Nanomechanical Sampling of Material for Nanoscale Mass Spectrometry Chemical Analysis

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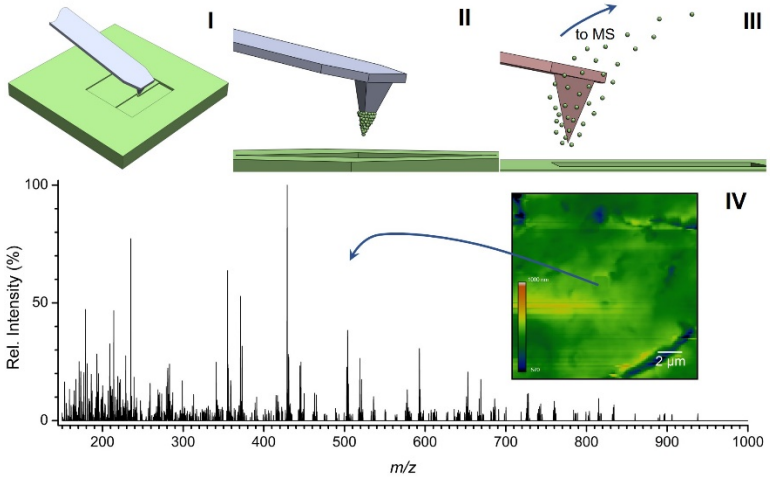
TOC FIGURE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ABSTRACT

The ability to spatially resolve the chemical distribution of compounds on a surface is important in many applications ranging from biological to material science. To this extent we have recently introduced a hybrid atomic force microscopy (AFM) - mass spectrometry (MS) system for direct thermal desorption and pyrolysis of material with nanoscale chemical resolution. However, spatially resolved direct surface heating using local thermal desorption becomes challenging on material surfaces with low melting points, because the material will undergo a melting phase transition due to heat dissipation prior to onset of thermal desorption. Therefore, we developed an approach using mechanical sampling and collection of surface materials on an AFM cantilever probe tip for real time analysis directly from the AFM tip. This approach allows for material to be concentrated directly onto the probe for subsequent MS analysis. We evaluate the performance metrics of the technique and demonstrate localized MS sampling from a candelilla wax matrix containing uv-stabilizers avobenzone and oxinoxate from areas down to 250 nm x 250 nm. Overall, this approach removes heat dissipation into the bulk material allowing for a faster desorption and concentration of the gas phase analyte from a single heating pulse enabling higher signal levels from a given amount of material in a single sampling spot.

Keywords: mechanical sampling, thermal desorption, nanometer scale, atmospheric pressure, atomic force microscopy, mass spectrometry, atmospheric pressure chemical ionization

INTRODUCTION

The structure-function relationship in systems ranging from additives in adhesives and soft materials to cellular function in biological systems are often defined by chemical segregation that occurs at the nanoscale. However, chemical composition in these materials is routinely studied using bulk analysis approaches like liquid and gas chromatography (LC and GC) coupled with mass spectrometry and optical spectroscopy that average the chemical information over the whole sample losing valuable information about chemical localization and local structure that can have profound effects on material functionality ranging from material elasticity to photovoltaic performance. Therefore, a lot of effort has been dedicated to developing spatially resolved techniques that allow to correlate physical and functional material properties with chemically sensitive approaches like infrared (IR), Raman and time-resolved optical spectroscopy, fluorescence and mass spectrometry.[1] However, spatially resolved mass spectrometry based chemical imaging techniques that provide localized chemical mapping, such as matrix assisted laser desorption ionization (MALDI)[2] and secondary ion mass spectrometry (SIMS)[3], are usually performed in vacuum environments requiring either complicated sample preparation or preclude certain volatile species from being analyzed all together. To circumvent the need to place a sample in a vacuum environment for spatially resolved chemical imaging, attention has focused on developing ambient mass spectrometry imaging approaches.[4] With this in mind we have demonstrated the use of proximal probe thermal desorption/ionization combined with mass spectrometry (TD/I-MS) for spatially resolved chemical profiling and imaging of surfaces under ambient conditions.[5-10] This surface sampling/ionization and analysis approach uses a heated probe tip placed in close proximity to or in contact with a surface

to locally desorb intact molecular species or pyrolysis products from a surface that are then ionized by an atmospheric pressure ionization source like electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) and analyzed using MS.

