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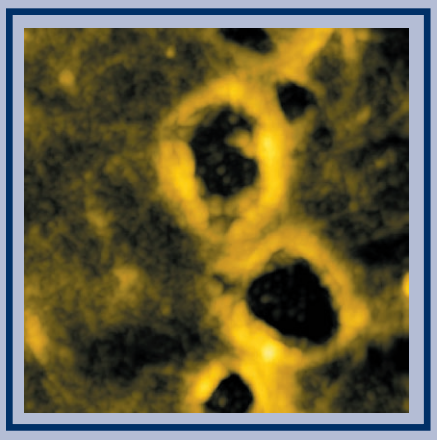

AFM Metrology and Analysis for Peptide, Nanoparticle and Their Complex

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1. Introduction

Three samples were prepared and submitted for AFM analysis. Sample #1 is peptide thin film, sample #2 is 5 nm gold nanoparticle, and sample #3 is laser-irradiated Au-peptide complexes. All three samples were prepared on mica substrates. A Nano-R2™ AFM system was used in this measurement and analysis. Multiple areas were scanned for each sample. This work is to show the capability of Pacific Nanotechnology's Nano-R2™ AFM in topographic imaging and quantitative analysis for the samples. Examples of measurement results are shown and discussed below.

2. Experiment

All sample measurement was carried out in air using the Close-Contact Mode of Nano-R2™ AFM equipped with a light lever scanner. The Close-Contact Mode was found the most suitable for these samples. Regular Close-Contact probes with a normalized radius of < 10 nm were used in all data acquisition.

The Nano-R2™ system was calibrated in the X, Y, and Z axes before measurement. We utilized the standard samples to calibrate Z-height channel, and resulting system' accuracy was ± 1 %. The resonant frequency of used probes was tested to comply with the manufacturers' datasheet. We minimized the drive amplitudes to enhance the measurement stability in the Close-Contact Mode. Raw scanning data were processed using the NanoRule+™ software. Different scanning rates, resolutions and angles were used to achieve optimal results.

3. Results and Discussion

3.1 Peptide (Sample #1)

We scanned many different localities of the sample, and found that mica surface was uniformly covered by micro-globes and their aggregates. Figure 1 shows a typical topography of the peptide thin film. The small globes have diameters of 20-100 nm (Figure 1B), and the film roughness is about 1.05 nm in RMS (Figure 1C), which are measured by NanoRule+™ (not shown).

