Combined Atomic Force Microscope-Based Topographical Imaging and Nanometer-Scale Resolved Proximal Probe Thermal Desorption/ Electrospray Ionization−Mass Spectrometry

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Proximal probe thermal desorption/secondary ionization−mass spectrometry (TD/SI−MS)[1,2] is a recently introduced approach, among the many other existing approaches, for atmospheric pressure (AP) surface sampling and ionization with mass spectrometric detection.[3−11] The TD/SI−MS approach uses a heated probe tip placed in close proximity to, or in actual contact with, a surface to locally desorb intact molecular species from that surface that are then ionized by an AP secondary ionization source like electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) and analyzed using MS. This approach offers a very simple and fast way to sample and analyze intact molecular species from a surface that are suited for thermal desorption. Though not yet demonstrated, it is expected that the proximal heated probe could be used for pyrolysis of intractable surface materials rather than for desorption of intact molecular species through the use of suitably elevated probe temperatures.

Initially, this AP-based TD/SI−MS technique was applied to the read out of the development lanes on thin layer chromatography (TLC) plates by performing line scans with a millimeter-sized chisel-shaped heated probe across compounds separated on the plates (*e.g.*, explosives, dyes, herbicides, and pharmaceuticals).[1] Using this setup,

ABSTRACT Nanometer- scale proximal probe thermal desorption/electrospray ionization mass spectrometry (TD/ESI−MS) was demonstrated for molecular surface sampling of caffeine from a thin film using a 30 nm diameter nanothermal analysis (nano-TA) probe tip in an atomic force microscope (AFM) coupled *via* a vapor transfer line and ESI interface to a MS detection platform. Using a probe temperature of 350 °C and a spot sampling time of 30 s, conical desorption craters 250 nm in diameter and 100 nm deep were created as shown through subsequent topographical imaging of the surface within the same system. Automated sampling of a 5×2 array of spots, with 2 μm spacing between spots, and real time selective detection of the desorbed caffeine using tandem mass spectrometry was also demonstrated. Estimated from the crater volume ($\sim 2 \times 10^6$ nm^3), only about 10 amol (2 fg) of caffeine was liberated from each thermal desorption crater in the thin film. These results illustrate a relatively simple experimental setup and means to acquire in an automated fashion submicrometer scale spatial sampling resolution and mass spectral detection of materials amenable to TD. The ability to achieve MS-based chemical imaging with 250 nm scale spatial resolution with this system is anticipated.

KEYWORDS: thermal desorption · nanometer scale · atmospheric pressure · atomic force microscopy · mass spectrometry · electrospray ionization · caffeine

millimeter scale spatial resolution in the sampling process was accomplished. Subsequent work with a 50 μm diameter circular cross section heated probe showed it was possible with optimized surface scan parameters to chemically image printed patterns on photopaper with ~50 μm spatial resolution through selected detection of the ink components thermally liberated from a paper surface.[2] As these reports established, the achievable spatial resolution
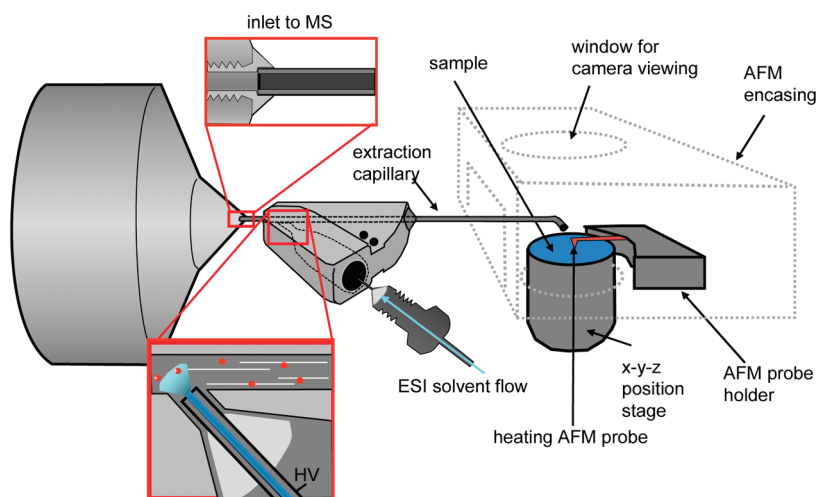
Figure 1. Schematic illustration of the AFM and proximal probe TD/ESI—MS experimental setup.

of the TD process was limited ultimately by the size of the heated probe tip. Thus, it was expected that spatial resolution and chemical image quality could be further improved by using probes of smaller contact dimensions. In addition, the results showed that reproducible mass spectral signal and imaging quality would be improved by incorporating a means to maintain a fixed real time optimal probe-to-surface distance, particularly in an imaging mode, continuously adapting to the changing topography of the surface during a line scan.