To improve on the spatial resolution of the sampling technique to the nanoscale and allow for corelated structure-chemical functionality analysis we employed the use of heated atomic force microscope (AFM) probes on a coupled AFM-MS platfrom.[6-8, 10] These works build on that of Reading and coworkers[11-15] where they used 5 μm tip diameter Wollaston wire heated probes to perform both point thermal desorption and pyrolysis, capturing the liberated vapor material then injecting it into a gas-chromatograph mass spectrometer (GC/MS) for separation, electron ionization (EI), and mass analysis from desorption craters approximately 6 μm in diameter and 1.7 μm deep. Using much smaller, ~30 nm diameter AFM probes we were able to demonstrate direct sampling on the AFM-MS systems from desorption craters 250 nm in diameter and 100 nm deep from caffeine and yellow-ink thin-films where the mass spectral signal from each desorption event recorded in real time.[10, 7] Most recently we demonstrated the ability to obtain co-registered mass spectral chemical images with AFM topographical as well as mechanical images from inked patterns on paper,[6] living bacterial colonies on an agar gel,[6] phase separated polymers,[8] additives in adhesives and active ingredients in personal care products directly on hair[16].

However, when performing direct thermal desorption or pyrolysis by the heated probe from certain material types it can be difficult to limit the sampling volume thus limiting the spatial resolution achievable in either a chemical profiling or imaging mode. This is because material surfaces with low melting points will undergo a melting phase

transition due to heat dissipation prior to the onset of thermal desorption.[17] This physical dissipation of heat into the material is a relatively slow process occurring on the time scale of milliseconds causing a much larger area than the diameter of tip to become damaged by the heating process. We have demonstrated that through the use of rapid tailored heating pulses to an AFM cantilever it is possible have a 381x improvement in the desorption efficiency and an 8x improvement in lateral spatial resolution compared to the conventional heating approaches on material surfaces like printed inks that undergo a melting transition prior to desorption.[10] An alternative approach to direct heating is to mechanically sample the surface with the AFM probe tip, remove the tip from close proximity of the surface, and subsequently thermal desorb material from the tip for mass spectral analysis. This enables very small areas of the surface to be analyzed, minimizing damage of the surface and maximizing the localization of chemical properties.

Mechanically, thermally and laser assisted nanosampling with scanning probes for subsequent chemical analysis on the probe tip or elsewhere by means of various optical spectroscopy and mass spectrometry approaches has been shown as successful route towards multimodal chemical imaging.[12, 18, 19] [20, 21] However, all pervious MS approaches required to mechanically transfer material from the surface of the AFM tip to a separate and subsequent mass spectrometry analysis platform before revealing the material that was captured on the probe.

Herein, we report on mechanical sampling and collection of surface materials on an AFM probe tip for real time analysis directly from the tip. This approach allows for material to be concentrated directly onto the probe from areas down to 250 nm x 250 nm enabling sampling of waxy and volatile materials that would not be amenable to direct

thermal desorption. We demonstrate the effect of force to maximize on sample collection. Additionally, this approach allows heat to be transferred faster into the sampled volume by minimizing on heat dissipation into the bulk material allowing for a faster desorption and concentration of the gas phase analyte from a single heating pulse enabling higher signal levels from a given amount of material in a single sampling spot.

MATERIALS AND METHODS

Chemicals

Candelilla wax matrix containing uv-stabilizers avobenzone 3% and oxinoxate 7.5% was purchased locally in the form of ChapStick® Lip Balm and was applied directly to a glass cover slide mounted on an AFM sample puck. A thin-film on the lip balm was created by wiping away excess wax with a Kimwipe. Neat avobenzone was purchased from Sigma Aldrich (St. Loui, MO) and headspace sampling of volatile vapor was performed to obtain the MS/MS spectra of the avobenzone standard. Healthy mouse kidneys were obtained from Maastricht University in accordance with protocols approved by the Animal Care and Use Committee under Animal Experiment Committee (DEC) number 2016-006 AVD107002016720. 12-µm thick sections were prepared using a cryo-microtome (Leica, Nussloch, Germany) at –20 °C and thaw-mounted on ITO-coated glass slides. Sample were stored at -80C until shipment to the US on dry ice.