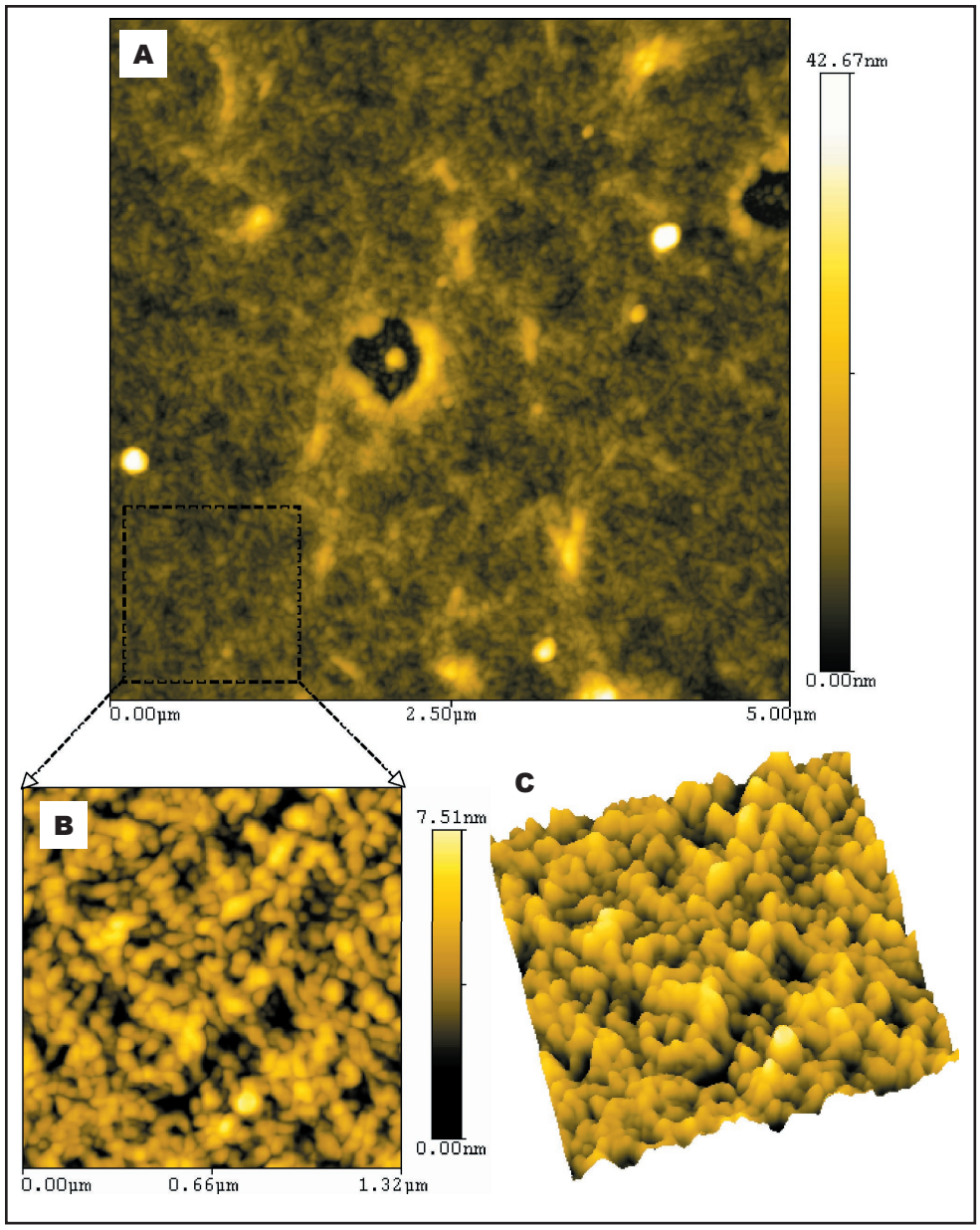


Figure 1: Typical Topography of the Sample #1 (Peptide): (A) 5 × 5 µm scan; (B) 1.32 × 1.32 µm; (C) 3D view of the structure (B). The measured surface roughness of the structure in (B) is ~1.05 nm in RMS (not shown).

We also found the surfaces were typically covered by micro-sized circular holes (Figure 1A). Figure 2 shows a close look for the hole structure. It is clear that there are small globes inside those holes. Figure 2B is a phase image.

