

Application Note

Nano Chemical Characterization Uncovers Coating Issues for Monolayer Surface Functionalization

Background

Modifying surfaces to influence subsequent molecular interactions is a powerful technique. By adding functional groups that specific molecules can bind to, researchers can manage how antibodies, enzymes, DNA, bacteria, and viruses interact with a surface [1]. Commonly referred to as surface modification or functionalization, this technique finds applications in diverse industries, including biotechnology, tissue engineering, biosensors, and the semiconductor industry. The crucial assumption is that functional groups form a critical monolayer for experimental success, as the chemical state of the surface ultimately determines its interactions with other molecules. Therefore, unsuccessful functionalization or the presence of contaminants can introduce confounding variables, making it incredibly challenging to troubleshoot unexpected experimental outcomes.

Analytical techniques can be very valuable in ruling out surface modification of the substrate as the cause of any subsequent steps not going as planned.

Analytical Techniques for Monolayers

While understanding the state of a modified surface is critical, characterization at the nanoscale remains a challenge even with advanced microscopy and spectroscopy techniques. Ideally, we need a technique that is non-destructive, operable in ambient conditions, boasts high spatial resolution to detect monolayer gaps, and offers monolayer sensitivity. Popular techniques that are used for materials like this are either ToF-SIMS or XPS. However, ToF-SIMS is a destructive process, and XPS, while also surface sensitive, requires a cumbersome vacuum envi-

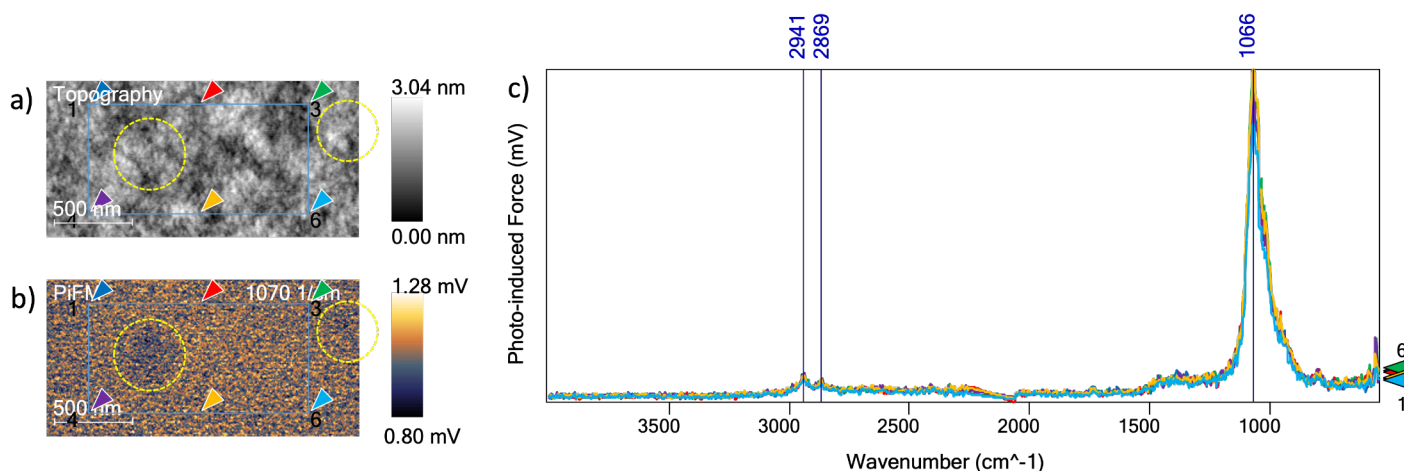


FIGURE 1: a) topographical results of the bare substrate show the physical features at a 2 μm x 1 μm scale. b) PiFM image at 1070 cm⁻¹, the chemical signature of glass. Spectra were taken at the locations indicated by the arrows. c) The six spectra acquired at the locations in the previous figure are shown in this graph.

ronment. Unfortunately, both techniques fall short in spatial resolution. Water contact angle, a common choice, provides a convenient success indicator, but its inability to identify specific surface molecules can lead to a false perception of success.