Experimental Setup

The AFM-MS experimental setup has been described in detail previously.[8, 10, 16] Here we used both a Veeco Multimode AFM (Bruker AXS, Santa Barbara, CA) equipped with a closed loop N-Point stage (N-Point, Madison, WI) and a Nanonis system

controller (SPECS Zurich GmbH, Zurich, Switzerland) for restive thermal desorption coupled to Thermo Fisher Scientific LTQ XL (Thermo Fisher Scientific, Bremen, Germany), as well as Asylum Research Cypher ES (Asylum Research, Goleta, CA) with Blue drive for photothermal desorption coupled with a Thermo Fisher Scientific Orbitrap Velo Pro (Thermo Fisher Scientific, Bremen, Germany) were used to obtain the nanometer scale topographical images and to control the nanoscale thermal desorption spot sampling. Material was thermally desorbed from the surface using VITA-MM-NANOTA-300 nano-thermal analysis (TA) AFM probes (Bruker AXS, Camarillo, CA) as well as NanoSensors PtSi-FM-SPL (NanoWorld, Neuchâtel, Switzerland) AFM probes. Temperature calibration of the AFM probes was carried out using a voltage ramp across standard polymeric samples polycarbolactone (PCL), high-density polyethylene (HDPE), and poly(ethylene terephthalate) (PET)) with known melting temperatures[22, 23] by calibrating the dynamic transfer function, a technique first introduced by Lee *et al.[24]*

Desorbed and ionized species were monitored with full scan mass spectra and MS/MS product ion spectra (avobenzone transition *m/z* 311 → ○, normalized collision energy 35%). For TD measurements over an array of points, the AFM was controlled using custom software that allowed for independent variation of the force-feedback set-point of the cantilever and the position of the tip across the sample surface. The mass spectrometer and the AFM where set-up with the AFM serving as the master controller for the timing sequences.

RESULTS

Materials like waxes and tissue samples that have large temperature differences between material damage and thermal desorption. As seen in Supplemental Figure S1 when

sampling directly using an AFM probe for thermal desorption from a candelilla wax matrix containing uv-stabilizers avobenzone 3% and oxinoxate 7.5%, the affected melt area is on the order of 50 μm, limiting the ability to perform spatially resolved imaging using an AFM-MS platform. To get around the heat dissipation problem of conventional thermal desorption surface sampling, where the AFM tip is engaged on the surface and heat is transferred from the probe into the material causing thermal desorption (Figure 1a) we have implemented nanomechanically assisted sample pick-up followed by thermal desorption with secondary ionization by APCI and mass spectral detection, "scratch and sniff" (Figure 1b). In the "scratch and sniff" process as depicted in Figure 1b the AFM tip is used to mechanically remove material from the surface with subsequent thermal desorption of the material from then tip when it is withdrawn from the surface. Using the AFM tip to mechanically collect material onto the tip allows the sampling of low melting point materials with high spatial resolution without physically altering a large area of the material surface as seen in Figure S1. In this process the area that is sampled, directly correlates to the area that AFM probe scanned on the surface. Figure 1b demonstrates the steps involved in the sampling process: engaging tip on the surface and raster scanning the tip over a surface (I), tip with material accumulated on it is withdrawn from the surface (II), and tip is heated to promote thermal desorption of material accumulated on the tip (III). To confirm material collection onto the probe, before and after nanomechanical surface sampling topography images were acquired for the candelilla wax surface, a very low melting point material. Figure 1c, d, and e show topography images of before, during and after nanomechanical sampling from a 2.5 μm x 2.5 μm area from the same candelilla wax matrix as in Figure S1 however now with a controllable collection process. As can be seen

in Figure 1e although the majority of material seems to be removed from the scanned area some material accumulation can be seen to the left and right boundaries of where the AFM tip scanned. The material build-up to the left and right of the scan direction supports the assumption that there is a limited amount of material that can be accumulated on the current AFM probe designs. However, tailored probe geometries could be envisioned that would enable more material to be collected on the probe during scanning.

Nanomechanical sample pickup relies on material being picked-up by the AFM tip caused by scanning the AFM tip across the surface in contact mode. Therefore, applying additional force onto the AFM tip as it scans across the surface improves the collection efficiency. Figure 2a shows the relative integrated mass spectral peak areas of *m/z* 135 and *m/z* 161, MS/MS products of uv-stabilizer avobenzone, m/z 311 → ○, CE 35, in a candelilla wax matrix as a function of force applied to the AFM cantilever. The increase in peak area is due to the change in intensity of the ion signal as the peak widths in the extracted ion chronograms are determined by the length of the heating pulses applied to AFM cantilever, that are kept constant with all experiments. Force applied to the scanning cantilever causes the AFM tip to penetrate deeper into the surface causing an increase in the amount of collected material that is proportional to the surface area of the tip is contact with the surface. Since the AFM tip has a pyramidal structure the amount of material collected onto the tip has a non-linear relationship with the applied force onto the cantilever. More importantly the data in Figure 2a, demonstrate that a certain amount of force is necessary to push the AFM tip into surface to improve on sample collection. For our sample surface a minimum of ~2 μN of force was required for the AFM tip to penetrate the surface versus just scanning over the surface.

