Electronic Supplementary Information (ESI)

Title: Water-Repairable Zwitterionic Polymer Coatings for Anti-Biofouling Surfaces

*Zhanhua Wang, Esther van Andel, Sidharam P. Pujari, Huanhuan Feng, Joshua A. Dijksman, Maarten M. J. Smulders, Han Zuilhof **

Experimental Section

Materials: Poly(ethylene glycol) dimethacrylate (PEGDMA) was received from Sigma-Aldrich and filtered through a basic alumina column to remove inhibitors before utilizing it for polymerization. Alexa Fluor® 488 conjugate Albumin from Bovine Serum was purchased from Thermo Fisher Scientific and fibrinogen (fraction I from porcine plasma, 78% protein) from Sigma-Aldrich. Acetone (Sigma-Aldrich, semiconductor grade VLSI PURANAL Honeywell 17617) and deionized water (resistivity 18.3 MΩ cm^{-1}) were used as received. Silicon wafers, with a 0.2° miscut angle along the ⟨112⟩ plane, were (111)-oriented, n-type, phosphorus-doped and with a specific resistance of 1−10 Ω cm^{-1} and purchased from Siltronix (France). Stoichiometric silicon nitride (Si_3N_4) surfaces (1 × 1 cm^2) prepared by LPCVD deposition, were received from Lionix BV. All other chemicals were purchased from Sigma-Aldrich and used as received.

Preparation of the zwitterionic polymer networks: To a 100 mL two-neck round-bottom flask, which was filled with argon and magnetic stirrer bar, 0.023 g 2,2′-azobis(2-methylpropionitrile), 0.5 g [2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide, poly(ethylene glycol) dimethacrylate (0.05– 0.25 mL, M_n = 550 g/mol) and 20 mL Milli-Q water were added. The mixture was purged with argon for 30 min, and then put into a 70 ^{o}C oil bath and allowed to react for 24 h. After cooling down to room temperature, the reaction solution was transferred into a dialysis tube and dialyzed in Milli-Q water for 72 h. The obtained solution was directly used for making coatings.

Surface modification: The silicon/silicon nitride surface was sonicated in acetone for 5 min and subsequently cleaned using air plasma (Harrick Scientific Products, Inc. Pleasantville, NY) for 5 min, and then quickly transferred to a one–necked flask which was charged with 1 mL 3-aminopropyl triethoxysilane and 60 ml anhydrous toluene. The reaction mixture was kept at 80 °C for 16 h. The sample was then removed from the flask and extensively rinsed with CH_2Cl_2, sonicated for 5 min in CH_2Cl_2 to remove physisorbed molecules, and blown dry with a stream of dry argon. The surfaces were directly used for preparing ZPN coatings.

Preparation of ZPN coatings: The ZPN coatings were obtained by spin-coating or drop-casting the polymer solution onto cleaned surfaces, which was subsequently dried at 75 ^{o}C for 20 min and 120 ^{o}C for 2 h. The as-prepared coatings were then immersed into PBS (pH 7.0) solution for 1 h to remove any low-molecular-weight or uncross-linked polymer chains and dried by argon.

Static contact angle measurements: The static contact angle measurements were conducted using a Krüss DSA 100 contact angle goniometer having an automated drop dispenser and image/video capture system. The static contact angles were measured at three different places on a modified surface by dispensing three small droplets (3.0 μL volume of deionized water) with the help of an automated drop dispenser. The tangent 1 fitting model was implemented for contact angle measurements with an accuracy of ± 2°.

X-ray photoelectron spectroscopy (XPS): The X-ray photoelectron spectra at ambient temperature were obtained using a JPS-9200 photoelectron spectrometer (JEOL, Japan) for all the samples used in the study of the anti-fouling experiment, unless otherwise specified. A monochromatic Al Kα X-ray source (h_v = 1486.7 eV, 12 kV and 20 mA) with an analyzer pass energy of 10 eV was used. A base pressure of 3×10^{-7} Torr was maintained in the XPS chamber during measurements and the spectra were collected at room temperature. The X-ray incidence angle and the electron acceptance angle was 10° to the surface normal. The intensity of the XPS core-level electron was measured as the peak area after standard background subtraction according to the linear procedure. The takeoff angle φ (angle between sample and detector) of 80° is defined to a precision of 1°. The typical sample size was 1 × 1 cm^2. All XPS spectra were evaluated using the Casa XPS software (version 2.3.15).

Ellipsometry: The ellipsometric thickness of the modified surfaces was measured using a rotating Sentech Instruments (Type SE-400) ellipsometer, operating at 632.8 nm (He−Ne laser), and an angle of incidence of 70°. The optical constants of a freshly etched H-terminated Si(111) surface were taken as n = 3.850 and k = 0.057. The thicknesses of the monolayers were determined with a planar three-layer (ambient, monolayer, substrate) isotropic model, with assumed refractive indices of 1.00 and 1.50 for ambient and the ZPN, respectively. The reported values are the average of at least 5 measurements.

