface. The resolution of the profilometry was less than AFM (Fig. 1). The commonly used height, functional, spatial, hybrid, functional volume, and functional (stratified surfaces) values of the roughness of the control group are presented in Table 2. Spatial and hybrid parameters of roughness were not able to obtain from profilometer analysis. Therefore the fineness of the material was unable to assess.

Overall, the AFM produced more precise value than profilometer, as AFM could give measurement in nanometer. AFM gave slightly lower roughness values (0.03426 ± 0.00183 μm) than profilometer (0.10 ± 0.02 μm) in the control (Figs. 3 and 4). The surface roughness measurement results with different polishing grit obtained using the AFM and profilometer are shown in Fig. 4. Based on the overall roughness evaluation, the AFM has lower value than profilometer (mean difference = −0.14, $p > 0.05$). When the Ti surface roughness was less than 0.2 μm, the measurement results of profilometry and AFM were similar (mean difference = 0.01 ± 0.03, $p = 0.81$). When the surface roughness was more than 0.3 μm, the measurement results of profilometry were higher than that of AFM (mean difference = 0.43 ± 0.15, $p = 0.04$) (Figs. 3 and 4).

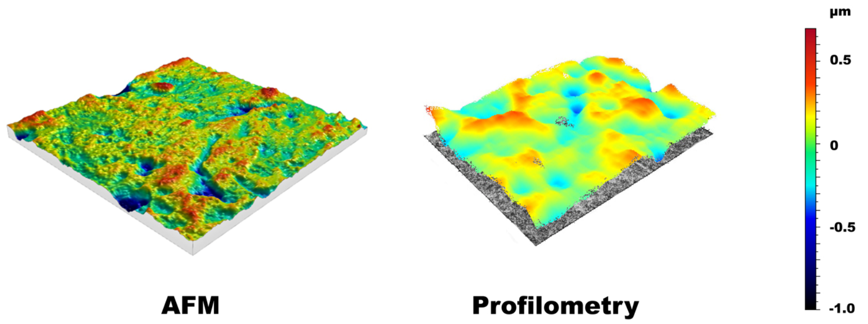

Figure 1 3D topographic images of AFM and profilometry of the titanium surface (control).

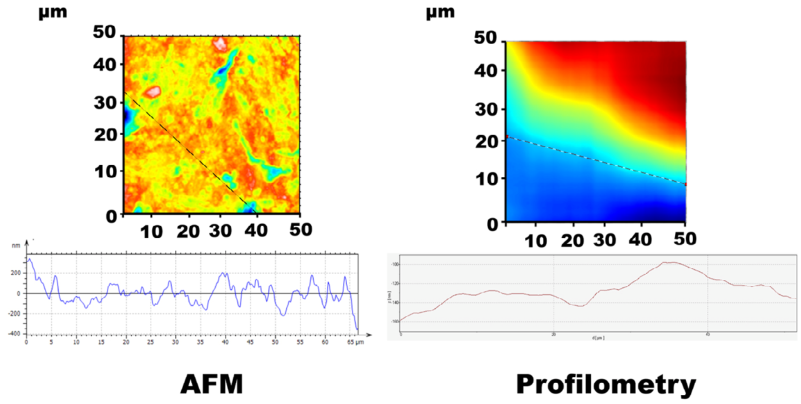

Figure 2 2D topographic images of AFM and profilometry of the titanium surface (control).