In addition to the effect of applied force on the AFM cantilever to the amount of material collected, the relationship between scanned area and mass spectral signal area of avobenzone was explored for scanned areas ranging 250 nm x 250 nm to 5 μm x 5 μm in size (Figure 2b). Although, a linear relationship between scanned area and relative mass spectral peak area would be expected, because the AFM tip has a limited surface area for material to collect on, saturation in the mass spectral response can be seen for sampled areas that are larger than 1 μm x 1 μm is size. However, as can be seen from the inset in Figure 2b for sampled areas between 250 nm x 250 nm to 1 μm x 1 μm a linear signal relationship is observed. Further supporting the notion that the surface area of the AFM tip in contact with the surface plays an important role in determining how much material can be accumulated on the probe. Therefore, if we assume that a standard AFM probes is pyramidal with a tip height of ~ 4 μm and each side width of ~ 2.5 μm the exposed surface area of a tip that is fully penetrated into a surface is ~ 21 μm^2. Since we expect the AFM probe to not sink completely into the material the actual exposed surface area will be less than 21 μm^2 and be proportional to the square of penetration depth of the tip into the surface. Furthermore, the limited surface area of the tip will also lead to greater uncertainty in the reproducibility of the sampling values. Once all the exposed surfaces are covered with material it is possible to accumulate multiple layers of material on the probe but such material accumulation would most likely be less stable and reproducible from scan to scan leading to large error bars seen in Figure 2b on the larger sampling areas. Overall, the volume of sampled material is likely to correlate directly with signal strength, however few factors that complicate this analysis are (i) unknown tip shape and the interplay between that and the sample plasticity and hardness, along with the milled material-tip adhesion

will all affect the amount of material extracted and therefor the signal strength and (ii) if the samples region is heterogeneous, it is not necessarily clear that increasing the sampling volume will increase the amount of analyte. Work on nano-milling of material and the correlation with depth and material removal, especially in complex materials is as of today still an active research area and deserves more exploration.[25, 26]

The sampling reproducibility for areas less than 1 μm^2 is demonstrated in Figure 3a from 3 subsequent samplings of 500 nm x 500 nm areas. Figure 3a shows the extracted ion chronogram for *m/z* 135 and *m/z* 161, the main fragments for avobenzone, which is the uv-stabilizer in candelillia wax matrix. As can be seen in Figure 3b the quality of the associated mass spectra for avobenzone that is 3% by weight matches very well with that of the avobenzone standard analyzed via head space sampling in Figure 3c. Additionally, the reproducibility of collecting material from three subsequent 500 nm x 500 nm samplings seen in Figure 3a points to the uniform distribution of the 3% avobenzone active ingredient in the wax matrix. Figure 3a and 3b also highlight that by minimizing on heat dissipation into the bulk material allowing for a faster desorption and concentration of the gas phase analyte from a single heating pulse enabling higher signal levels from a given amount of material in a single sampling spot i.e clear mass spectra of 3% avobenzone from a 500 nm x 500 nm sampling. The minimum sampling volume for avobenzone in the candelilla wax was calculated using three different analytical approaches shown in Figure S2 and Table S1 using volume data calculated from the AFM images of the removed material and integrated signal for avobenzone, and was found to be between 0.0205 μm^3 and 0.108 μm^3. Using the 0.108 μm^3 volume estimate and the known 3% concentration of avobenzone in the candelilla wax matrix produced a limit of detection (LOD) of 7.4 amol

for avobenzone (Table S2). It should be noted that this number is in good agreement other thermal desorption and laser vaporization ambient surface sampling mass spectrometry approaches with a 4 amol LOD for agrochemical ametryn reported for pin-to-capillary flowing atmospheric-pressure afterglow ion source (FAPA)[27] and 2 amol LOD for lysine using laser desorption/ionization droplet delivery mass spectrometry (LDIDD-MS)[28]. An in-depth review of quantitation using various ambient desorption and ionization mass spectrometry approaches by Shelly et al[29] provides a summary of the limits of detection for all the techniques and a perspective on the challenges to take ambient mass spectrometry from a qualitative screening to an accurate quantification tool.