Reflectometry: ZPN coated surfaces, FIB solution and the reflectometry measurements were treated, prepared and performed as previously described by our group.[1] In short, a self-made reflectometer was used, equipped with a monochromatic linearly polarized light beam (He-Ne laser; 632.8 nm) and a glass prism which results in an incidence angle of 68° between the solvent-substrate interface. The reflected light is split into its *p*- and *s*-polarized components by a beam splitter. The ratio between the intensities of the *p*- and *s*-polarized components results in the output signal *S*. The adsorbed amount of protein could then be calculated using the following equation[2]:

$$\Gamma = Q_f \cdot \frac{\Delta S}{S_0}$$

where Γ is the adsorbed amount (mg/m^2), Q_f is the sensitivity factor (mg/m^2), S_0 is the initial signal given by the reflectometer before introducing protein solutions (mV), ΔS is the difference between the output signal and S_0 (mV). The sensitivity factor Q_f is dependent on, among other parameters, the thickness of each layer of the substrate. The thickness used for the ZPN coating was 80 nm, as measured by ellipsometry. The other parameters are listed in the Supporting Information. The sensitivity factor was calculated using Prof. Huygens software. The binding curves were smoothened using the 50 percentile filtering option of Origin version 8.

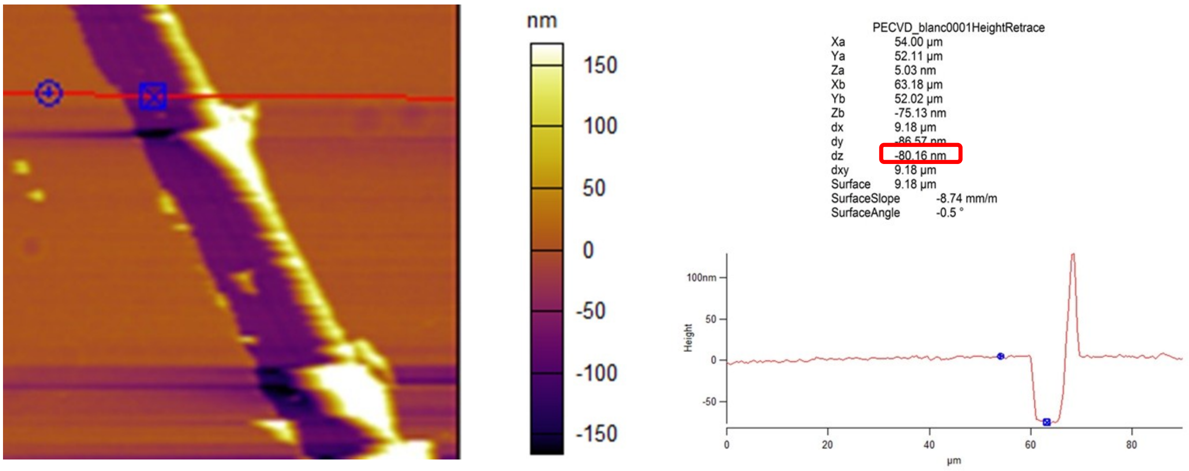
The ZPN coated surfaces were glued on one side of a 1×4 cm silicon strip, the other side was clamped into the reflectometer. The surfaces were before use pre-wetted for 1 hour in PBS to avoid artifacts. All measurements were performed at room temperature. Each measurement started with a 400 sec injection of PBS, followed by injecting for 1000 sec with an 0.5 mg/mL FIB solution and ended by another >400 sec PBS washing phase.

AFM characterization: AFM images (256 × 256 pixels) were obtained with an MFP3D AFM (Asylum Research, Santa Barbara, CA). The imaging was performed in tapping mode in air using OMCL-AC240 silicon cantilevers (Olympus Corporation, Japan) with a stiffness of 1.54 N/m. Images were flattened with a first-order flattening procedure using the MFP3D software. The root-mean-square (RMS) roughness was calculated from the fluctuations of the surface height around the average height in the image. In this way the RMS value describes the topography of the surface.

Dynamic light scattering: The particles size is measured by using an ALV goniometer and correlator equipped with a 632.8 nm 22 mW Uniphase 1145P HeNe laser using avalanche photodetector (Exelitas Technologies). Measurements are performed at a fixed scattering angle of 150°. The measured decorrelation functions are fitted using the standard cumulant methods.

Mechanical property testing: We tested the mechanical properties of the coating with a home-built sphere indentation tester in air at room temperature.[3] The indentation device consists of a force sensor (Futek LSB200, 250 gram capacity) with an acrylic sphere (0.3125" diameter, Engineering Laboratories) attached to it. The force sensor is mounted on a closed loop motorized vertical stage (PI M-451.1PD) that allows us to indent coatings with micrometer steps at a rate of 2 micron per second. The stage and force sensor are interfaced with LabVIEW. The sensor-sphere assembly is not infinitely stiff, so we calibrate its combined stiffness constant before the experiments. This calibration was used to compute the actual amount of indentation of the coating from the stage travel and measured force. The stiffness calibration was checked before and after completion of the experiments. Sphere indentation tests were used to probe the macroscopic mechanical properties of the ZPN coatings.[3] By consistently applying the same sphere indentation test on our samples, it was possible to obtain semi-quantitative measures for the mechanical performance of the coatings. We did not aim to measure the elastic constants of the material, as the coating properties are not linearly elastic; they exhibit plastic flow and are very thin, so will likely experience boundary stresses upon deformation. The indentation tests were performed with an approximately 7.9 mm diameter acrylic sphere on a ZPN layer cast on a Si wafer. One side of the cast layer was damaged by making several shallow incisions, with random orientation, by a surgical blade. This damaged part was subsequently repaired by covering it with a drop of DI water. The entire coated Si wafer, with both undamaged and repaired piece, was dried in ambient conditions for approximately 1.5 days. We then performed the indentation tests on the undamaged and a repaired layer. After completing the indentation tests on the undamaged layer, we damaged the ZPN coating with the same procedure, and immediately measured the hardness of the damaged layer with the same indentation tests under the same ambient conditions.