Table 1 Parameters of surface characterization from profilometry and AFM

	Parameters							
	Height	Functional	Spatial	Hybrid	Functional volume	Feature	Functional (stratified surfaces)	Feature (watershed, shape)
Profilometry	Sq, Ssk, Sku, Sp, Sv, Sz, Sa	Smr, Smc	Sal, Str, Std	Sdq, Sdr	Vmp, Vmc, Vvc, Vvv	**N/A**	Sk, Spk, Svk, Smr1, Smr2, Spq, Smq, Svq	**N/A**
AFM	Sq, Ssk, Sku, Sp, Sv, Sz, Sa	Smr, Smc, **Sdc**	Sal, Str, Std, **Ssw**	Sdq, Sdr	**Vm, Vv,** Vmp, Vmc, Vvc, Vvv	**Spd, Spc, S10z, S5p, S5v, Sda, Sha, Sdv, Shv, Svd, Shh, Shhx, Shhq, Shax, Shaq, Shvx, Shvq, Sdd, Sddx, Sddq, Sdax, Sdaq, Shn, Sdn**	Sk, Spk, Svk, Smrk1, Smrk2, Spq, Svq, Smq, **Sak1, Sak2, Spkx, Svkx**	**Shrn, Shrnx, Shrnq, Shff, Shffx, Shffq, Shed, Shedx, Shedq, Shar, Sharx, Sharq, Sdrn, Sdrnx, sdrnq, Sdff, Sdffx, Sdffq, Sded, Sdedx, Sdedx, Sdarx, Sdarq**

Bold: the different between AFM and profilometry analysis

Table 2 Roughness parameters (ISO 25178) in control between profilometry and AFM

ISO 25178	Profilometry	AFM
Height		
Sa	0.10 ± 0.02 μm	0.03426 ± 0.00183 μm
Sq	0.13 ± 0.02 μm	0.04561 ± 0.00299 μm
Sz	1.09 ± 0.10 μm	0.83767 ± 0.11849 μm
Sp	0.43 ± 0.09 μm	0.55623 ± 0.07873 μm
Sv	0.66 ± 0.04 μm	0.28143 ± 0.05637 μm
Sku	3.64 ± 0.81 μm	6.11267 ± 1.25056 μm
Functional		
Smc	0.43 ± 0.09 μm	0.60583 ± 0.05273 μm
Spatial		
Sal	N/A	0.60583 ± 0.05273 μm
Str	N/A	0.85310 ± 0.03952
Hybrid		
Sdq	N/A	0.20193 ± 0.01046
Sdr	N/A	1.34017% ± 1.15656%
Functional volume		
Vvv	17.59 ± 3.45 μL/m^2	5.59100 ± 0.25422 μL/m^2
Vvc	156.68 ± 37.12 μL/m^2	50.22000 ± 2.58529 μL/m^2
Vmp	5.9 ± 2.30 μL/m^2	2.91433 ± 0.40824 μL/m^2
Vmc	120.54 ± 25.26 μL/m^2	37.00667 ± 1.63121 μL/m^2
Functional (stratified surfaces)		
Sk	0.34 ± 0.06 μm	0.10038 ± 0.00492 μm
Spk	0.11 ± 0.04 μm	0.05784 ± 0.00765 μm
Svk	0.16 ± 0.03 μm	0.05170 ± 0.00336 μm
Smr1	0.08 ± 0.01	0.10960 ± 0.00436
Smr2	0.89 ± 0.01	0.87893 ± 0.00333

*Profilometry only can export two decimals at μm scale. AFM can export data to 5 decimals at μm scale.

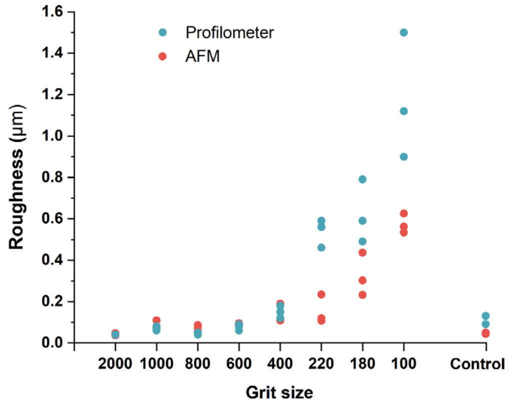

Figure 3 Surface roughness of the titanium treated with different grits of sandpapers characterized by profilometer and AFM.