To demonstrate the universality of the "scratch and sniff" to a wider class of materials and thermal desorption approaches. We performed nanomechnical sampling directly from a mouse kidney thin-tissue sample followed by direct photothermal desorption from the AFM tip. Figure 4a shows the AFM topography of the kidney tissue prior to sampling while Figure 4b and 4c show the topography of the surface after a 1 μm x 1 μm box was scanned to remove material. As with the wax sample surface some material build-up can be seen along the edges of the scanned area indicating that methodologies that enable higher collection efficiencies need to be explored in the future. The corresponding full scan background subtracted mass spectra from the 1 μm x 1 μm sampled area in Figure 4c can be seen in Figure 4d. The rich mass spectra that is seen in Figure 4d illustrate the benefit of TD of material from a small volume on the tip versus directly on the tissue. The abundance of higher mass ion above 500 Da indicate the ability to preserve intact molecular species be avoiding melting and decomposition of the material that occurs during slow heat transfer processes when heating a sample directly as seen in Figure S1.

DISCUSSION

In this work we report on a new method of nanomechanical sampling of materials from a surface using an AFM probe tip for real time analysis directly from the tip using thermal desorption with secondary ionization on an AFM-MS platform. This approach allows for material to be concentrated directly onto the probe from areas down to 250 nm x 250 nm and enables sampling of waxy and volatile materials that would not be amenable to direct thermal desorption or chemical analysis in a vacuum environment. We demonstrate the optimization metrics of the approach such the collection efficiency as a function of cantilever force and scanned area. Overall, this approach allows heat to be transferred faster into a smaller sampled volume by minimizing on heat dissipation into the bulk material. This allows for a faster desorption and concentration of the gas phase analyte from a single heating pulse, thereby enabling more efficient sampling. Furthermore, improvements in heating pulses applied directly to the tip could enable the use of rapid heating of the material on the tip to improve on the spatial resolution to sub-100 nm and desorb a much broader range of materials intact with molecular weight exceeding the 500 Da limit seen in standard thermal desorption experiments. Although, currently the approach is more suited for profiling experiments due to the average data acquisition time per sampling one could envision fast AFM scanning routines where each area could be scanned several times in the under 10 s per sampling event to allow for mapping experiments in the future.

ACKNOWLEGMENTS

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare no competing interests.

FIGURE CAPTIONS

Figure 1. Schematic illustration of experimental flow for (a) direct heating thermal desorption (I) a non heating AFM probe is positioned above the surface and subsequently is engaged onto the surface (II) voltage is applied to the AFM tip causing the tip to heat up (III) thermal diffusion from the heated AFM tip into the substrate produces a thermal desorption of the material. (b) Nanomechanical assisted sample pickup and removal followed by thermal desorption (I) a non heating AFM probe is rastered across a surface to collect materials onto the AFM tip (II) the AFM tip is subsequently removed from the surface bringing with it material collected on the surface (III) voltage is applied to the AFM tip causing it to heat up and thermally desorbing the material that was collected on the probe. Topography images of uv-stabilizer avobenzone in candelilla wax matrix (c) before, (d) during and (e) after nanomechanical sample pick-up. Material was removed during the raster scanning processes, dashed square highlights area of removed material.

Figure 2. Optimization of parameters for uv-stabilizer avobenzone in a candelilla wax matrix using nanomechanical sample pick up followed by TD relative to the integrated mass spectral peak areas of *m/z* 135 and *m/z* 161, MS/MS products of uv-stabilizer avobenzone, m/z 311 → ○, CE 35 as a function (a) of force applied to the cantilever while scanning 500 nm x 500 nm area, (b) size of scanned area while applying 3μN of force to the tip. Scan speed was 0.5 Hz per line with 128 lines for each area, TD probe temperature was 450°C. Error bars represent ± 1 from 3 replicate samplings.

Figure 3. Spatially resolved sampling of uv-stabilizer avobenzone in a candelilla wax matrix using nanomechanical sample pick followed by TD (a) extracted ion current of m/z 161 for three replicate 500 nm x 500 nm using 3 μN of force applied to the tip, (b) background subtracted MS/MS (m/z 311 → ○, CE 35) spectra from sampling "2" shown in panel (a), inset shows structure of avobenzone, (c) background subtracted MS/MS spectra of avobenzone standard.

Figure 4. Spatially resolved nanomechanical sampling of material from a mouse kidney thin-tissue sample followed by photothermal desorption. Topography of kidney tissue (a) before and (b) after nanomechanical pick-up from 1 μm scan area with 3μN of force applied to the tip, (c) zoomed-in area from (b) designated by dashed red square, (d) background subtracted (2 min before laser firing) full scan mass spectra from material collected on the thin-tissue sample from the 1 μm scanned area.

Figure 1.