Local fouling experiments: ZPN coatings were covered by a drop of a 0.1 mg/mL Alexa Fluor 488-labeled BSA solution for 10 min, and then washed by 10 mM PBS solution for 5 times and then dried by argon. The surfaces were studied using a confocal laser scanning microscope (Zeiss LSM 510 Meta) which was outfitted with an argon ion laser at 488 nm. To allow comparison of the fluorescent images, the same settings were used in all measurements.



```
PECVD_blanc0001HeightRetrace
Xa           54.00 µm
Ya           52.11 µm
Za           5.03 nm
Xb           63.18 µm
Yb           52.02 µm
Zb           -75.13 nm
dx           9.18 µm
dy           -86.57 nm
dz           -80.16 nm
dxy          9.18 µm
Surface      9.18 µm
SurfaceSlope       -8.74 mm/m
SurfaceAngle       -0.5 °
```

Figure S1. AFM image and surface profile of the damaged ZPN coatings. As determined from the AFM images, we can see that the thickness of the coating is about 80 nm.

Table S1. Thickness of the ZPN coating determined by ellipsometry.

	Spot 1	Spot 2	Spot 3	Spot 4	Spot 5	Average
Thickness (nm)	75.18	76.30	81.93	78.68	79.43	78.30

Table S2. Overview of parameter values that were used to calculate the amount of adsorbed FIB in reflectometry measurements.

Parameter	Description	Value
n_1	Si real refractive index	3.877[a]
k_1	Si imaginary refractive index	0.0196[a]
n_2	Si_3N_4 real refractive index	2.012[a]
d_2	Si_3N_4 layer thickness	162 nm[a]
d_3	ZPN coating thickness	80 nm
n_3	Refractive index ZPN coating	1.45[b]
d_4	Assumed protein layer thickness	5 nm[b]
n_5	Solution (PBS) refractive index	1.33[b]
$\mathrm{d}n_{FIB}/\mathrm{d}C$	Fibrinogen differential refractive index	0.185 ± 0.003 L/kg[b]
θ	Laser incident angle with the surface	68°
λ	Laser wavelength	632.8 nm[b]

[a] As provided by the silicon nitride supplier (Lionix BV)

[b] As described and used by Nguyen *et al.*[4]