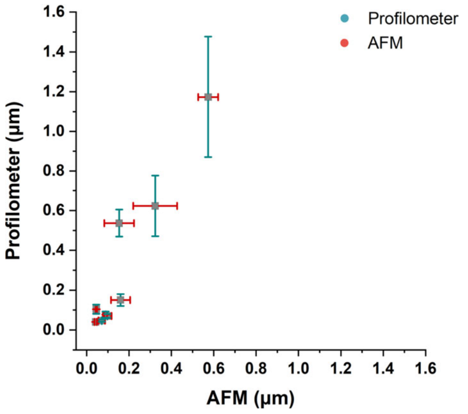

Figure 4 Correlation of the surface roughness characterized by profilometer and AFM.

4 Discussion

Surface characteristics, such as roughness and topography, have significant effect on the cell response, for example, Ti surface significantly enhances osseointegration [16]. In the literature, biomaterial surfaces are often characterized using different microscopy technologies, such as profilometry, AFM, scanning electron microscopy, and confocal laser scanning microscopy [17–19]. Among these technologies, profilometry and AFM can provide both quantitative and quantitative information of the material surfaces. The current study compared the profilometry and AFM for surface characterization of Ti and found that the profilometry scanned faster than the AFM but provided relatively less detailed information about surface topography. The AFM provided images with atomic level resolution for measuring surface topography when comparing with profilometry. Both technologies provided similar measurement when the roughness was less than 0.2 μm. When the Ti surface roughness was more than 0.3 μm, the surface roughness measured by profilometry was slightly higher than that by AFM.

Profilometers utilise a variety of optical concepts test the surface roughness of materials. AFM is a very effective tool for measuring and recording the structural properties of a material, and it enables the visualisation of the surface topography of Ti with a high spatial resolution. Given the benefits of their imaging properties and the fact that they do not alter the surface of the sample, these instruments are increasingly utilised to evaluate the surface characteristics of dental materials [20]. The most used parameter for measuring the surface roughness of Ti is Sa

In this study, the Ti surface roughness and topography were measured with two different methods and the findings obtained by the two devices are compared. Due to differences in the measurement sensitivity and working processes of the two devices, the identical plates may provide different surface roughness values. In this investigation, the overall roughness was found to be higher in profilometer than AFM in our study, which is consistent with other studies [8, 21]. Regarding the Sa parameter, the profilometer and AFM have shown extremely comparable patterns when the roughness is less than 0.2 μm. They have both demonstrated that certain polished surfaces had no discernible variation from the control, which is consistent to other studies [6]. Variations in AFM measurements may be attributable to the fact that the AFM device gave a better resolution picture and conducted nanometric measurement, and a more sensitive measurement may produce more distinguishable findings [15].

The nature of surface topography is 3D. Hence, the measurement of 3D surface topography can reflect the intrinsic properties of surface. 3D parameters are more realistic than 2D profiles. The information that obtained from 3D measurement provides a more detailed representation of surface topography than 2D measurement [21]. AFM could be used for qualitative measurements and delivers data in 3D. The differences found between AFM and profilometer suggest that AFM could offer more detailed definition of surface topography. This may indicate that there is a difference in sensitivity between profilometer and AFM. AFM requires accurate measurement to examine the surface topography of the Ti at the high spatial resolution. Hence, it is more sensitive to small topographic changes. Compared to profilometer, the AFM with a 20 nm tip radius allows for more precise tracings. Therefore it may result in more distinctive results.

Apart from the surface roughness measurement, AFM was developed and has been widely used as a method for imaging biomolecules with atomic level resolution under real-time physiological condition. It has been used to investigate the nanomechanical characteristics of cells, tissues, microorganisms, and biological macromolecules like proteins, lipids, mRNA, and DNA [19, 22–24]. Profilometer on the other hand is an easy to use and compact optical profiler. The main advantages of the profilometer are its fast scanning speed, which results in a reaction time of the distance sensor that is less than 0.1 ms, high bandwidth, a compact size, and low cost [25, 26].