This is where IR-PiFM shines. PiFM's sub-5 nm spatial resolution and sub-monolayer sensitivity can precisely measuring the IR absorption of organic and inorganic surfaces allows it to excel in characterizing both unprocessed and functionalized surfaces [2]. Its effectiveness is further illustrated by two surface modification case studies, where IR-PiFM successfully confirms the presence of monolayers and shed light on the underlying surface interactions.

Case Study 1: Aminosilane Glass Functionalization

Objective:

A group working on using aminosilane to functionalize glass substrates contacted Molecular Vista to check the quality of their coating processes. This particular material appears to be quite popular in the industry as we have also worked with customers using similar substrates that are commercially available. The samples prepared by this customer were checked before and after functionalization using PiFM.

Results:

A $2\ \mu\text{m} \times 1\ \mu\text{m}$ (Figure 1A) topographical image of the bare glass substrate was acquired, and by using the chemical signature of glass ($1070\ \text{cm}^{-1}$), a PiFM image is generated, providing a map of the chemical composition across the

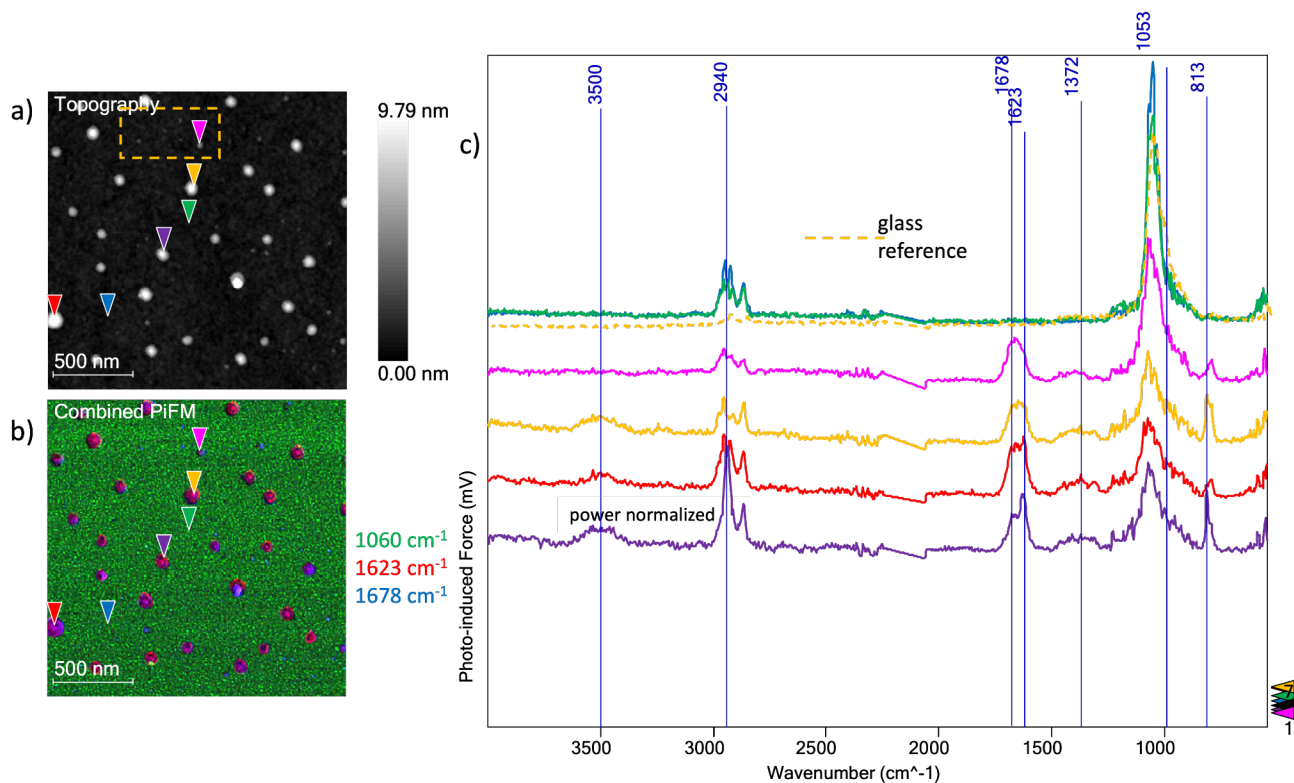


FIGURE 2: a) Aminosilane coated glass substrate topography. b) combined PiFM image. c) six spectra acquired on locations indicated by triangles in the images along with the averaged bare glass spectrum.

surface (Figure 1B). Six PiF-IR spectra were then acquired at specific points indicated in the images (Figures 1A,B). These spectra, representing the IR spectra of the molecular species at each location, offered invaluable insights into the true state of the supposedly bare surface. Notably, the spectra exhibited consistent repeatability (Figure 1C).