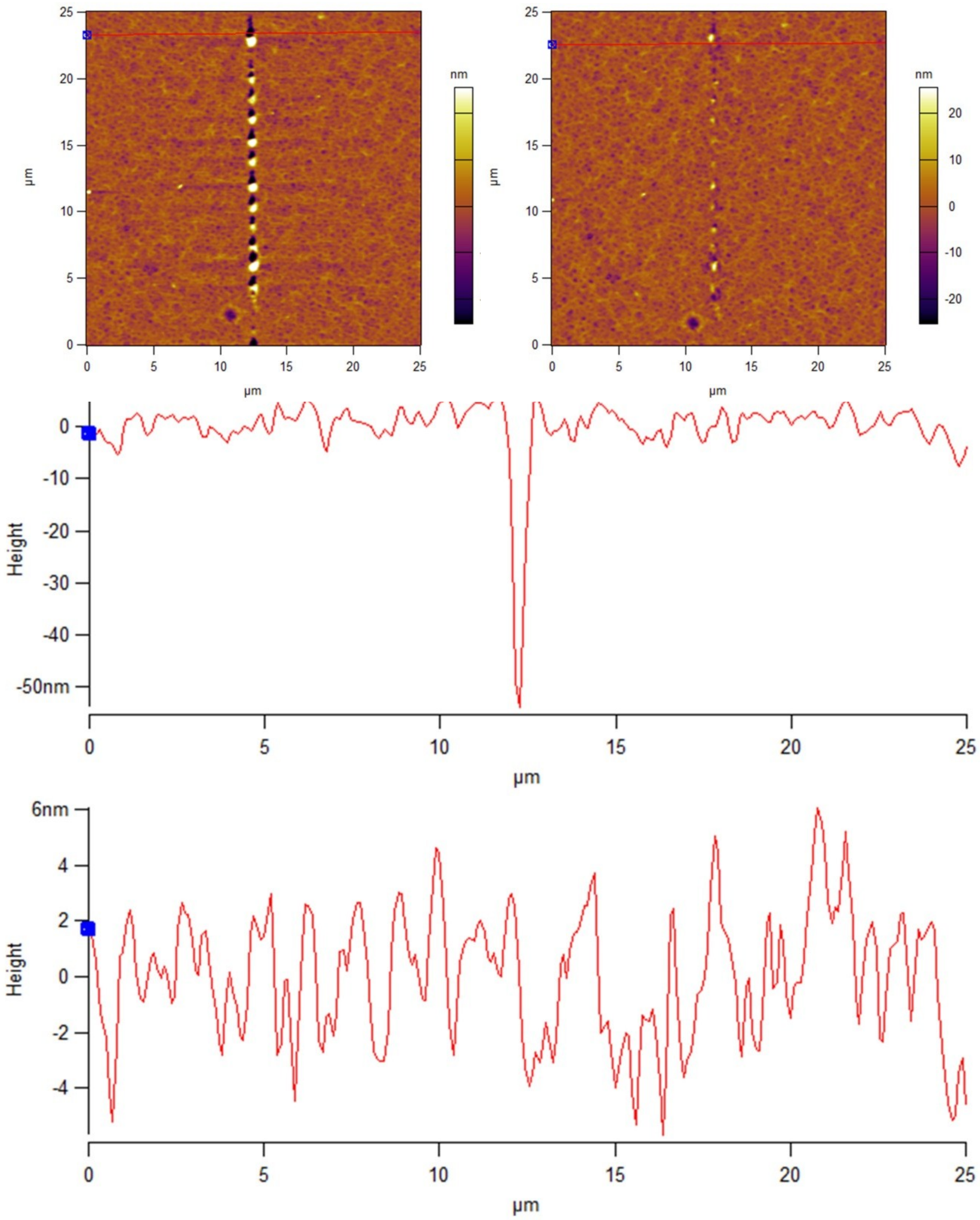


Figure S2. Tapping-mode AFM images and surface profile of the damaged and repaired ZPN coatings corresponding to spots a and a’ in Figure 4.

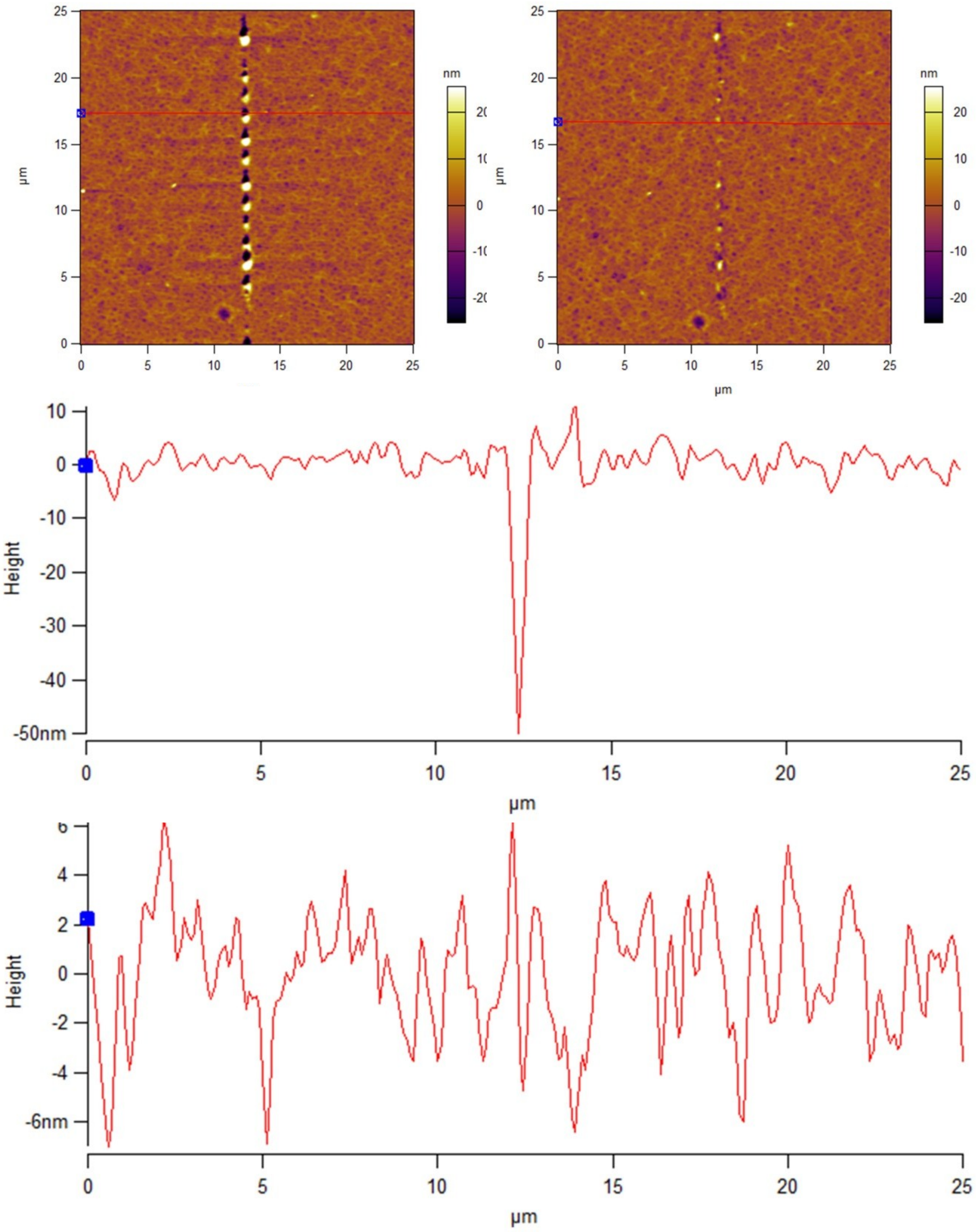


Figure S3. Tapping-mode AFM images and surface profile of the damaged and repaired ZPN coatings corresponding to spots b and b’ in Figure 4.