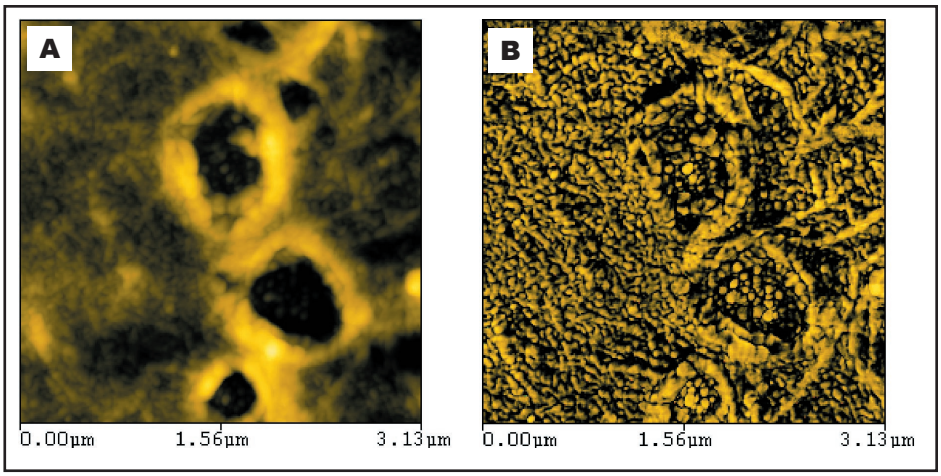
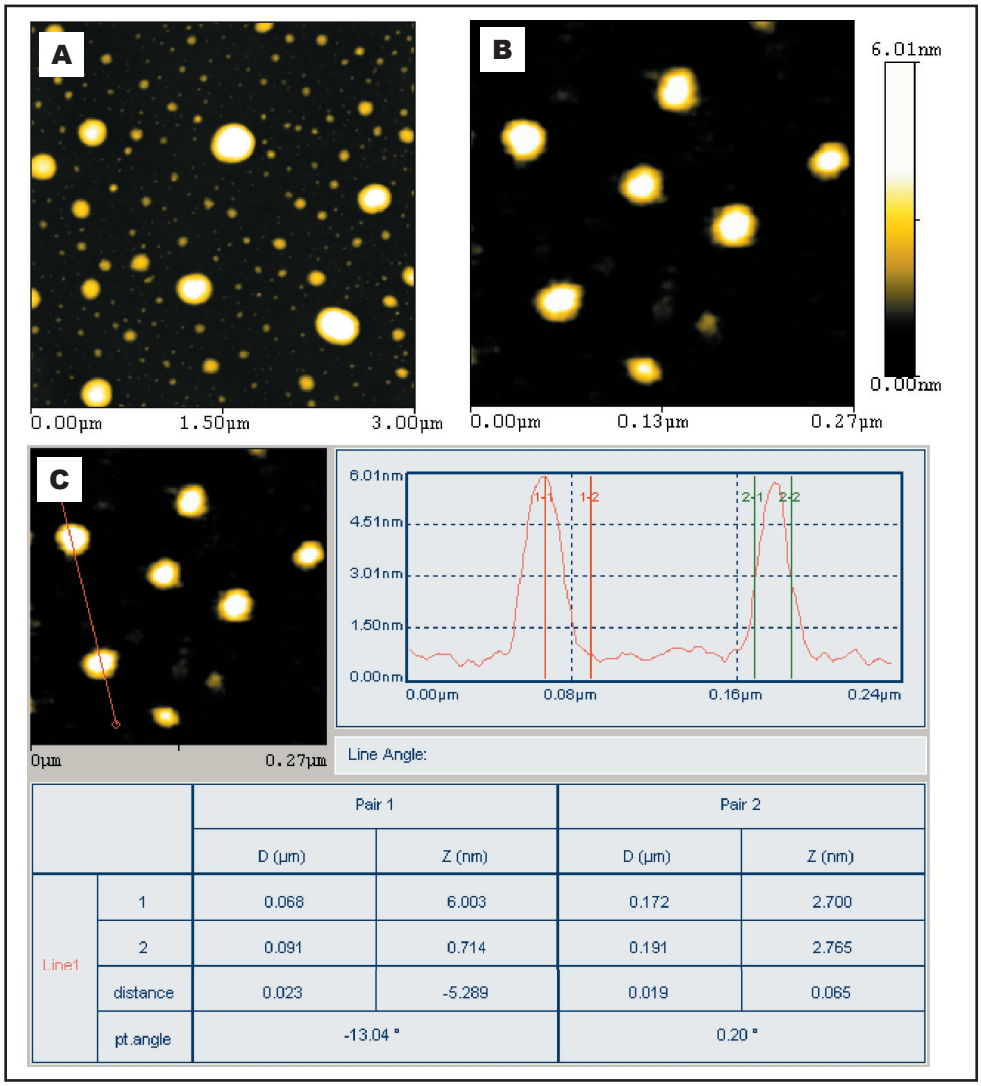


Figure 2: A Close-look of hole structures which is typical on the Sample #1. (A) Topography; (B) Phase Image of the same structure.



		Pair 1		Pair 2	
		D (µm)	Z (nm)	D (µm)	Z (nm)
Line1	1	0.068	6.003	0.172	2.700
	2	0.091	0.714	0.191	2.765
	distance	0.023	-5.289	0.019	0.065
	pt.angle	-13.04 °		0.20 °	

Figure 3: Topography and Dimension Analysis of the Sample #2 (5 nm Au nanoparticle). (A) 3 × 3 µm scan with the height scale of 102.74 nm; (B) 270 × 270 nm in the area with small size distribution; (C) Dimension Analysis for (B): The pair 1 shows the particle height is 5.3 nm; the pair 2 represents the particle diameter as 19 nm, resulting from the tip broadening effect.