Low micrometer to submicrometer size heated probes and built-in capabilities for real time probe-to-surface positioning are available with appropriately configured atomic force microscope (AFM) platforms. For example, Reading and co-workers in a series of reports have demonstrated varied uses of 5 μm tip diameter Wollaston wire heated AFM probes.[12−16] They were able to perform both point thermal desorption and pyrolysis, capturing the liberated vapor material and then injecting it into a gas-chromatograph mass spectrometry (GC/MS) for separation, electron ionization (EI), and mass analysis. Within that work they also showed the ability to directly sample the vapors generated at AP into the vacuum-based EI source using a heated transfer capillary. Single point sampling from the surface of polymers and plant tissue was demonstrated. The use of force-feedback on the AFM system allowed for the precise positioning of the Wollaston wire probes on the surface during the heating process as well as for subsequent topographic imaging of the thermal desorption craters. The smallest desorption craters achieved were conical in shape, approximately 6 μm in diameter and 1.7 μm deep.

Herein, we expand on the prior AP proximal probe TD/SI—MS work[1,2] by using a 30 nm diameter heated AFM probe tip and an AFM control system in the same genre as that used by Reading, *et al.*[12−16] Using a probe temperature of 350 °C and a spot sampling time of 30 s, conical desorption craters 250 nm in diameter and

100 nm deep were created in a caffeine thin film and AFM topographical images of the surface were acquired within the same system. Automated sampling of a 5 × 2 array of spots, with 2 μm spacing between spots, and real time selective detection of the desorbed caffeine (∼10 amol per spot) using tandem mass spectrometry demonstrated the system reproducibility and potential for automated surface sampling and analysis.

RESULTS

To couple the ambient AFM-based TD experiment with mass spectrometric detection required the ability to efficiently transport the desorbed material to the ionization region, ionize that material, then transport the ions into the mass analyzer. High efficiency for each step was required due to the low attomole quantities of material liberated into the gas phase during the nanometer scale desorption process (see below and Supporting Information, Figure S1). These analyte levels would pose a challenge for detection even if 100% of the material sampled could be ionized and transported to the mass analyzer. Here, desorbed material was transported through a transfer tube from the region of the heated AFM tip into an ESI region *via* the vacuum draw from the mass analyzer (Figure 1). A 90° curve at the sampling end of this interface allowed very close positioning of the capillary to the TD region. A "Y" intersection of the ESI plume and vapors from the desorption region provided an effective way for the secondary ionization of the desorbed species (see inset in Figure 1). Because this extractor/ESI adaptor was fitted into the normal heated capillary interface of the mass spectrometer (typically held at 200 °C), it was kept substantially above ambient temperature minimizing condensation of the desorbed analyte in the transport region.

The ability to detect mass spectrometric signal from material thermally desorbed with the AFM is
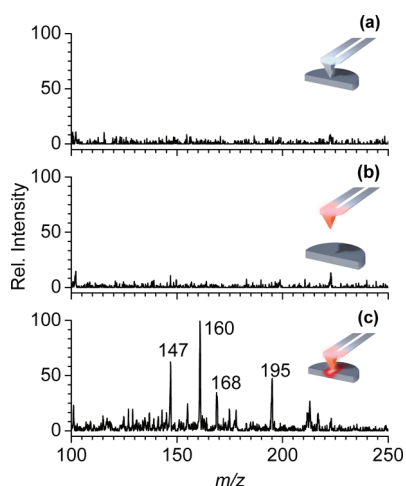
Figure 2. Averaged full scan mass spectra obtained from a thin film caffeine surface while (a) the AFM tip was engaged on surface and not heated, 25 °C, (b) the AFM tip was removed from surface 10 μm and heated to 350 °C, and (c) the AFM tip was engaged on surface and heated to 350 °C. The relative intensity in panels a and b is normalized to the intensity of the base peak *m/z* 160 in panel c.

