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Nanoscale silicon surface-assisted laser desorption/ionization mass spectrometry: Environment stability and activation by simple vacuum oven desiccation

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Nanoscale silicon surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS) is an emerging matrix-free, highly sensitive MS analysis method. An important challenge in using nanoscale silicon SALDI-MS analysis is the aging and stability of the silicon after storage in various environments. No proper nanoscale silicon SALDI-MS activation procedure was reported to solve this issue. This study investigated the sensitivity, wettability, and surface oxidation behavior of nanoscale silicon surface SALDI-MS in a room, an inert gas atmosphere, and a vacuum environment. A simple vacuum oven desiccation was proposed to activate the SALDI-MS surface, and the limit of detection was further enhanced 1000 times to 500 attomole level using this approach. The long-term stability and desorption/ionization mechanism were also investigated.

Introduction

Advances in nanoscale silicon materials have been made in many fields for various applications including uses pertaining to energy, biosensors, and biological diagnostics.^{1–4} In mass spectrometry (MS) analysis, nanoscale silicon materials can be employed as surface-assisted laser desorption/ionization (SALDI) materials for high sensitivity, matrix-free MS analysis. Desorption/ionization on silicon (DIOS) is the first and one of the most well-known silicon-based SALDI technique that utilized porous silicon material as a MS target substrate.⁵ The silicon-based SALDI-MS method utilizes high surface-to-area ratio nanoscale porous silicon surfaces to replace the organic chemical matrix in matrix-assisted laser desorption/ionization (MALDI) MS analysis. Because the organic matrix is removed in the DIOS method, noise background issues present in MALDI-MS analyses are eliminated. However, it is only limit to analysis small molecules (<4,000 Da). Compare to other SALDI-MS technique, the DIOS method doesn't require further chemical surface modification for matrix-free and high sensitivity MS analysis. If DIOS substrate require better sample selectivity or sensitivity, the silicon-based surface can be further enhanced by tailoring with various surface functional groups for better functionality.⁶ Owing to the matrix-free and high sensitivity advantages of the DIOS method, various nanoscale silicon materials, such as nanowires,^{7, 8} nanopillars,⁹ nanocones,¹⁰

nanostructured silicon,^{11, 12} nanowells,¹³ and a nanopost array¹⁴ have been proposed. Other non-silicon materials, such as diamond nanowires,¹⁵ graphene¹⁶, porous aluminum,¹⁷ and carbon nanodots,¹⁸ have also been demonstrated to be effective highly sensitive matrix-free nanostructured surface assisted SALDI-MS substrates. These silicon-based SALDI-MS analysis methods demonstrate its application in protein characterization,^{19, 20} MS imaging,^{21–23} polymer analysis,²⁴ forensics,²⁵ and drugs of abuse.²⁶ A recent review paper has summarized the use of nanomaterials in SALDI-MS analysis.²⁷

As the nanoscale silicon material exhibits great potential and usefulness in MS analysis, Bruker Corp. and Waters Corp. have launched commercialized nanoscale silicon material products, NALDI[®] and MassPREP DIOS-target[™], for high sensitivity SALDI-MS applications. With the success and advancement of nanoscale silicon materials in MS, more and more studies have investigated the nanoscale silicon material desorption/ionization mechanism. In the early stages, it was realized that porous silicon acts a UV absorber to absorb the laser energy from the mass spectrometer and transfer the energy to the molecules for desorption/ionization.⁵ Recently, it was proved that the nanoscale silicon material mass spectrometry was driven by thermal and proton transfer mechanism for MS desorption/ionization.^{28–32} In particular, Alimpiev *et al.*³² reported extensive results exploring the silicon-based SALDI mechanism. The silicon substrate formed hole/free electron pairs through laser irradiation, and the holes became trapped at localized electronic states near the surface. The hole-trapping near the surface resulted in the increased acidity of surface Si–OH groups, which enabled proton transfer to the analyte. The protonated molecules desorbed from the surface by laser-induced thermal