3.2 Au Nanoparticle (Sample # 2)

We scanned at least 7 different localities of the sample #2. Au nanoparticles and their aggregates are all over the surface. Figure 3A shows a typical topography where Au nanoparticles present a large size distribution. We run the Particle Analysis function in NanoRule+™ to count and analyze the total of 620 Au particles larger than 4 nm in Figure 3A. As shown in Figure 4, the average of diameter was 8.1 nm with the standard deviation of 8.04 nm, and the size ranged from 4.11 nm up to 97.66 nm. This suggests that the 5 nm Au nanoparticles have been aggregated.

Figure 3B shows a 270 x 270 nm scan on the sample #2, where 7 nanoparticles shows a narrow size distribution. Figure 3C shows a dimension analysis for two individual nanoparticles. The pair 1 shows the particle height is 5.3 nm; the pair 2 represents the particle diameter as 19 nm, resulting from the tip broadening effect (apparent lateral dimension is greater than the actual size when the measurement object is comparable in size to the probe used). One can see that particles are not perfect round in Figure 3C, because a lithography-fabricated silicon probe is not geometrically symmetric in all-directions.

Total Objects

Item	Area (μm²)	Perimeter (μm)	Volume (μm³)	Height (nm)	Max_Height (nm)	Radius (μm)
Total	1.22	51.23	0.025	4301.8	5020.7	11.23
Avg.	0.00197	0.083	4.05e-005	6.94	8.10	0.018
Max.	0.094	0.99	0.00407	43.11	97.66	0.36
Min	3.44e-005	0.00586	1.41e-007	4.11	4.11	0.0
Range	0.094	0.98	0.00407	39.00	93.55	0.36
St.	0.00735	0.11	2.69e-004	4.01	8.04	0.025

Total Particles: 620

Figure 4: The Particle Analysis function of NanoRule+™ for the particles in Figure 3A.