Both measurement methods provide comparable and distinct outcomes, they somewhat complement one another. In general, AFM provided relative more comprehensive surface characterization than profilometry but relatively slower speed. Since both methods only look at a small part of the surface of the sample, measurements that analyse the whole surface could give more accurate results.

5 Conclusion

Profilometry and AFM are both useful techniques for the characterization of biomaterial surfaces. Both techniques yield equivalent and unique results. They complement one another to some extent. In general, AFM produced a more exhaustive surface characteriszation than profilometry, albeit at a slower rate. Both technologies provided similar measurement when the roughness was less than 0.2 μm. When the Ti surface roughness was more than 0.3 μm, the surface roughness measured by profilometry was slightly higher than that by AFM. As both approaches only examine a small portion of the sample's surface, measurements that examine the entire surface may yield more accurate findings.

Acknowledgements

This research were funded by 2021 SJWRI PhD Research Grant (No. 118940), and 2022 University of Otago Research Grants (No. 0122-0323).

Data availability statement

Data is available on request due to privacy/ethical restrictions.

Competing interests

The authors declare that they have no competing interests.

Ethics approval statement

Not required.

References

[1] Portan, D. V.; Kroustalli, A. A.; Deligianni, D. D.; Papanicolaou, G. C. On the biocompatibility between TiO_2 nanotubes layer and human osteoblasts. *J. Biomed. Mater. Res. A.* **2012**, *100A*, 2546–2553.

[2] Elias, C. N.; Meirelles, L. Improving osseointegration of dental implants. *Expert Rev. Med. Devices* **2010**, *7*, 241–256.

[3] Méndez-Vilas, A.; Donoso, M. G.; González-Carrasco, J. L.; González-Martín, M. L. Looking at the micro-topography of polished and blasted Ti-based biomaterials using atomic force microscopy and contact angle goniometry. *Colloids Surf. B: Biointerfaces* **2006**, *52*, 157–166.

[4] Canabarro, A.; Figueiredo, F.; Paciornik, S.; De-Deus, G. Two- and three-dimensional profilometer assessments to determine titanium roughness. *Scanning* **2009**, *31*, 174–179.

[5] Kournetas, N.; Spintzyk, S.; Schweizer, E.; Sawada, T.; Said, F.; Schmid, P.; Geis-Gerstorfer, J.; Eliades, G.; Rupp, F. Comparative evaluation of topographical data of dental implant surfaces applying optical interferometry and scanning electron microscopy. *Dent. Mater*. **2017**, *33*, e317–e327.

[6] Tholt, B.; Miranda-Júnior, W. G.; Prioli, R.; Thompson, J.; Oda, M. Surface roughness in ceramics with different finishing techniques using atomic force microscope and profilometer. *Oper. Dent*. **2006**, *31*, 442–449.

[7] Beitia, C.; Sater, M. A.; Cordeau, M.; Godny, S.; Petitgrand, S.; Alliata, D. Optical profilometry and AFM measurements comparison on low amplitude deterministic surfaces. In *30th Annual SEMI Advanced Semiconductor Manufacturing Conference*, Saratoga Springs, USA, 2019, pp 1–4.

[8] Vieira, T. I.; de Sousa Andrade, K. M.; Cabral, L. M.; Valença, A. M. G.; Maia, L. C.; Batista, A. U. D. Linear and areal surface roughness assessments for the study of tooth wear in human enamel. Clin. Oral Investig. **2023**, 27, 329–338.

[9] Yılmaz, C.; Kanık, Ö. Investigation of surface roughness values of various restorative materials after brushing with blue covarine containing whitening toothpaste by two different methods: AFM and profilometer. *Microsc. Res. Technol*. **2022**, *85*, 521–532.

[10] Binnig, G.; Quate, C. F.; Gerber, C. Atomic force microscope. *Phys. Rev. Lett*. **1986**, *56*, 930–933.

[11] Pantić, M.; Mitrović, S.; Babić, M.; Jevremović, D.; Kanjevac, T.; Džunić, D.; Adamović, D. AFM surface roughness and topography analysis of lithium disilicate glass ceramic. *Tribol. Ind*. **2015**, *37*, 391–399.