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activation. With better understanding of the nanoscale silicon SALDI mechanism, different nanoscale silicon SALDI methods using a thin silicon-on-insulator (SOI) layer were also developed as an effective mode for SALDI-MS. As the nanometer-thin SiO₂ insulator layer can also effectively heat the surface by laser, and the Si–OH groups on the SOI provide protons for ionization, the 2D SOI layer can be used as a SALDI-MS substrate even without the 3D nanoscale structures on the surface.³³

One important issue in nanoscale silicon SALDI-MS analysis is the aging and stability of the silicon surface. Storage and stability issues for the use of nanoscale silicon SALDI have been reported but not investigated thoroughly. Finding a proper approach to activate the SALDI surface for enhanced MS detection sensitivity is also challenging. Storage of nanoscale silicon in a room environment for different periods of time was found to cause a decay in MS detection sensitivity. This was due to oxidation of the silicon surface, which created a shorter shelf life for commercial products or the incapability of obtaining the optimum MS detection sensitivity for lab use unless the silicon surface was “freshly-made.” The most common manner by which to activate the surface is double etching in hydrofluoric acid or utilizing buffered oxide etch to remove the oxide layer on the silicon surface.³⁴ This procedure recovers a portion of the MS detection sensitivity. However, the MS performance degrades compared to a fresh surface, and it necessitates a hazardous chemical etching step, which requires cleanroom facilities and specific training. Here, we report the nanoscale silicon SALDI surface stability for different storage conditions. Furthermore, we propose a simple, non-hazardous vacuum oven desiccation method to activate and further enhance the nanoscale silicon SALDI-MS. The detection limit of the nanoscale silicon SALDI surface can be enhanced 1000 times by using the vacuum oven desiccation method. The long-term stability and vacuum oven desiccation performance for up to 3 months are also presented.

Experimental

Materials and reagents

P-type <100> 6 inch single side polish silicon wafer (resistivity 1–100 Ω·cm) was purchased from Summit-Tech Resource Corp. (Hsinchu, Taiwan). Acetone (electronic grade), isopropyl alcohol (electronic grade), hydrofluoric acid (HF, electronic grade, 49%), and hydrogen peroxide (H₂O₂ electronic grade, 31%) were purchased from BASF Corp. (Ludwigshafen, Germany). Buffered oxide etch (BOE) and MS grade water were purchased from J.T. Baker (NJ, USA). Des-Arg⁹-Bradykinin (BK) and fluorescein sodium salt were purchased from Sigma-Aldrich (MO, USA). Hydrogen tetrachloroaurate(III) trihydrate (HAuCl₄, 99.99%) was purchased from Alfa Aesar.

Nanoscale silicon surface preparation

Nanoscale silicon surfaces were created by a metal-assisted etching process^{35,36} to create the nanostructured silicon (nSi) that was used as the nanoscale silicon substrate for SALDI-MS in this study. The silicon substrate was first cleaned with acetone, isopropyl alcohol, and a water bath in an ultrasonic cleaner for 5 min followed by N₂

blow-drying to clean the surface. The silicon substrate was then immersed in a HF/HAuCl₄ (0.01 M/2.4 M) solution for 30 s to deposit Au particles on the silicon surface by a galvanic displacement reaction. The silicon was removed from the HF/HAuCl₄ mixture and rinsed with methanol for 3 min to complete the Au deposition procedure. The Au-deposited silicon substrate was then immersed in a H₂O₂/EtOH/HF (1:1:1 v/v/v) solution for 2 min to create the nSi surface. The nSi surface was fully rinsed with methanol solution to remove the etchant out of the nanostructured surface in the last step of nSi fabrication process, which may have caused the solvent to be trapped in the nanostructure pores even with N₂ blow-drying right after etching. The top and cross-section FESEM nSi surface images are shown in Figure. The wire-like nSi nanostructures measured approximately 9 μm in height and 50–150 nm in diameter.

Figure 1. (a) Top (Magnification: 30K) and (b) cross-sectional (Magnification: 10K) FESEM images of nSi surface.