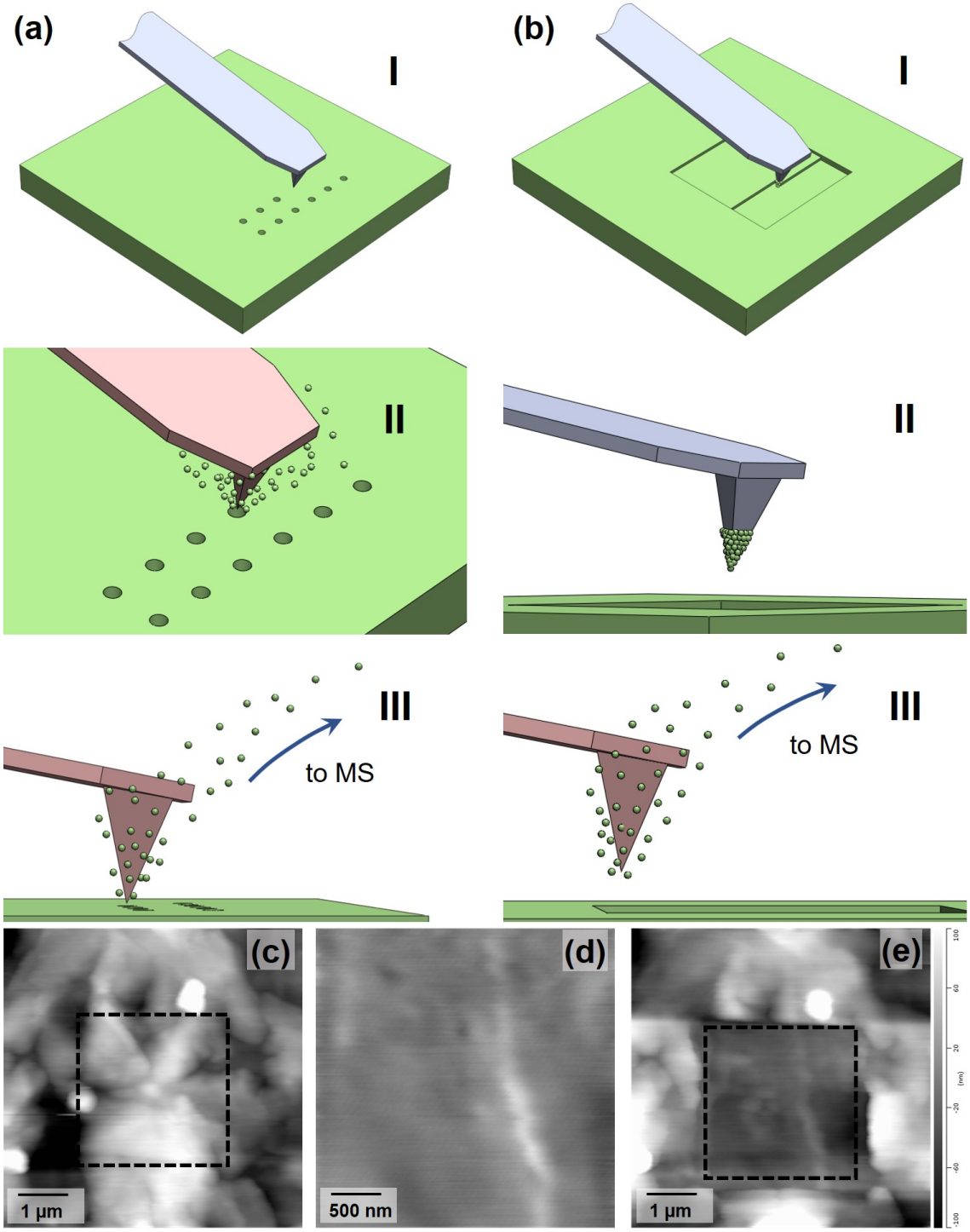
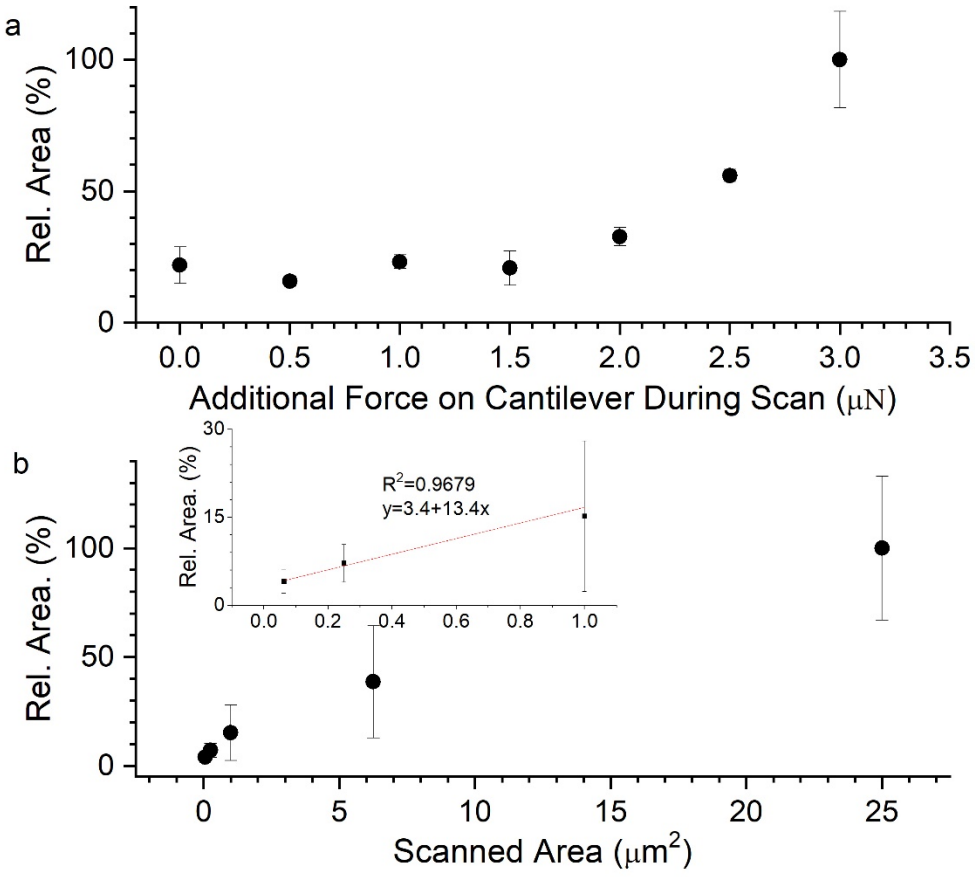