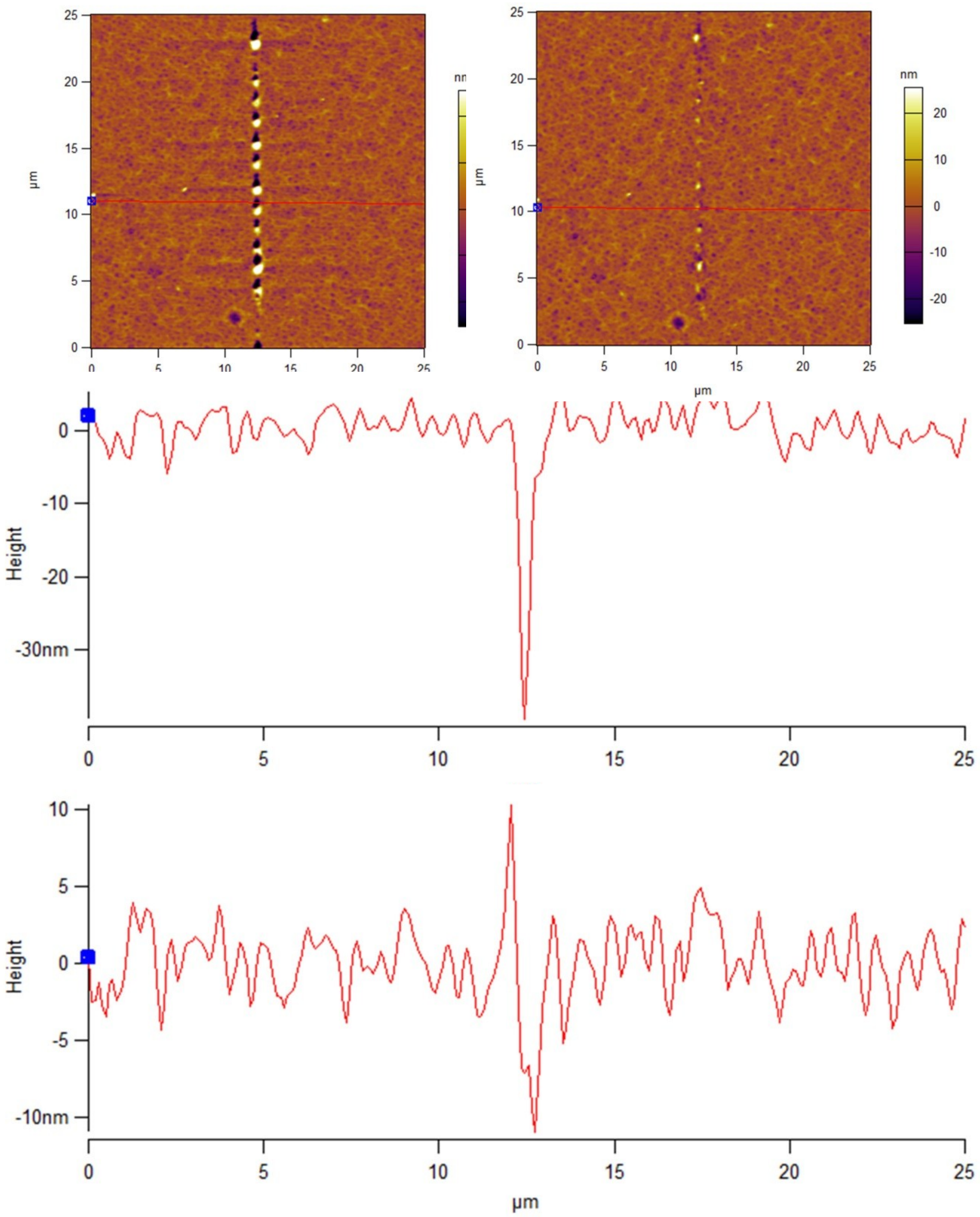


Figure S4. Tapping-mode AFM images and surface profile of the damaged and repaired ZPN coatings corresponding to spots c and c' in Figure 4.

