analytical chemistry

LETTER

pubs.acs.org/ac

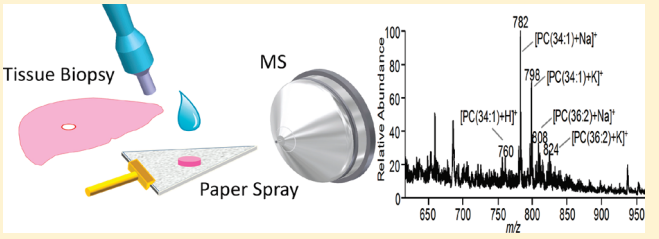
Direct Analysis of Biological Tissue by Paper Spray Mass Spectrometry

He Wang,[†] Nicholas E. Manicke,[‡] Qian Yang,[†] Lingxing Zheng,[§] Riyi Shi,[§] R. Graham Cooks,[‡,⊥] and Zheng Ouyang*[,†,⊥]

[†]Weldon School of Biomedical Engineering, [‡]Department of Chemistry, [§]Department of Basic Medical Science, School of Veterinary Medicine, and [⊥]Center for Analytical Instrumentation Development, Purdue University, West Lafayette, Indiana 47907, United States

Supporting Information

ABSTRACT: Paper spray mass spectrometry (PS-MS) is explored as a fast and convenient way for direct analysis of molecules in tissues with minimum sample pretreatment. This technique allows direct detection of different types of compounds such as hormones, lipids, and therapeutic drugs in short total analysis times (less than 1 min) using a small volume of tissue sample (typically 1 mm^3 or less). The tissue sample could be obtained by needle aspiration biopsy, by punch biopsy, or by rubbing a thin tissue section across the paper. There exists potential for the application of paper spray mass spectrometry together with tissue biopsy for clinical diagnostics.



Rapid, quantitative, and specific chemical information on biological tissue is of importance to biomedical research and clinical diagnosis. Such information not only can advance the discovery of biomarkers for disease diagnosis[1−3] but also can help to determine the distribution of therapeutic drugs and their metabolites in the course of new drug development.[4] Tissue biopsy is a routine clinical procedure which is applied to tissue samples of all types for assessment of disease state, including the presence and nature of tumors.[5,6] Needle aspiration biopsy (NAB), for instance, involves aspiration of tissue sample into a syringe through a thin, hollow needle and its expulsion onto a glass slide as a tissue smear for examination. Typically, histochemical or immunohistochemical staining of the tissue smear is performed followed by microscopic examination by a pathologist. While this method is time-tested and well established, a label-free, generally applicable, and molecularly specific method of chemical analysis applicable to the biopsied sample could potentially significantly improve the information content of the analysis and consequently improve the accuracy of diagnosis.[7,8] If such a method were also rapid and easily implemented, its value would be considerable.

Mass spectrometry (MS) provides a highly sensitive, molecularly specific, and in some forms, high-throughput approach to chemical analysis.[9−17] The challenge in applying MS to tissue analysis includes the cumbersome sample preparation normally required before the MS analysis step. In addition to analysis using electrospray ionization (ESI)-MS of analytes separated from tissue samples,[18] a series of desorption−ionization methods has been applied to imaging of the analytes within tissue sections. These methods have included matrix-assisted laser desorption ionization (MALDI),[19] desorption electrospray ionization (DESI),[20] electrospray laser desorption ionization (ELDI),[21] laser ablation electrospray ionization (LAESI),[22] and secondary ion mass spectrometry (SIMS).[23,24] Depending on the sampling and ionization method, preparation of the tissue samples is done in different ways, from simple sectioning of frozen tissues[22,25] to pretreatment with addition of appropriate matrixes.[19,26] A variety of compounds from tissue have been examined using these methods, including drugs,[27,28] lipids,[25] peptides, and proteins.[29]

Tissue biopsy can be performed quickly at outpatient clinics, and the tissue can be characterized by morphological, histological, and histochemical methods. The application of MS analysis to tissue biopsy requires sampling and ionization methods suitable for small volume samples, preferably with no requirement for sample preparation and, hence, with high throughput characteristics. As an example, probe electrospray ionization (PESI) sampling tissue using a needle with subsequent analysis by ESI.[30] The ambient ionization methods, especially DESI, have seen significant use in tissue analysis. Quantitative capabilities using rapid screening sampling methods would significantly increase the value of applying MS analysis to clinical tissue biopsy analysis. In this study, we explore a method based on paper spray ionization for rapid tissue biopsy molecular analysis.