Contact angle measurement

The surface wettability was determined by measuring the water contact angle on the nSi surface. A 5-μL water droplet was pipetted onto the nSi surface, and the droplet side-view image was captured by a custom-made optical goniometer, which consisted of a high resolution digital camera (Canon EOS 450D/TAMRON Macro 90 mm F2.8 lens), a z-axis precision stage, and a light source. Then the images were processed using AutoCAD 2013 software to determine the contact angles.

Nanoscale silicon SALDI-MS analysis

A Bruker autoflex speed MALDI TOF/TOF mass spectrometer equipped with a 1 kHz 355 nm SmartbeamTM laser was used for the SALDI-MS analyses. A 5-μL sample was first pipetted onto the nSi surface and left for 2 min to allow sufficient sample absorption to the nSi surface; afterwards, the droplet was displaced from the nSi surface by N₂-blowing. The nSi surface was then mounted to a custom-made holder and loaded into the mass spectrometer for MS analysis conducted in positive-ion mode at 3×10^{-6} torr. The optimized laser energy was set to 32%. Like MALDI-MS, nSi-MS require search for the sweet spot for optimized performance and each MS spectrum in this study was averaged from 150 laser pulse shots. We defined a MS peak as indiscernible when the signal-to-noise (S/N) ratio was less than 3. It is noted that nSi-MS method is a very high sensitivity approach for small molecules. Some unpredicted surface contaminate peaks from the sample, containers, or during fabrication procedure may be observed in the low m/z region.

Buffer oxide etch (BOE) cleaning

BOE cleaning was used to remove the nSi surface oxidation. As nSi surfaces exhibit very high hydrophobic surface behavior, the BOE may not have fully rinsed the high aspect ratio nSi surface. We used O₂ plasma cleaner (PDC-32G, Harrick plasma) prior to BOE cleaning to treat the nSi surface (7.5×10^{-1} torr, 18 W) for 15 s to change the nSi surface contact angle from hydrophobic to hydrophilic ($\sim 5^\circ$) for good BOE penetration into the nanostructures for effective cleaning performance. The nSi surface was immersed in BOE solution for ~ 10 s to remove the nSi surface oxidation then rinsed with the BOE solution for 120 s to complete the BOE cleaning.

Results and discussion

Environmental effects on nanoscale silicon SALDI-MS desorption/ionization efficiency

The nanoscale silicon SALDI surface stability was first evaluated, and the nSi chip MS desorption/ionization efficiency was characterized by the MS signal intensity and S/N ratio of the BK (MW: 904) peptide sample. BK was selected as the standard test sample for MS analysis because it is the most commonly tested standard sample in most nanoscale silicon-based SALDI studies. Thus, the MS test data presented in this study can be more easily compared with other published investigational results. Detection of small molecule other than BK such as abused drugs²⁶ or various peptides³⁷ on nSi surface has been reported previously. The BK sample concentration used for nSi SALDI-MS was 10^{-6} M unless described elsewhere. Three storage conditions, a room, an inert gas (nitrogen) environment, and vacuum storage, were studied. Placement of nSi chips in a room (air) environment is the most common way to store the surface. In this study, we put the nSi surface on a petri dish expose to the room (air) environment as shown in Figure 2a. In addition to typical room storage, another method suggested storage in inert gas may reduce the oxygen and water vapor exposure that leads to silicon surface oxidation.³⁸ Therefore, we placed the nSi chip in a nitrogen-filled bag and fully sealed it using a conventional household food sealer as our nitrogen storage condition (Figure 2b). Finally, we also investigated vacuum storage for nSi (Figure 2c) where the nSi chip was placed in a vacuum box (PC-250, Yeong-shin, Taiwan) under 70-cm-Hg gauge pressure.

Figure 2. nSi surface stored in (a) room (b) nitrogen and (c) vacuum environment. It is noted average annual temperature and humidity level in Taoyuan, Taiwan is 23 °C and 78.4% (data from central weather bureau, <http://www.cwb.gov.tw>).