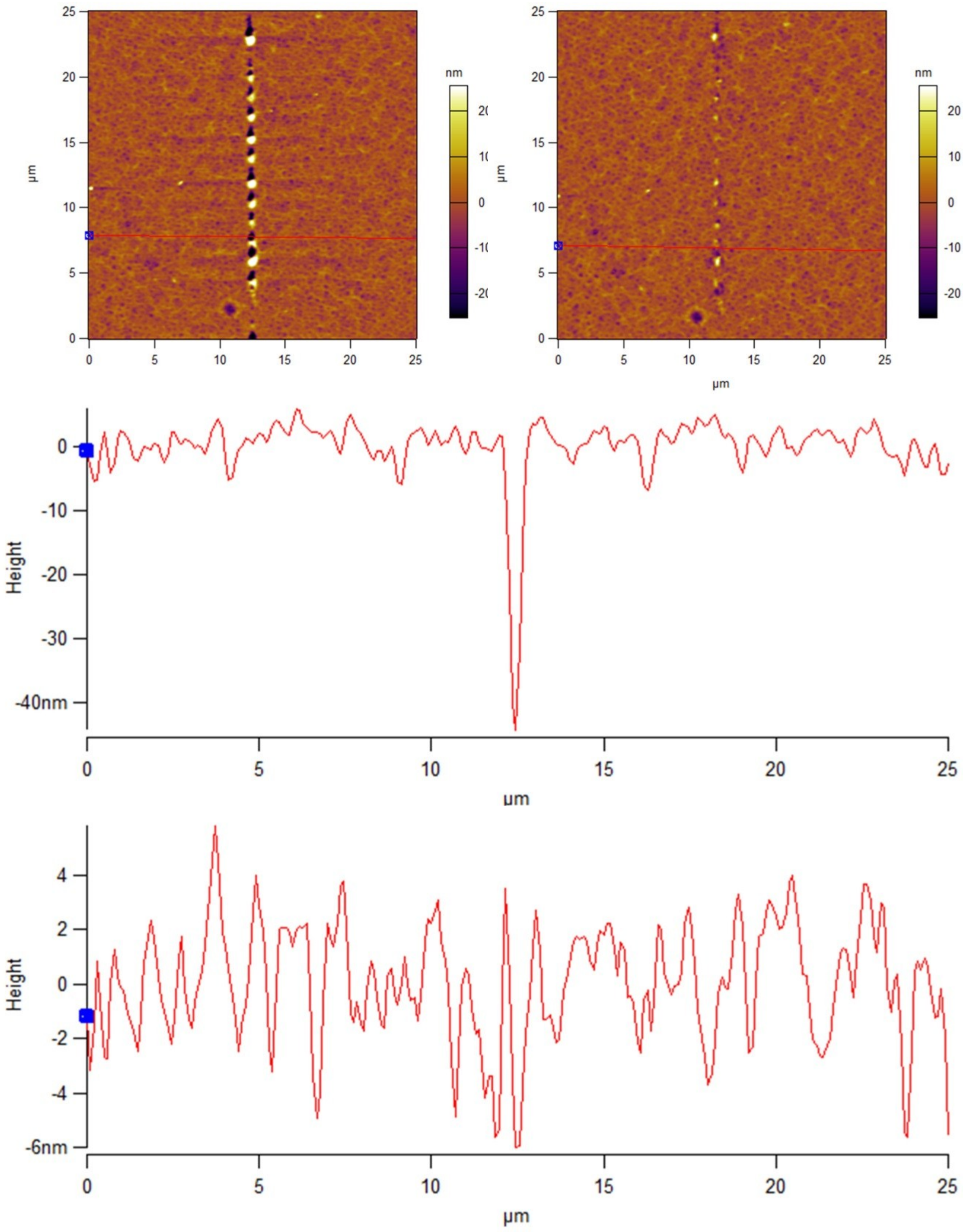


Figure S5. Tapping-mode AFM images and surface profile of the damaged and repaired ZPN coatings corresponding to spots d and d’ in Figure 4.