The topography image hints at height variations with longer spatial range, most of which are not represented in the PiFM image because the surface is chemically identical. Variations in the PiFM image (shown in yellow dashed circles) where the PiF signal is slightly lower, are most likely due to a slightly thicker intervening contaminant layer between the tip and the glass substrate. The small peaks at $\sim 2900\text{ cm}^{-1}$ (correlating to the C-H stretch modes at 2941 cm^{-1} and 2869 cm^{-1}) and $\sim 1400\text{ cm}^{-1}$ (correlating to the C-H bending modes) indicate that the entire surface is covered with an ultrathin layer of organic contaminant. In

this way, PiFM reveals the complexities beneath the apparent simplicity of "bare" materials.

Next, a glass substrate functionalized with amino silane was analyzed to compare to the first sample of bare glass. The results in Figure 2A, show the $2\text{ }\mu\text{m} \times 2\text{ }\mu\text{m}$ image of topography, which is the first step. Then, from the composite of three PiFM images at 1060 cm^{-1} (glass), 1623 cm^{-1} , and 1678 cm^{-1} , a combined PiFM image is generated (Figure 2B).

To round out the analysis, the six spectra from Figure 1C are averaged and displayed as a dashed spectrum in Figure 2C for comparison purposes, in addition to six PiF-IR spectra that were acquired at points indicated in the images on the aminosilane-coated glass substrate. A combination of the topography and PiFM image indicates the aminosilane molecules have aggregated into clusters instead of forming a monolayer assembly.

The topography in Figure 2A shows aggregates of various sizes on the surface. To understand what is on both the glass substrate and in these islands, six spectra were taken. Two spectra (dark blue and green) were captured on the bare substrate, away from the aggregates. These spectra show the main glass peak at $\sim 1060\text{ cm}^{-1}$, indicating that most of the surface is pure glass. We also see the previously identified contaminant peaks at $\sim 2900\text{ cm}^{-1}$ (C-H stretches).

The remaining four spectra, each corresponding to a specific aggregate (pink, red, gold, and purple in Figure 2B, C) show prominent peaks at $\sim 1623\text{ cm}^{-1}$ (NH_2 Scissoring), $\sim 1678\text{ cm}^{-1}$ (unknown), and a peak at 813 cm^{-1} , (NH_2 wagging mode). For the three taller aggregates, there also exists a broad peak at 3500 cm^{-1} (NH Stretch). The peak at 1623 cm^{-1} for these three is quite prominent compared to the shortest aggregate, the pink spectrum. A summary of these results is shown in Table 1.

The existence of these amine groups indicates that these are aminosilane aggregates. We took a closer look at these islands in a $600\text{ nm} \times 300\text{ nm}$ region shown in the dashed gold rectangle in Figure 2A, the result of which is seen in Figure 3.

Location	Spectrum Color	Key Peaks
Bare Substrate	Dark Blue, Green	1060 cm^{-1} (glass), 2900 cm^{-1} (contaminant)
		1623 cm^{-1} (NH_2 Scissoring), 1678 cm^{-1} (unknown), 813 cm^{-1} (NH_2 wagging mode)
Short Aggregate	Pink	1623 cm^{-1} (NH_2 Scissoring), 1678 cm^{-1} (unknown), 813 cm^{-1} (NH_2 wagging mode)
Tall Aggregates (Red, Gold, Purple)	Red, Gold, Purple	1623 cm^{-1} (NH_2 Scissoring), 1678 cm^{-1} (unknown), 813 cm^{-1} (NH_2 Wagging mode), 3500 cm^{-1} (NH stretch)

TABLE 1: Summary of Infrared Spectra Peaks for Different Locations on the Surface.