Paper spray (PS) is a recently developed ionization method that has been shown to be effective in the analysis of complex biological fluid samples, including compounds in whole blood and raw urine.[31,32] Chromatographic paper or other porous substrates are cut into a triangular shape and then loaded with biological samples. When solvent is applied and a high voltage is supplied to the paper, a spray of charged droplets is induced at the tip of the paper triangle. Internal standards have been used for accurate quantitation, and they are applied with the solvent or by

Received: November 30, 2010
Accepted: January 11, 2011
Published: January 19, 2011

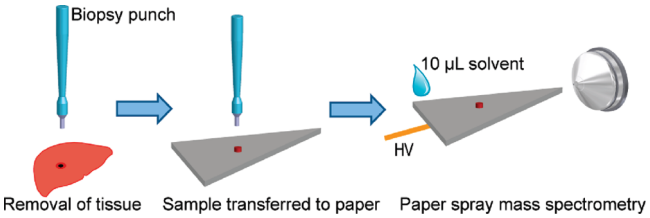


Figure 1. Procedure for combining tissue biopsy with paper spray mass spectrometry.

preprinting them onto the paper. Limits of quantitation (LOQ) as low as 1 ng/mL have been achieved for therapeutic drug monitoring with dried blood spots.[32,33] One unique feature of paper spray is the low consumption of sample as well as consumables required for the analysis, a feature which is particularly suitable for on-site clinical analysis. In this study, we demonstrate that paper spray can also be used for direct analysis of biological tissue. Less than 1 μL of tissue sample is enough for direct detection of hormones, lipids, and therapeutic drugs without any sample pretreatment. This method can be coupled with tissue biopsy as a point-of-care (POC) medical test to probe the chemical information of biological tissues.

EXPERIMENTAL SECTION

Porcine adrenal gland and mouse liver tissue were purchased from Pel-Freez (Rogers, AR). Human prostate tumor and normal tissue sections (ca. 3 cm^2 × 15 μm) obtained from the School of Medicine at Indiana University and previously studied using DESI imaging[34] were used. The procedure for preparing rat tissues and tissue homogenates containing hydralazine is described in the Supporting Information. For the punch biopsy experiment, a homemade biopsy punch with a stainless steel tubing (i.d. 1 mm) tip was inserted into the biological tissue to remove a fixed volume of tissue sample (ca. 0.8 μL). The phospholipid 1-palmitoyl-2-glutaryl-sn-glycero-3-phosphocholine(16:0−05:0 (COOH) GPCho) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Other reagents were purchased from Sigma-Aldrich (Milwaukee, WI). Mass analysis using paper spray was performed using TSQ Quantum, LTQ ion trap, and Exactive Orbitrap mass spectrometers (Thermo Scientific Inc., San Jose, CA), and the detailed experimental conditions are described in the Supporting Information.

RESULTS AND DISCUSSION

Figure 1 shows in schematic form an experiment in which paper spray ionization is used for MS analysis of biological tissue. The experiment couples tissue biopsy with MS analysis. The punched tissue sample was transferred onto the paper triangle for paper spray ionization. Other methods were also used to obtain the tissue samples such as needle aspiration biopsy from bulk tissue or by smearing tissue section on a glass slide.

Hormones. To demonstrate that paper spray can be used for hormone detection, porcine adrenal gland, the gland mainly responsible for releasing hormones under stress, was analyzed using the LTQ ion trap mass spectrometer with paper spray. Porcine adrenal gland tissue (≈1 mm^3) was placed onto the paper surface using the NAB sampling procedure; MeOH/water (v/v, 1:1; 10 μL) was added, and a DC potential of 4.5 kV was applied to the paper to produce a spray. Hormones epinephrine (EP)