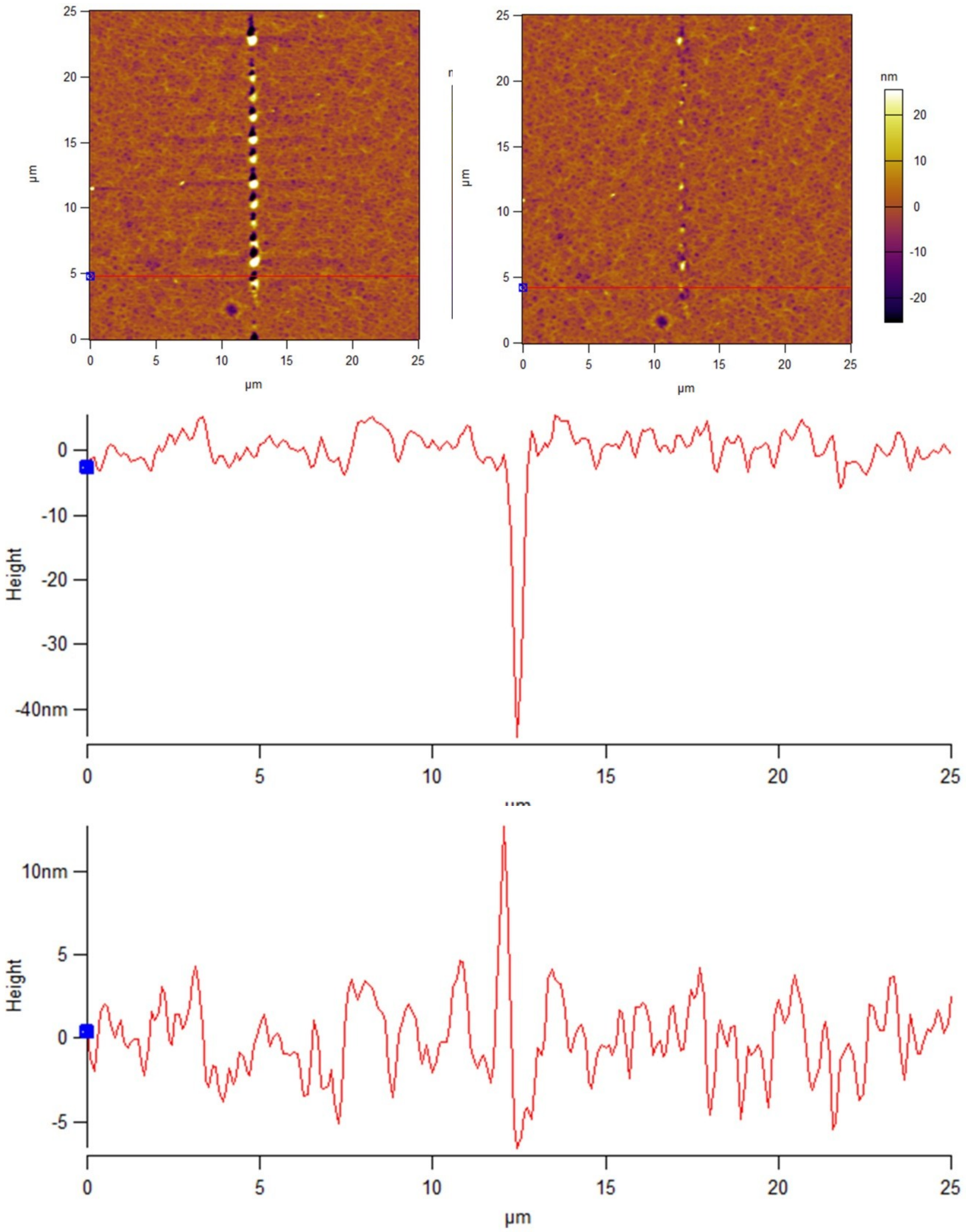


Figure S6. Tapping-mode AFM images and surface profile of the damaged and repaired ZPN coatings corresponding to spots e and e’ in Figure 4.

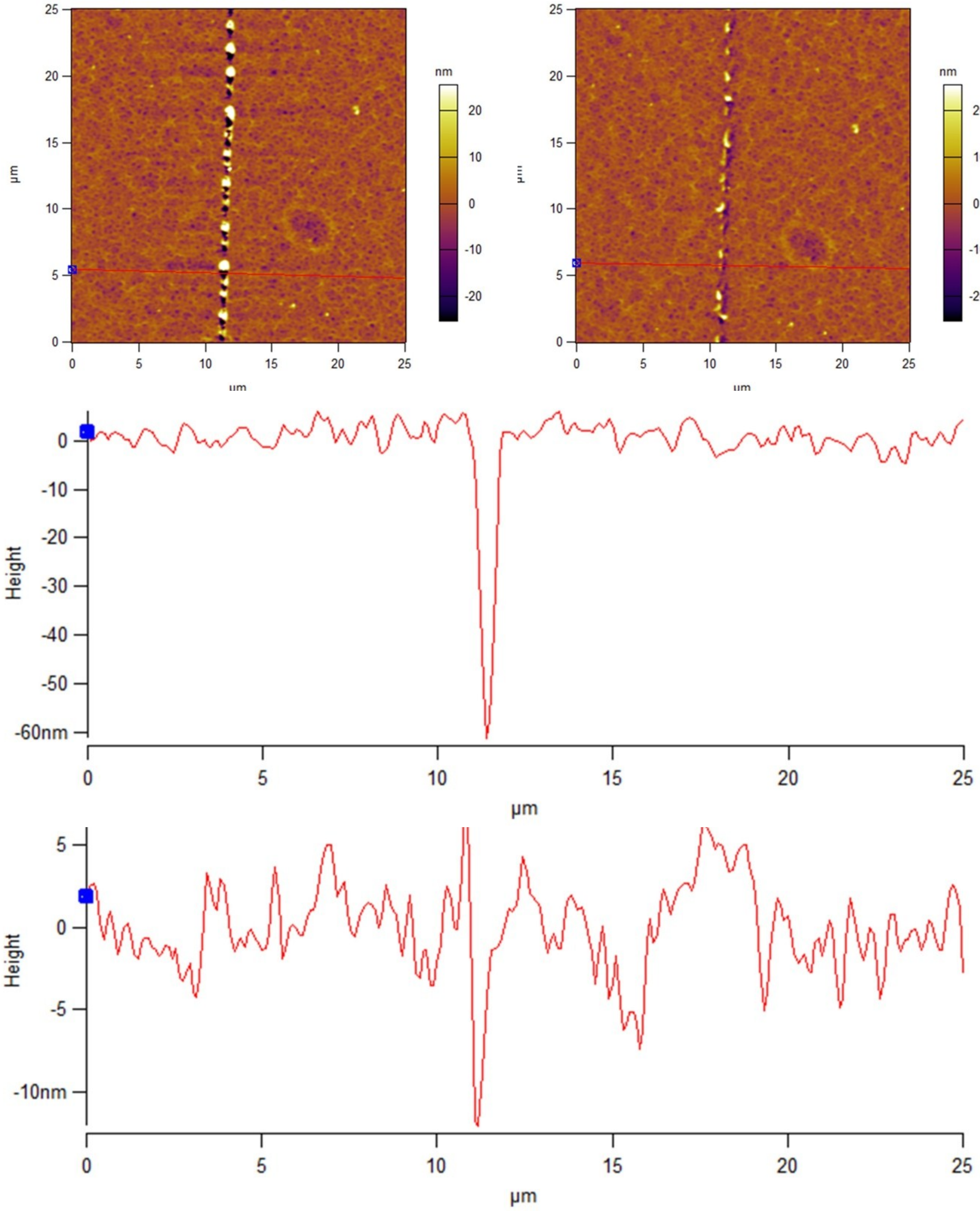


Figure S7. Tapping-mode AFM images and surface profile of an 80 nm ZPN film with a narrow (less than 0.5 micron), 60 nm deep scratch, after damage (to) and repair (bottom).