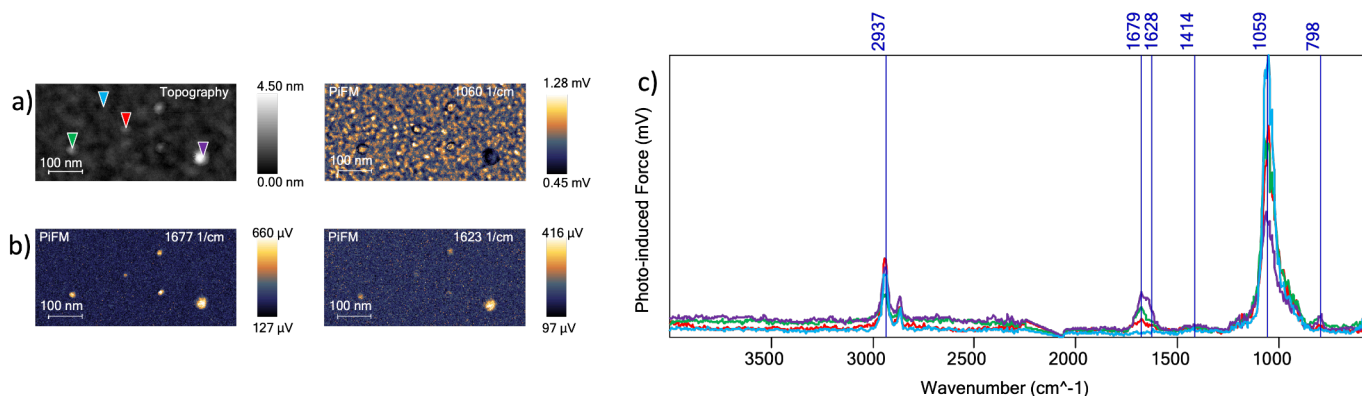


FIGURE 3: a) 600 nm x 300 nm region defined by the dashed golden rectangular box in Figure 2A, topography and PiFM. b) PiFM images at 1060 cm^{-1} , 1623 cm^{-1} , 1677 cm^{-1} . c) Four PiF-IR spectra acquired at the locations indicated by triangles in the topography image.

In the topographical image, Figure 3A, we can see several smaller clusters with heights ranging from ~ 0.7 nm to ~ 1.4 nm (excluding the aggregate from where the purple spectrum is acquired). These heights are better estimates of the cluster size, since the tip radius will dilate the lateral dimensions. Notably, we can clearly see the amine peaks even on these small clusters due to the high spatial resolution of PiFM; the red spectrum is acquired on

a cluster that is only ~ 0.7 nm thick, most likely a monolayer or a bilayer.

Intriguingly, on these thinner clusters, both the spectra and the image suggest a stronger peak at 1679 cm^{-1} compared to 1628 cm^{-1} . This might be due to the molecules arranging themselves in a more ordered, two-dimensional layer. This is valuable information because PiFM, due to its tip orientation, is more sensitive to out-of-plane vibrations, giving us clues about molecule align-