3.3 Laser-irradiated Au-Peptide Complex (Sample #3)

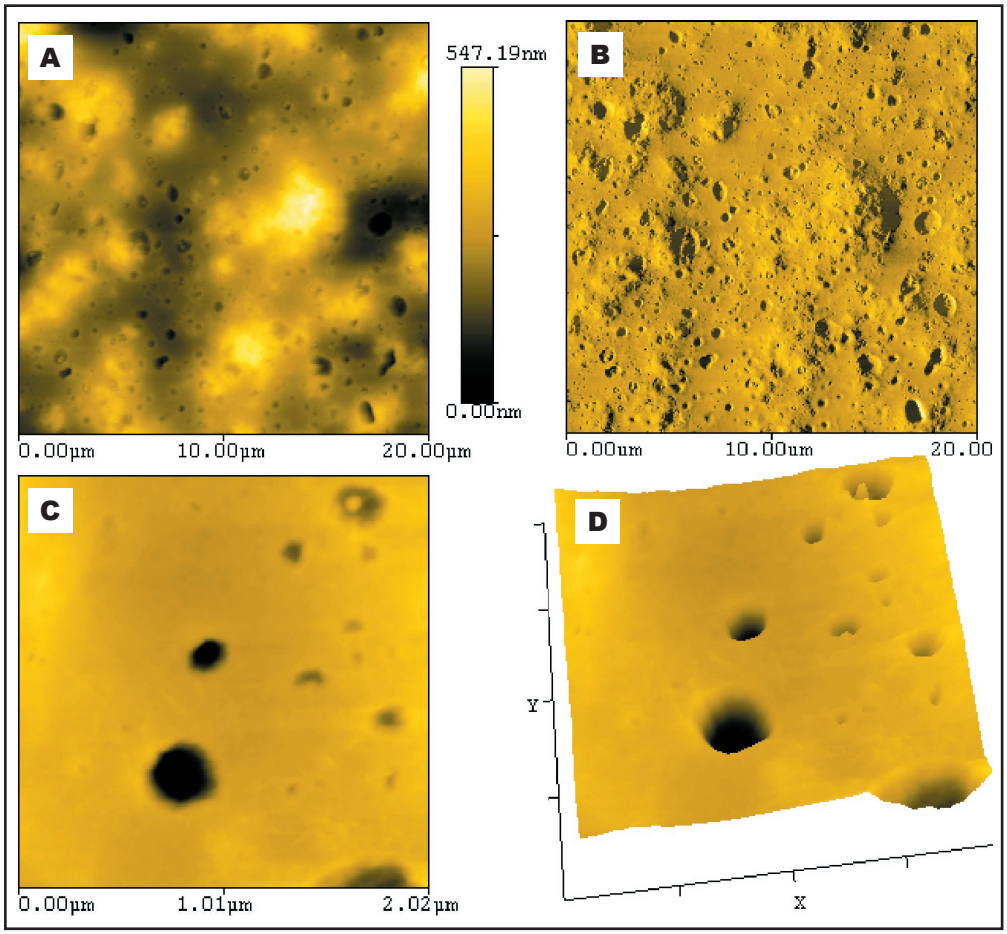


Figure 5: Typical Topography of the Sample #3 (Laser-irradiated Au-Peptide Complex). (A) 20 × 20 µm Scan; (B) Phase image of the same area; (C) 2 × 2 µm Scan with Z-height scale of 77.54 nm; (D) 3D view of the structure of C. No fiber-like structure was found.

We scanned at least 10 different localities of sample #3, and found that the typical topography is different from sample #1 and sample #2. As shown in Figure 5, we did not find either small globes (peptide in Figure 1) or nanoparticles (Figure 3). The surface is very smooth except for hole structures (Figure 5C), which have been also found on sample #1. Interestingly, the small globes inside holes in Figure 2A or Figure 1A were not visible in sample #3 (Figure 5C). Figure 5 represents the most typical topography of the sample #3, where we did not find fiber-like structures. However, this micron-sized structure was found occasionally (Figure 6). Phase image offers the most sensitive way to detect this filament. In the phase image of Figure 5B which is the most typical topography, we did not find these fibers in this entire 20 × 20 µm scan patch.

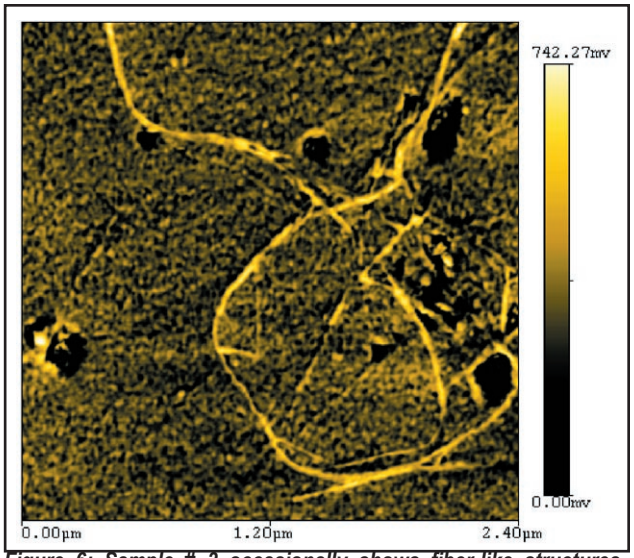


Figure 6: Sample # 3 occasionally shows fiber-like structures (phase image).

4. Conclusion

This measurement shows example results of topography and phase images of peptide thin film, (sample #1) Au nanoparticles (sample #2) and laser-irradiated Au-peptide complex (sample #3). Dimension analyses of individual nanoparticles were given using the Particle Analysis and Line Analysis function of NanoRule+™. Sample #1 is a uniform film composed by nanometer-sized globes. Sample #2 is nanoparticles and their aggregates. The topography of the sample #3 is different from the sample #1 and #2 because we did not find small globes (sample #1) and nanoparticle (sample #2) on the surface. No fiber-like structure was typically visible on the sample #3, although we found micron-sized filaments on occasion. It is clearly that AFM can provide very detailed information on all these samples.

Pacific Nanotechnology's Nano-R2™ AFM is shown fully capable for analysis and study of nanoscale biomaterials and nanoparticles.