Figure 2a~c shows the MS signal intensity and S/N ratio of nSi surface storage in a room environment from 1 week to 1

month. Results showed that the MS peak of BK decayed with increased storage time. Similar to the other reports regarding surface oxidation, the MS signal intensity decayed with room (air) storage. Low desorption/ionization efficiency with a signal intensity of 35.3 and S/N of 3.8 were observed in the first 3 weeks; the nSi surface could not be used for SALDI-MS analysis when it was subjected to a room (air) environment for more than 1 month. The BK MS peak was indiscernible for the 1 month condition (intensity: 7 and S/N: 1.7). Surface oxidation has been widely accepted as the major reason for the nSi desorption/ionization efficiency decay.³⁴ In Figure 2~f, the dashed blue line shows the O1s electron intensity measured by ESCA. The O1s electron intensity increased with air storage time, which indicates the extent of the surface oxidation increase on the nSi surface. The surface oxide saturation was approximately 23500 counts/s after 3 weeks. This finding agrees with previous observations that the surface oxidation presented negative effects on the MS desorption/ionization efficiency. Based on the nanoscale silicon SALDI desorption/ionization mechanism, it is known that the Si–OH groups on the silicon surface promotes proton transfer to the analyte. Thus, other factors caused by surface oxidation may play an important role in affecting the MS desorption/ionization efficiency. The distribution of the analyte inside the nanoscale structure is determined by the wettability of the surface. From the surface wettability results, the water contact angle decreased from $127.3 \pm 1.2^\circ$ to $13.3 \pm 3.5^\circ$ from fresh nSi to 1-month room (air) environment storage as shown in Figure 2, which was mainly caused by surface oxidation. The oxidized nSi silicon surface became more hydrophilic, thereby resulting in droplet spreading on the nSi surface. The sample droplet spreading on the nSi surface lowered the local concentration resulting in desorption/ionization efficiency decay during nSi SALDI-MS analysis. This can be proved by the high correlations between the MS signal intensity (Figure 3a) and surface contact angle (Figure 3d, solid line).

Nitrogen (Figures 3b) and vacuum storage (Figures 3c) conditions showed better nSi SALDI-MS desorption/ionization efficiency and stability than the room (air) storage condition. As storage in nitrogen gas or vacuum reduced the surface oxidation and water moisture absorption on the silicon surface, the extent of silicon surface oxidation was reduced. The surface oxidation linearly increased with the storage time but did not reach the saturation value of 23500 counts/s within a month. Moreover, owing to the relatively dry environment in nitrogen gas and vacuum storage, the nSi surface remained highly hydrophobic with a water contact angle above 120° (Figures 3e, 3f solid line). As result, the nSi-MS signal intensity did not decrease with storage time. On the contrary, the desorption/ionization was enhanced with increased storage time in some conditions.

Nanoscale silicon surface SALDI-MS activation and enhancement by vacuum oven desiccation

Figure 3. MS signal intensity and S/N ratio (a,b,c) and water contact angle and O1s electron intensity (d,e,f) under room/air, inert gas/nitrogen gas, and vacuum storage conditions. The error bars in the figure represent the standard deviations obtained from three different measurements. With each measurement averaged from 200 MS scans.

Based on the abovementioned results, we found that the MS signal intensity was highly correlated with the nSi surface wettability, and nSi surface oxidation exhibited less pronounced nSi-MS dependence in MS detection sensitivity. For the room (air) storage condition, because water moisture in the room environment tended to trap water molecules inside the nSi nanopores by capillary condensation, the nSi surface became relatively wet resulting in water contact angle reduction with increased storage time. For the “dry” storage methods, nitrogen and vacuum storage conditions, owing to the dry silicon nanostructure surfaces as well as the Lotus effects from the silicon nanostructure morphology, the nSi maintained its high surface hydrophobicity of around 125°. As laser-induced surface heating in addition to proton transfer from surface Si–OH groups are the major factors for sample desorption/ionization in nSi SALDI-MS, we found that the MS signal intensity was enhanced under nitrogen and vacuum storage conditions with increased surface oxidation while maintaining its surface wettability. Air moisture and solvent removal from silicon nanostructures is important for good SALDI-MS sensitivity. The water moisture and solvent trapping inside the nSi surface may have not only reduced the nSi surface hydrophobicity, thus lowering the sample local concentration during MS analysis, but also reduced the nSi-MS thermal absorption efficiency (and the silicon-based SALDI-MS desorption/ionization mechanism is mainly driven by thermal processes). Therefore, in both the nitrogen and vacuum storage conditions, the dry nitrogen or vacuum environment aided in aqueous solution removal from the nSi surface; the enriched Si–OH groups provided more proton sources for

sample ionization. These results showed an overall nSi SALDI-MS signal enhancement after nitrogen and vacuum storage.