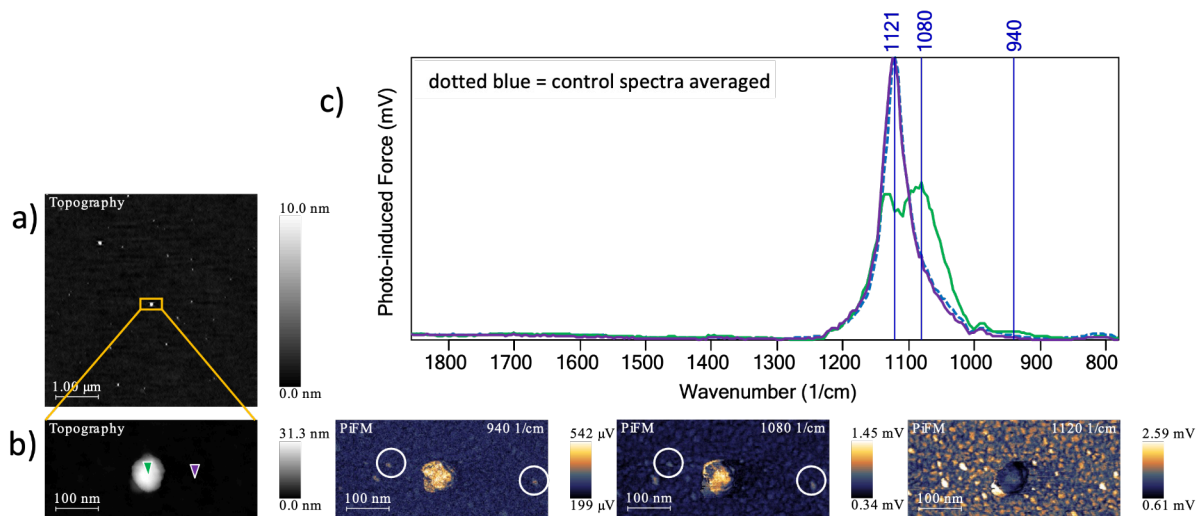


FIGURE 4: a) SiO_2/Si treated with an epoxide-terminated methoxy silane topography. b) PiFM image and topography of zoomed in section. c) PiF-IR spectra.

ment. You can read more about the orientation-dependent PiFM signal on crystalline samples on this application [note](#).

Moving away from the clusters, the light blue spectrum (Figure 2C) shows only the glass peak and contaminant peaks, with no aminosilane signatures. This confirms that most of the surface isn't covered by aminosilane. While this might be enough for some applications, achieving a more uniform monolayer could significantly improve the yield and reliability of final products. Identifying any gaps in this monolayer using a tool like PiFM could also aid in troubleshooting any subsequent processes that do not go as expected.

Case Study 2: Silicon Substrate Functionalization

Objective:

Our second example investigates the application of an epoxide-terminated methoxy silane on a silicon substrate with native oxide. Specifically, we wanted to assess the uniformity of this treatment using PiFM analysis.

Results:

Figure 4A shows a 5 μm x 5 μm topography that shows similar aggregates of molecules to the first case study. The green marker in Figure 4B shows the location where the green spectrum in Figure 4C was collected. This spectrum shows extra peaks at 1080 cm^{-1} and 940 cm^{-1} when compared to spectrum acquired on the substrate (purple spectrum). We attribute the peak at 1080 cm^{-1} and 940 cm^{-1} to the Si-O-Si and Si-OH vibrations, respectively [3]. The PiFM images at these wavenumbers highlight the aggregate prominently. Additionally, we can notice fainter aggregates (white circles), whose heights are ~ 0.5 nm thick (not shown), again a monolayer or bilayer aggregate, most likely.

Our observations are somewhat consistent with the model proposed by Naik et al., who suggest that silanes, instead of forming a uniform monolayer (Figure 5A), cluster into structures resembling snow moguls (Figure 5B).

These clusters are likely held together by Si-O-Si linkages, with only a few of the silane molecules actually attaching to the surface.

While Naik et al. attribute the cluster formation primarily to internal strain within the silane molecules caused by the mismatch of alkyl-chain van der Waals radius and Si-O-Si bond distance, we believe the density of -OH bonds in relation to the cleanliness of the oxide surface will also play a significant role.

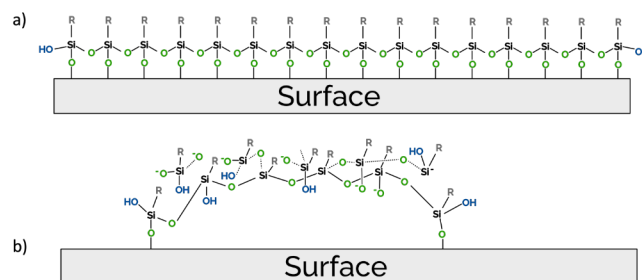


FIGURE 5. (a) Classical view of the silane coated silicon oxide surface. (b) Proposed new model of silane coated silicon oxide surface, adapted from Naik et al. [4]

Summary

Our studies reveal that both treatments, instead of forming the expected uniform monolayer, assemble into clusters across the substrate. This leaves significant portions of the surface bare, potentially impacting the performance of final products relying on interactions with these functional groups. Further investigation is necessary to understand the factors governing cluster formation and explore strategies for achieving a more uniform, potentially more reliable, surface modification, that can then be examined with PiFM.

References

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