demonstrated by the data in Figure 2. In this case, the sample surface was a thin film of caffeine on a glass microscope coverslip. Figure 2a shows the full scan mass spectrum obtained when the heated AFM probe tip was placed on the surface without turning on the heater (probe temperature ~25 °C). Figure 2b shows the corresponding full scan mass spectrum when the AFM tip was lifted to a position 10 μm above the surface with the tip heated to 350 °C. In neither case were ions corresponding to caffeine or any other species apparent in the mass spectra. This showed that mechanical or other nonthermal processes did not contribute to the liberation of caffeine from the surface. Figure 2c shows the full scan averaged spectrum obtained when the AFM tip was engaged on the caffeine surface and heated to 350 °C. In this case, ions corresponding to the protonated caffeine molecule were observed at *m/z* 195. Other unidentified ionic species were also observed including those at *m/z* 147, 160, and 168. These other species presumably arose from materials in the sample, from solvents used to prepare the thin film, and/or potentially from species in the laboratory air that condensed onto the thin film surface before analysis and are not discussed further here. Regardless, this spectrum demonstrates that caffeine was thermally desorbed during heated probe contact with the surface, transported into the ionization region, ionized, and then transported into the mass spectrometer and detected.

A TD crater created under identical experimental conditions used to acquire the data shown in Figure 2c was imaged with the same AFM tip used for the TD. The topographical image (Figure 3a) and profile cross section across the crater (Figure 3b) show the desorption crater is conical in shape, approximately 250 nm
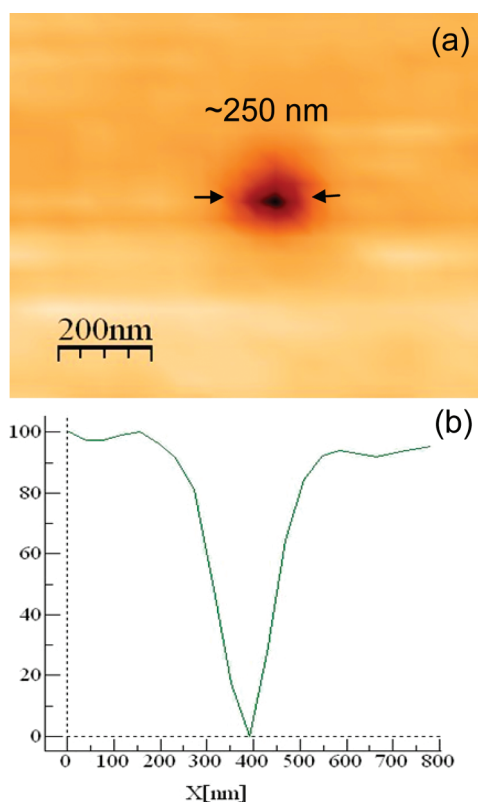

Figure 3. (a) AFM topographical image and (b) line profile of single desorption spot created by heating a thin film caffeine surface with the AFM probe tip engaged to the surface for 30 s at 350 °C.

across and 100 nm deep. This corresponds to a volume of 1.6×10^6 nm^3 removed from the thin film, which equates to ~10 amol (2 fg) of caffeine (see Supporting Information for calculation details).

To demonstrate the reproducibility and the potential for automated surface sampling and analysis, a grid experiment was performed in which the AFM probe tip was brought into contact with the same caffeine thin film surface for 30 s while heat was applied to 350 °C. The tip was then removed from the surface, and the heater was turned off for 20 s between subsequent grid points. During this sequence the mass spectrometer was continually operating in MS/MS mode, monitoring the well-known *m/z* 195 → 138 precursor to product ion transition characteristic for caffeine.[17] Figure 4a shows the topographic image of the caffeine surface acquired prior to carrying out the TD grid spot sampling experiment and Figure 4b shows the topographic image after the experiment. After this spot sampling experiment the image shows a 5 × 2 array of desorption craters each ~250 nm across by 100 nm deep, spaced 2 μm apart. The corresponding mass spectrometric chronogram shown in Figure 4c reveals the presence of 10 separate increases in the signal for caffeine corresponding to the 10 sampling events. The signal observed, though clearly distinct from the background noise, was at a relatively low intensity due to
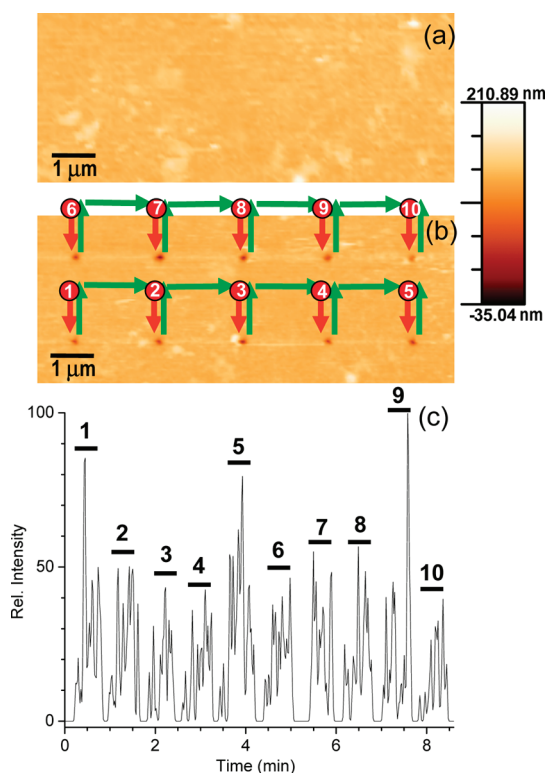
and the relative mass spectral signal area (Supporting Information, Figure S2). It was not possible to determine definitely from the present data set if the extremes of variation observed between crater size and mass spectral signal at spots 3, 5, and 9 were the result of actual surface composition variation or the result of experimental variability in the gas phase sampling, ionization, and mass spectral detection. Additional studies with well characterized homogeneous and heterogeneous model surfaces will be required.