Figure 2.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Additional Force on Cantilever During Scan (μN)
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Scanned Area (μm²)
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Figure 3.

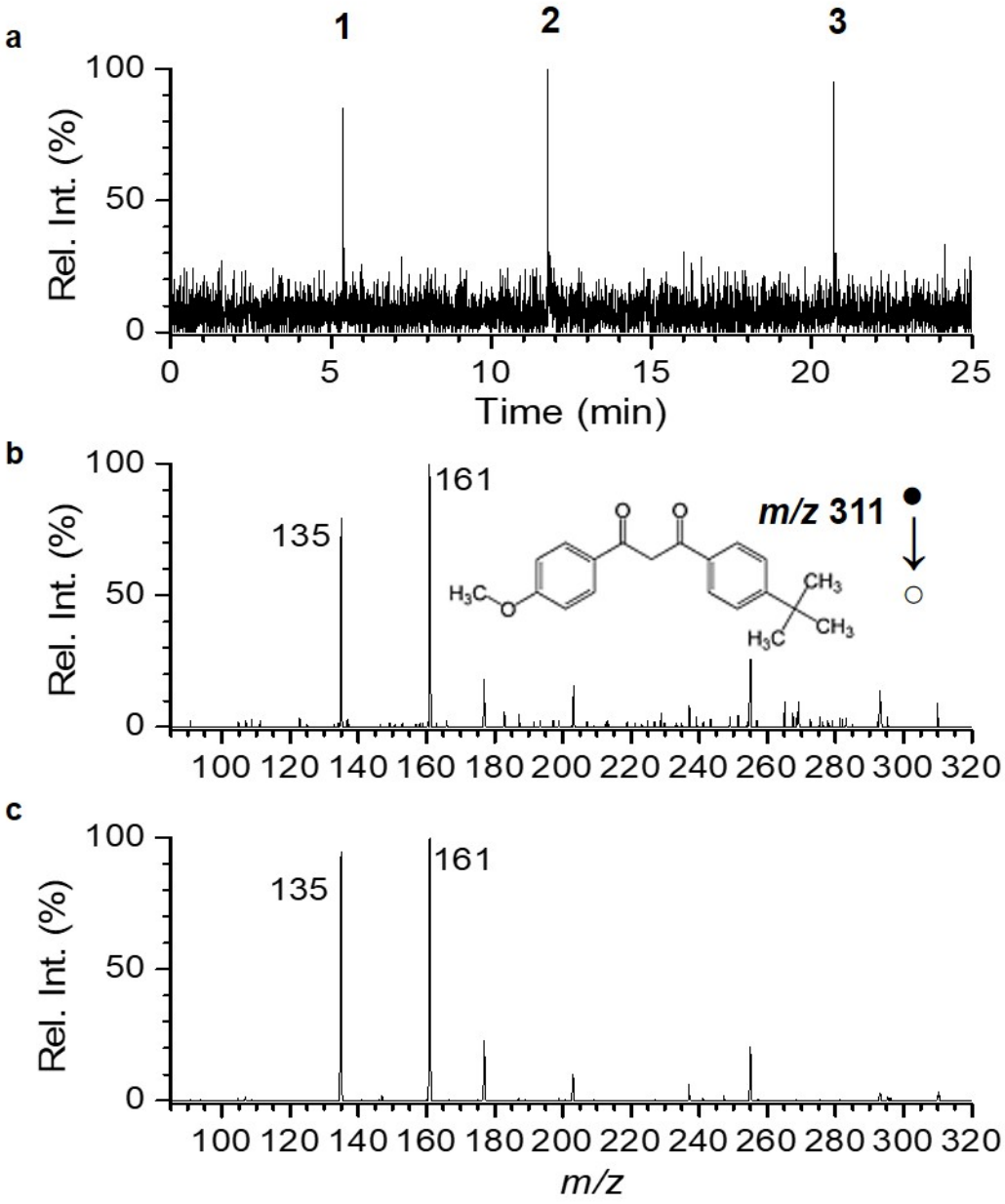
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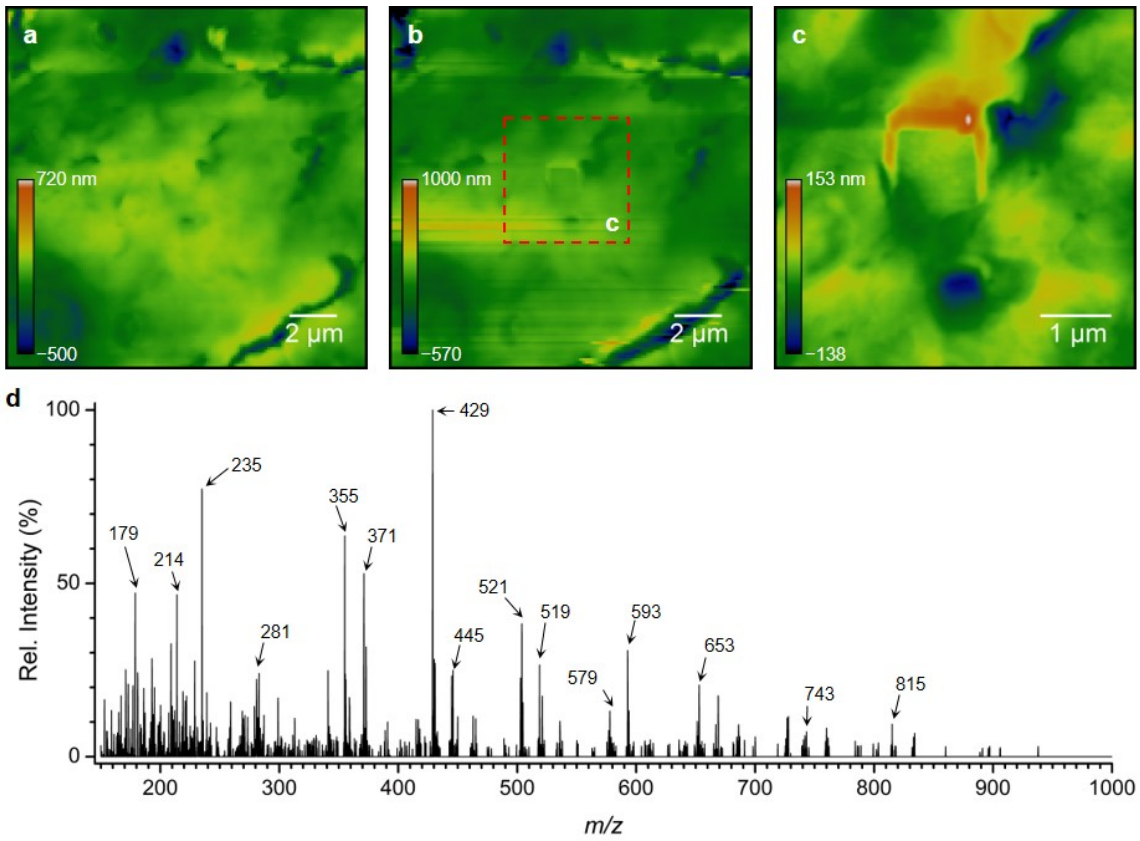
Figure 4.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