We proposed a simple method using a vacuum oven to desiccate the nSi surface. We postulated that the nSi-MS signal intensity can be enhanced by aqueous solution removal from the silicon nanostructure pores inside the nSi surface to increase the surface wettability and also enhance nSi thermal absorption capability during MS analysis. The vacuum oven desiccation was performed by putting the nSi chip inside the conventional vacuum oven (OV-27, Paymo Inc., Taiwan), heating, and vacuuming under 70-cm-Hg for 10 hours. Figure shows the nSi-MS signal intensity of nSi chip storage in a room (air) environment for 1 month (control) and the nSi-MS spectrum after 10-h vacuum oven heating at 35, 85, and 130 °C. The nSi-MS control experiment exhibited a very weak MS signal (53.67 a.u.). Under the 35 and 85 °C vacuum oven desiccation, the nSi-MS signal intensity enhanced to 252 and 720 a.u., respectively, because the elevated temperature in the vacuum oven promoted water/solvent removal from the nanostructured silicon surface. At the vacuum oven treatment condition of 135 °C, the MS signal was enhanced more efficiently to 2465.67 a.u., an approximate 46-fold MS signal enhancement compared to the control experiment. This dramatic MS signal enhancement was presumably because of the heating temperature being elevated above the water boiling temperature of 100 °C causing the water trapped inside the nanostructure pores to evaporate more effectively than other cases. The blue line in Figure 4 shows the increase in nSi surface hydrophobicity under the vacuum oven desiccation. The nSi surface shows hydrophilic behavior 13.3° at 1 month room storage environment (control). The nSi surface

hydrophobicity dramatically increase to 99° and 128° at 85, and 130 °C vacuum heating conditions.

The vacuum oven desiccation proved to be a simple activation method for nSi SALDI-MS. The conventional approach to recover nanoscale silicon SALDI-MS sensitivity is by removing the surface oxidant in a hydrofluoric acid or BOE solution. We evaluated the MS intensity, surface wettability, and O1s intensity of the nSi surface after BOE and compared it with vacuum oven desiccation for the three different storage conditions. The highest (compared to the other cases) O1s intensity of 23355.1 counts/s., shown as the blue-dash line in Figure 5a, resulted from the nSi surface under air storage being exposed to the most oxygen and water moisture. With BOE cleaning, the surface oxidant was effectively removed by the oxide etchant, and the O1s intensity was reduced by approximately 6000 for all three storage conditions. Comparing the vacuum oven desiccation process to BOE cleaning, the O1s intensity don't decrease under vacuum oven desiccation as there is no oxidant removing mechanism in the vacuum oven desiccation procedure.

Figure 4. nSi surface wettability and SALDI-MS intensity for 1 month air storage nSi surface under vacuum oven desiccation at 35°, 85° and 130°C. The error bars in the figure represent the standard deviations obtained from three different measurements.

For the nSi SALDI-MS sensitivity test shown in Figure 5, the MS signal intensity from the 3-week air storage chip (Figure 5a) increased from 358.67 to 1398.30 a.u. after BOE cleaning. However, for the nitrogen (Figure 5b) and vacuum (Figure 5c) storage conditions, the MS detection sensitivity decreased to 1941 and 1503 a.u., respectively. One of the reasons for the MS decay may be the change in surface wettability. Though both nitrogen- and vacuum-stored silicon surface maintained high hydrophobicity within the storage conditions, the surface contact angle decreased to approximately 80° after BOE etching. However, vacuum oven desiccation effectively removed moisture and enhanced surface wettability as well as

MS sensitivity, as shown in Figure 4. The MS detection sensitivity was enhanced approximately 14 times for the air storage condition. Even for the “dry” storage conditions, nitrogen (Figure 5b) and vacuum storage (Figure 5c), the MS detection sensitivity was also enhanced 1.5 and 1.9 times, respectively, because of the high surface wettability and thermal efficiency for nSi SALDI-MS desorption/ionization. It is important to note that all of the nSi MS detection was enhanced over the “fresh” native nSi chip (double dash green line in Figure 5) after the caused a decay in the nSi-MS sensitivity.