On the basis of the baseline signal separations between sampling events, carry-over contamination from spot to spot did not appear to be an issue. Though the signal levels observed in experiments of this type might be compound dependent, caffeine is a relatively volatile molecule and ionizes well by ESI. Consequently, one can expect that improvements in the efficiency of sampling, ionization, transport, and mass spectral detection might be necessary to further improve spatial resolution.

CONCLUSIONS

In this report, we demonstrated a combined proximal probe TD AFM/ESI−MS system for nanometer scale resolved surface spot sampling and mass spectral detection of the model analyte caffeine desorbed from a thin film on a glass surface. Topographies of the surface were acquired in the same system using the AFM. This analytical platform is shown to provide both spatially resolved chemical information and physical characterization of the sample. Such a combined AFM/MS platform might be used to coregister local nanomechanical measurements and topography using AFM with MS-based chemical profiling of specific surface features. The ability to achieve MS-based chemical imaging with submicrometer scale spatial resolution with this system would be expected. Potential improvements to the system that would increase the mass spectral signal are currently being investigated and include the heating of the transfer line to higher temperatures as well as the modeling of the transfer of both neutrals and ions through the vapor transport capillary.

Figure 4. AFM topography of a caffeine thin-film surface (a) preheating and (b) postheating the surface for 30 s per spot in a 5 × 2 array. (c) MS/MS ion current chronogram (5 pt Gaussian smooth) for caffeine (m/z 195 → 138, normalized collision energy 35%) recorded during the 30 s per spot sampling of the 5 × 2 array. The red and green arrows in panel b correspond to the movement of the AFM probe while the probe is at 350 °C, and at ambient temperature 25 °C, respectively. Crater numbers in panel b correspond to MS chronogram peaks in panel c with the line below the numbering in panel c representing the 30 s heating interval during which the AFM probe is on the caffeine thin-film surface.

the relatively small amount (∼10 amol (∼2 fg)) of caffeine desorbed to form each crater. While the surface composition at each of the 10 sampled spots was assumed to be uniform, we noted that there was a 38% relative standard deviation (RSD) in the average volume of the TD craters and a 28% RSD in the mass spectral signals for caffeine. However, except for sampling spots 3, 5, and 9, there was good correlation between the corresponding relative crater volume

METHODS

Chemicals. Caffeine (99%) and analytical reagent grade methanol (99.9%) were obtained from Fisher Scientific (Fisher Scientific, Pittsburgh, PA). Formic acid (99.9%) was obtained from Sigma-Aldrich (Aldrich, St. Louis, MO). Caffeine solutions were prepared by dissolving the appropriate amount of caffeine in methanol to make 1 μM solutions. The caffeine thin film was formed by freely dispersing 70 μL of 1 μM caffeine solution onto a glass coverslip that was attached to a steel puck using cyanoacrylate adhesive. The ESI solvent was 99.9% methanol with 0.1% (v/v) formic acid.