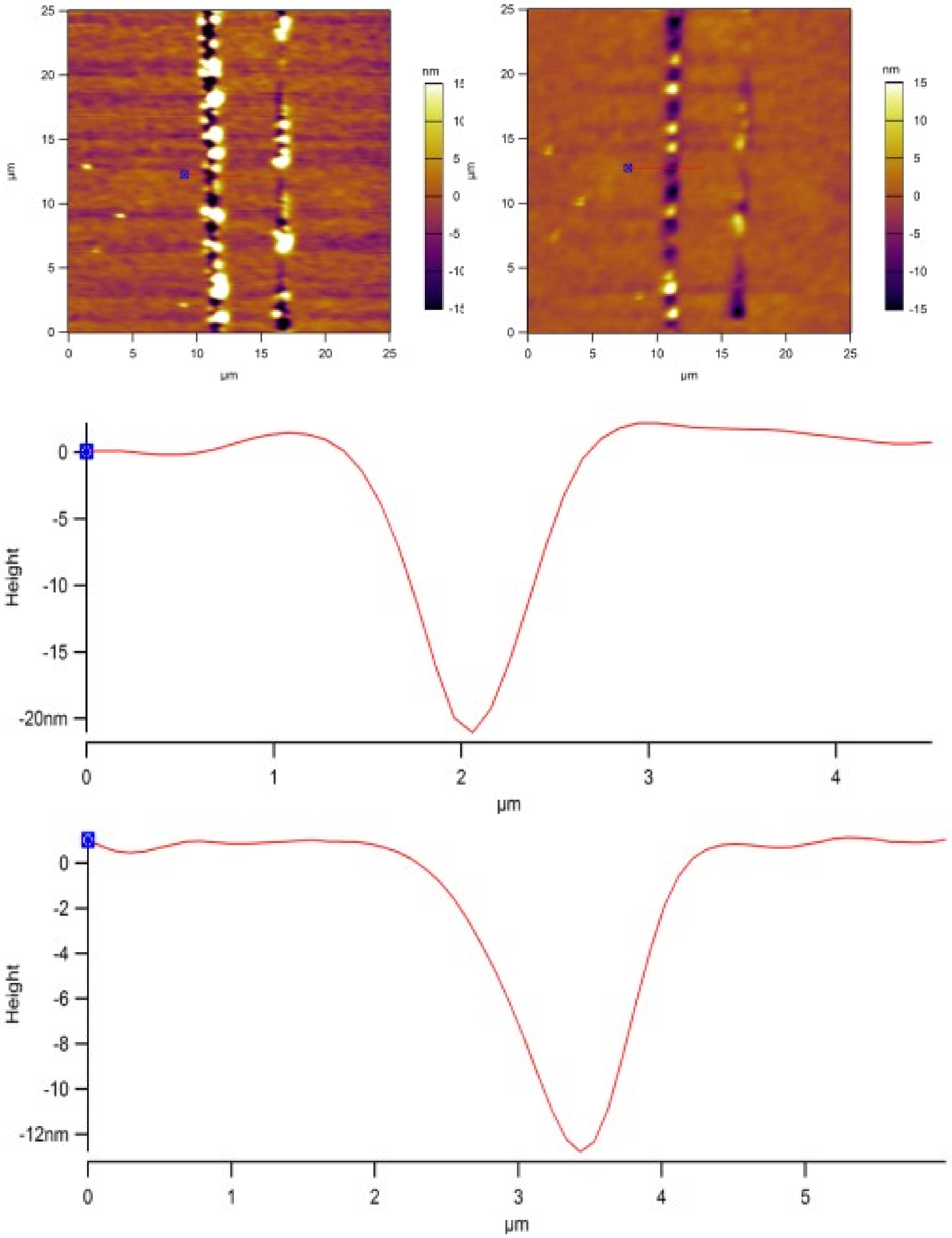


Figure S8. Tapping-mode AFM images and surface profile of an 80 nm ZPN film with a micrometer wide, 20 nm deep scratch, after damage (top) and repair (bottom).

References

[1] S. Lange., E. v. Andel., M. M. J. Smulders., H. Zuilhof, *Langmuir* **2016***, 32,* 10199.

[2] J. C. Dijt, M. A. C. Stuart, G. J. Fleer, *Adv. Coll. Interf. Sci.* **1994**, *50,* 79.

[3] N. Huber, D. Munz, C. Tsakmakis, *J. Mater. Res.* **1997**, *12,* 2459.

[4] A. T. Nguyen, J. Baggerman, J. M. J. Paulusse, C. J. M. van Rijn, H. Zuilhof, *Langmuir* **2011**, *27,* 2587.