Figure 5. nSi SALDI-MS signal intensity, contact angle (red line) and O1s intensity (blue dash line) for BOE cleaning and vacuum oven desiccation for nSi chips storage in (a) air/room, (b) nitrogen, and (c) vacuum environment. The vacuum oven desiccation process condition is 10 hr vacuum oven heating at 130°C. The error bars in the figure represent the standard deviations obtained from three different measurements.

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The nSi-MS detection sensitivity enhancement after vacuum oven desiccation is a multiphysics phenomenon. We have investigate the silicon nanostructure pore size, height and morphology effect to the MS efficiency in our previous investigation.¹² Results shows that the nanoscale silicon structure surface morphology is a critical factor for good SALDI-MS detection sensitivity. Higher surface-to-area ratio or rougher silicon surface nanostructures enable more surface sample absorption for an enhanced detection sensitivity. Figure 6a and b shows the FESEM images of a fresh nSi surface before and after vacuum oven desiccation. The nSi pore size, height, and surface morphology remained unchanged after the vacuum oven treatment. Therefore, the vacuum oven desiccation will not affect the nanoscale silicon surface morphology and change the nSi-MS sensitivity due to morphology variations. The nSi chip storage (Figure 3) and the vacuum oven treatment (Figure 5) experiment results also showed that the nSi surface hydrophobicity is an important indicator of nSi-MS sensitivity. High surface hydrophobicity was found to effectively increase the local concentration on the SALDI-MS substrate and promote detection sensitivity, which is one approach for enhancing MALDI-MS sensitivity—modification of the target substrate.^{39, 40} Hydrophobicity increase after vacuum oven desiccation not only reduced the local concentration for MS detection but also changed the sample absorption on the surface. Sample absorption was measured using fluorescent absorption tests. We found that the sample was uniformly absorbed on the fresh nSi surface as shown in Figure 6c. Under the same nanostructure silicon morphology, the “dried” nSi surface after vacuum oven treatments tended to form <10 μm size highly-concentrated focusing dots on the nSi surface, as shown in Figure 6d. These concentrated sample dots on the nSi surface were a highly concentrated spot for LDI-MS laser shots and therefore resulted in an enhanced MS detection sensitivity.

Figure 6. FESEM nanoscale silicon surface morphology (a) before and (b) after vacuum oven desiccation. Fluorescent absorption test (Fluorescein sodium salt, 10^{-2} M) (c) before and (d) after vacuum oven desiccation.

Figure 7. nSi SALDI-MS spectrum for fresh nSi and vacuum oven desiccated nSi surface with 5 μL 10^{-7} to 10^{-10} M BK sample.

Limit of detection and long-term stability tests

As the vacuum oven desiccation process can effectively enhance the nSi MS sensitivity, the MS limit of detection (LOD) can also be further improved for better nanoscale silicon surface SALDI-MS analysis. Figure 7 shows the SALDI-MS spectrum of a 10^{-7} – 10^{-10} M BK sample on both a fresh nSi chip and a nSi chip after vacuum oven desiccation. The contact angle of native nSi surface is measured $127.3 \pm 1.2^\circ$. After vacuum oven desiccation, the surface hydrophobicity increase to $133.0 \pm 1.1^\circ$. The MS results in Figure 7 demonstrated that the fresh nSi chip detected the BK sample at 10^{-7} M with no discernible peak observed under 10^{-7} M. With the additional vacuum oven desiccation procedure, the LOD of the nSi surface enhanced from 10^{-7} to 10^{-10} M, which showed a 1000-fold LOD increment for the nSi surface. Highest detection sensitivity of 500 attomole ($5 \mu\text{L}$, 10^{-10} M) was found using vacuum oven desiccation method. The LOD enhancement was due to the vacuum oven desiccation process not only enhance the nSi surface hydrophobicity for increase local sample concentration, but also form highly concentrated sample spots (Figure 6d) to enhance the nSi SALDI-MS detection sensitivity as well as LOD.