Experimental Setup. The experimental setup is shown in Figure 1. 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) were used to obtain the nanometer scale topographical images and to control the nanoscale TD spot sampling. Material was thermally desorbed from a caffeine thin film surface using VITA-MM-NANOTA-300 nanothermal analysis (nano-TA) AFM probes (Bruker AXS, Camarillo, CA). Temperature calibration of the nano-TA 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[18,19] by calibrating the dynamic transfer function, a technique first introduced by Lee et al.[20]

Thermally desorbed material was transferred to the mass spectrometer from the AFM tip area using a vapor transfer line, or extractor, with a "Y" geometry intersecting ESI source. This extractor/ESI source, fabricated from stainless steel and 1.27 mm o.d. by 0.84 mm i.d. stainless steel capillary, was press fit into a counter-bored sampling capillary of an LCQ Deca XP interface mated with a LCQ Deca quadrupole ion trap mass spectrometer (Thermo Scientific, San Jose, CA). The vacuum draw from the mass spectrometer ($\sim$7 mL/min) pulled the vapors from the AFM region into this interface and on into the mass spectrometer. ESI solvent was delivered into the device through a metal capillary emitter (500 μm o.d. x 127 μm i.d) at a rate of 2 μL/min using a syringe pump (Harvard Apparatus, Holliston, MA). The emitter was held in a PEEK fitting/ferrule assembly that screwed into the extractor unit. The position of the ESI emitter inside the extractor/electrospray device was adjusted to achieve a constant ESI current. The PEEK fitting/ferrule assembly provided electrical isolation between the metal emitter held at high voltage (3.5 kV) and the body of the extractor, which was connected to the interface heated capillary (200 °C), which was held at an experimentally optimized value of 23 V. The tube lens was optimized at 55 V. Ionization of the vaporized materials took place via interaction with the ions and charged droplets produced from the ESI emitter (see inset in Figure 1). The sampling end of the device had a 90° bend to allow for positioning of the capillary within 1 mm of the AFM tip given the AFM mounting configuration. The total length of the extractor/ESI adaptor was 160 mm. The AFM assembly was positioned on a Zaber NA14B60 (Zaber Technologies Inc., Vancouver, Canada) motorized linear actuator and a Newport 9064-X-P motorized translation stage (Newport, Irvine, CA) to allow for the close positioning of the extractor with the AFM tip. The position of the AFM tip relative to the extractor was visualized using a Navitar telescope with a 12X objective (Navitar Inc., Rochester, NY) connected to a Costar SI-C400N CCD camera (Costar Inc., Anaheim, CA) and a Marshall V-ASL8080 LCD monitor with a 4:3 aspect ratio (Marshall Electronics Inc., El Segundo, CA). This setup provided a 500 μm horizontal field of view at the highest (12X) magnification, allowing for accurate monitoring of the position of the probe relative to the extraction capillary. Desorbed and ionized species were monitored with full scan mass spectra and MS/MS product ion spectra (caffeine transition m/z 195 → 138, normalized collision energy 35%). For TD measurements over an array of points, the AFM was controlled using custom software which utilized components of the Nanonis Programming Interface in the Labview programming environment. This programming interface allowed, from a Labview virtual instrument, the independent variation of the force-feedback set-point of the cantilever, the position of the tip across the sample surface, as well as control of analog output channels (AO1 and AO2) on the SC4 module of the Nanonis controller. One analog output was used to drive the temperature change of the nano-TA cantilever, and the second channel was used to trigger the mass spectrometer.

Supporting Information Available: Detail of the calculation of the amount of caffeine liberated from a desorption crater and a comparison plot of crater volume and mass spectral signal. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES AND NOTES