[12] Santos, S.; Barcons, V.; Christenson, H. K.; Font, J.; Thomson, N. H. The intrinsic resolution limit in the atomic force microscope: Implications for heights of nano-scale features. *PLoS One*. **2011**, *6*, e23821.

[13] Guo, T.; Wang, S. M.; Dorantes-Gonzalez, D. J.; Chen, J. P.; Fu, X.; Hu, X. T. Development of a hybrid atomic force microscopic measurement system combined with white light scanning interferometry. *Sensors* **2012**, *12*, 175–188.

[14] Kroeger, M. E.; Sorenson, B. A.; Thomas, J. S.; Stojković, E. A.; Tsonchev, S.; Nicholson, K. T. Atomic force microscopy of red-light photoreceptors using peakforce quantitative nanomechanical property mapping. *J. Vis. Exp*. **2014**, e52164.

[15] Karatas, O.; Gul, P.; Gündoğdu, M.; Iskenderoglu, D. T. An evaluation of surface roughness after staining of different composite resins using atomic force microscopy and a profilometer. *Microsc. Res. Technol*. **2020**, *83*, 1251–1259.

[16] Deligianni, D. D.; Katsala, N.; Ladas, S.; Sotiropoulou, D.; Amedee, J.; Missirlis, Y. F. Effect of surface roughness of the titanium alloy Ti-6Al-4V on human bone marrow cell response and on protein adsorption. *Biomaterials* **2001**, *22*, 1241–1251.

[17] Elias, C. N.; Oshida, Y.; Lima, J. H. C.; Muller, C. A. Relationship between surface properties (roughness, wettability and morphology) of titanium and dental implant removal torque. *J. Mech. Behav. Biomed. Mater*. **2008**, *1*, 234–242.

[18] Liu, Y. P.; Zhu, D. K.; Gilbert, J. L. Sub-nano to nanometer wear and tribocorrosion of titanium oxide-metal surfaces by *in situ* atomic force microscopy. *Acta Biomater*. **2021**, *126*, 477–484.

[19] Guan, G. Z.; He, Y.; Mei, L. Atomic force microscopy: A nanobiotechnology for cellular research. *Nano TransMed* **2022**, *1*, e9130004.

[20] Fandridis, J.; Papadopoulos, T. Surface characterization of three titanium dental implants. *Implant Dent*. **2008**, *17*, 91–99.

[21] Kakaboura, A.; Fragouli, M.; Rahiotis, C.; Silikas, N. Evaluation of surface characteristics of dental composites using profilometry, scanning electron, atomic force microscopy and gloss-meter. *J. Mater. Sci. Mater. Med*. **2007**, *18*, 155–163.

[22] Takechi-Haraya, Y.; Usui, A.; Izutsu, K. I.; Abe, Y. Atomic force microscopic imaging of mRNA-lipid nanoparticles in aqueous medium. *J. Pharm. Sci.* **2023**, *112*, 648–652.

[23] Ridolfi, A.; Caselli, L.; Montis, C.; Mangiapia, G.; Berti, D.; Brucale, M.; Valle, F. Gold nanoparticles interacting with synthetic lipid rafts: An AFM investigation. *J. Microsc*. **2020**, *280*, 194–203.

[24] Pyne, A. L. B.; Hoogenboom, B. W. Imaging DNA structure by atomic force microscopy. In *Chromosome Architecture*. Leake, M. C., Ed.; Springer: New York, 2016; pp 47–60.

[25] Semenov, D. V.; Nippolainen, E.; Kamshilin, A. A. Accuracy and resolution of a dynamic-speckle profilometer. *Appl. Opt*. **2006**, *45*, 411–418.

[26] Liao, H. S.; Cheng, S. H.; Hwu, E. T. Method for film thickness mapping with an astigmatic optical profilometer. *Sensors* **2022**, *22*, 2865.