Figure 8. nSi SALDI-MS signal intensity and surface contact angle for nSi surface stored in air (a,d), nitrogen (b,e) and vacuum (c,f) environment from fresh nSi (0 day) to 3 months storage. The error bars in the figure represent the standard deviations obtained from three different measurements.

Long-term storage is an important issue as freshly making nSi surfaces is not a convenient approach for research lab use or commercialized products. Figure 8 shows the native nSi surface MS sensitivity and contact angle under air (Figure 8a and 8d, black line), nitrogen (Figure 8b and 8e, black line), and vacuum storage (Figure 8c and 8f, black line) conditions from fresh native surfaces to 3-month storage. Under the room (air) storage condition, the MS sensitivity decayed with time approaching 0 a.u. within 3 months. The nSi SALDI-MS lose the SALDI-MS performance presumably caused by couple important factors. It may due to the hydrophobicity decay with increasing time (Figure 8d) lowering the local sample concentration. Other reasons may due to the excessive surface contaminations when it storage in air (as shown in Figure 9) or the nanostructured silicon surface oxidation. With the vacuum oven desiccation for the nSi chip storage in room (air) environment, the nSi surface maintained high hydrophobicity around 130°, and the MS sensitivity was enhanced 4–30 times from 1 week to 3 months under air storage condition. nSi chip storage in inert gas (nitrogen) and vacuum provided better long-term storage stability compared with room (air) storage. The surface maintained its hydrophobic behavior with long-term storage for both cases. The MS detection sensitivity also exhibited equivalent or better signal intensity than the fresh nSi surface (Figure 8, green dash time). With the vacuum oven desiccation process, the MS signal intensity can be further improved to a 6000–7000 a.u. signal intensity level.

Nitrogen and vacuum storage provided good nSi long-term stability of the surface hydrophobicity and MS signal intensity. Moreover, those storage conditions also preserved the nSi surface from contamination. Figure 9 shows the MS spectrum for nSi surface storage under air, nitrogen, and vacuum conditions for 1 month. As the nSi surface was exposed to the air without sealing, the possibility of contaminating the surface and reducing the sensitivity increased. Numerous surface contaminant peaks were found in the MS spectrum below m/z 500. This surface contamination issue may also relate to the

Figure 9. Noise backgrounds for nSi surface storage in air, nitrogen, and vacuum environment for 1 month.

MS detection sensitivity decrease under the air storage condition. Under nitrogen storage, fewer surface contaminant peaks were found in the spectrum; these peaks may have come from the nitrogen gas or the package bags. For vacuum storage, very few contaminant peaks were found except for the BK signal peak at m/z 904.

Conclusion

Storage and environmental stability is an important consideration for the use of nanoscale silicon SALDI-MS. We investigated the nSi SALDI-MS detection sensitivity, surface wettability, and oxygen levels on the nSi surface under room (air), inert gas (nitrogen), and vacuum conditions. From the analyses, we found that the SALDI-MS signal intensity was closely related to the surface wettability for all of the storage conditions. Nitrogen gas and vacuum storages provided better MS sensitivity and environmental stability for preservation of the nanoscale silicon SALDI-MS surface, which inhibited nSi surface oxidation and maintained the high surface wettability for better MS desorption/ionization efficiency.

A vacuum oven desiccation process was developed to activate the SALDI-MS surface. This process was a low-cost, simple, and

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hazardous reagent-free method to activate and further enhance SADLI-MS detection sensitivity. The LOD was improved up to 1000 times after the vacuum oven desiccation method, and its mechanism was explained by the local concentration model. The vacuum oven desiccated surfaces were found to have good SADLI-MS sensitivity for up to 3 months.

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