1. Ovchinnikova, O. S.; Van Berkel, G. J. Thin-layer Chromatography and Mass Spectrometry Coupled Using Proximal Probe Thermal Desorption with Electrospray or Atmospheric Pressure Chemical Ionization. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 1721–1729.
2. Ovchinnikova, O. S.; Kertesz, V.; Van Berkel, G. J. Molecular Surface Sampling and Chemical Imaging Using Proximal Probe Thermal Desorption/Secondary Ionization Mass Spectrometry. *Anal. Chem.* **2011**, *83*, 598–603.
3. Van Berkel, G. J.; Pasilis, S. P.; Ovchinnikova, O. Established and Emerging Atmospheric Pressure Sampling/Ionization Techniques for Mass Spectrometry. *J. Mass. Spectrom.* **2008**, *43*, 1161–1180.
4. Harris, G. A.; Nyadong, L; Fernandez, F. Recent Developments in Ambient Ionization Techniques for Analytical Mass Spectrometry. *Analyst* **2008**, *133*, 1297–1301.
5. Venter, A.; Nefliu, M.; Cooks, R. G. Ambient Desorption Ionization Mass Spectrometry. *Trends Anal. Chem.* **2008**, *27*, 284–290.
6. Chen, H. W.; Gamez, G.; Zenobi, R. What Can We Learn from Ambient Ionization Techniques?. *J. Am. Soc. Mass. Spectrom.* **2009**, *20*, 1947–1963.
7. Alberici, R. M.; Simas, R. C.; Sanvido, G. B.; Romao, W; Lalli, P. M.; Benassi, M.; Cunha, I. B. S.; Eberlin, M. N. Ambient Mass Spectrometry: Bringing MS into the "Real World". *Anal. Bioanal. Chem.* **2010**, *398*, 265–294.
8. Huang, M. Z.; Yuan, C. H.; Cheng, S. C.; Cho, Y. T.; Shiea, J. Ambient Ionization Mass Spectrometry. *Ann. Rev. Anal. Chem.* **2010**, *3*, 43–65.
9. Weston, D. J. Ambient Ionization Mass Spectrometry: Current Understanding of Mechanistic Theory; Analytical Performance and Application Areas. *Analyst* **2010**, *135*, 661–668.
10. Ifa, D. R.; Wu, C.; Ouyang, Z.; Cooks, R. G. Desorption Electrospray Ionization and Other Ambient Ionization Methods: Current Progress and Preview. *Analyst* **2010**, *135*, 669–681.
11. Chen, H. W.; Hu, B.; Zhang, X. Fundamental Principles and Practical Applications of Ambient Ionization Mass Spectrometry for Direct Analysis of Complex Samples. *Chin. J. Anal. Chem.* **2010**, *38*, 1069–1088.
12. Price, D. M.; Reading, M.; Hammiche, A.; Pollock, H. M. Microthermal Analysis: Scanning Thermal Microscopy and Localized Thermal Analysis. *Int. J. Pharm.* **1999**, *192*, 85–96.
13. Price, D. M; Reading, M.; Hammiche, A.; Pollock, H. M. New Adventures in Thermal Analysis. *J. Therm. Anal. Calorim.* **2000**, *60*, 723–733.
14. Price, D. M.; Reading, M.; Lever, R. J.; Hammiche, A.; Polluck, H. M. Microthermal Analysis and Evolved Gas Analysis. *Thermochim. Acta* **2001**, *367–368*, 195–202.
15. Price, D. M.; Reading, M.; Smith, R. M.; Pollock, H. M.; Hammiche, A. Localized Evolved Gas Analysis by Microthermal Analysis. *J. Therm. Anal. Calorim.* **2001**, *64*, 309–314.
16. Reading, M.; Price, D. M.; Grandy, D. B.; Smith, R. M.; Bozec, L.; Conroy, M.; Hammiche, A.; Pollock, H. M. MicroThermal Analysis of Polymers: Current Capabilities and Future Prospects. *Macromol. Symp.* **2001**, *167*, 45–62.
17. Ford, M. J.; Deibel, M. A.; Tomkins, B. A.; Van Berkel, G. J. Quantitative Thin-Layer Chromatography/Mass Spectrometry Analysis of Caffeine Using a Surface Sampling Probe Electrospray Ionization Tandem Mass Spectrometry System. *Anal. Chem.* **2005**, *77*, 4385–4389.
18. Nikiforov, M. P.; Gam, S.; Jesse, S.; Composto, R. J.; Kalinin, S. V. Morphology Mapping of Phase-Separated Polymer Films Using Nanothermal Analysis. *Macromolecules* **2010**, *43*, 6724–6730.
19. Nikoforov, M. P; Jesse, S.; Morozovka, A. N.; Eliseev, E. A.; Germinario, L. T.; Kalinin, S. V. Probing the Temperature

Dependence of the Mechanical Properties of Polymers at the Nanoscale with Band Excitation Thermal Scanning Probe Microscopy. *Nanotechnology* **2009**, *20*, 395709.

20. Lee, J.; Beechem, T.; Wright, T. L.; Nelson, B. A.; Graham, S.; King, W. P. Electrical, Thermal, and Mechanical Characterization of Silicon Microcantilever Heaters. *J. Microelectromech. Syst.* **2006**, *15*, 1644–1655.

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