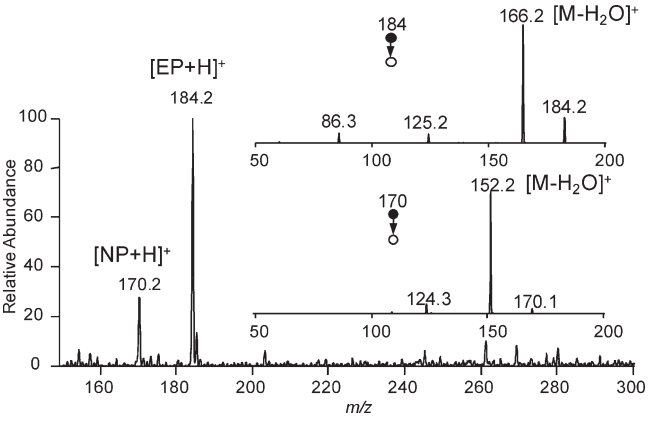


Figure 2. Direct analysis of hormones in animal tissue by PS-MS after needle aspiration biopsy. Porcine adrenal gland tissue (1 mm^3) was placed on the paper surface; MeOH/water (1/1 v:v; 10 μL) was added, and a DC potential of 4.5 kV was applied to the paper to produce a spray. Hormones epinephrine and norepinephrine were identified and confirmed with MS/MS (spectra shown as insets). Spectra recorded using LTQ ion trap mass spectrometer.

(m/z 184) and norepinephrine (m/z 170) were observed in the mass spectrum, and their identification was confirmed by tandem mass spectra (Figure 2). The most abundant fragments occur at m/z 166 which arises from m/z 184 and m/z 152 which arises from m/z 170, corresponding to H_2O loss from the parent protonated molecules, respectively.

Lipids. Lipids play important roles in energy storage and as the principal components of cell membranes, and they act as signaling molecules. In recent years, lipids have been found to be potential biomarkers for cancer diagnosis,[34−37] and their role in cardiovascular disease is also well established.[38,39] A fast and accurate method for analysis of lipid composition in tissue is highly desirable. Such information is obtainable by DESI mass spectrometry, which allows tissue imaging, but alternatives are desirable.

Human prostate tumor tissue and adjacent normal tissue were analyzed using paper spray ionization. Tumor and adjacent normal tissue sections (15 μm thick) were thaw mounted onto a glass slide. A metal needle was used to take 1 mm^2 × 15 μm volumes of tissue from the tumor and normal regions, as determined by earlier pathological examination. The removed tissue was smeared onto the surface of a paper triangle for paper spray experiments. Methanol/water (v/v, 1:1; 10 μL) was added to the paper as solvent, and then, 4.5 kV positive DC voltage was applied to produce the spray. As shown in Figure 3, phospholipids such as phosphatidylcholine (PC) and sphingomyelin (SM) were identified in the spectra, with distinctive patterns observed for normal and tumor tissue samples. The peak corresponding to $[PC(34:1) + K]^+$ at m/z 798 was significantly higher in the tumor tissue (Figure 3a), and the signals due to sphingomyelin, $[SM(34:1) + Na]^+$ at m/z 725, $[SM(36:0) + Na]^+$ at m/z 756, and $[SM(36:4)+Na]^+$ at m/z 804, were significantly lower in comparison with normal tissue (Figure 3b). It should be noted that the signal intensity of phospholipids obtained by paper spray was higher than that recorded in a typical DESI analysis on the same tissue section.[34] This is presumably due to the longer solvent extraction time and the larger sample volume examined for paper spray.

Rat brain tissue sections were also tested using paper spray ionization. The tissue samples were removed using a metal

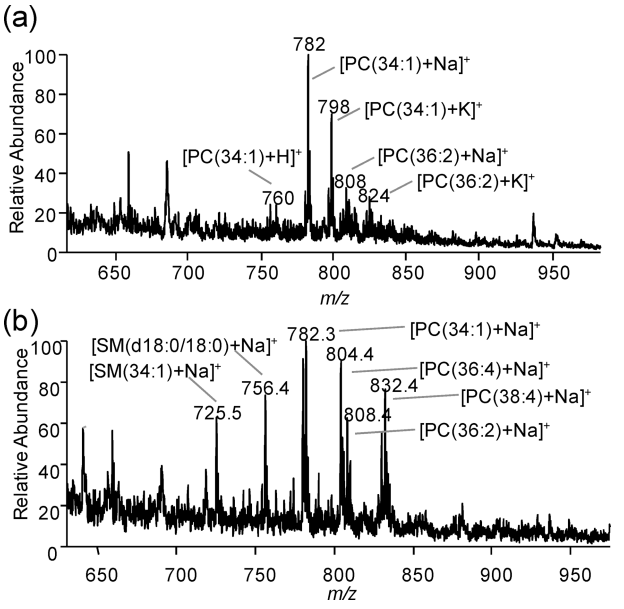


Figure 3. Analysis of tumor (a) and normal sections (b) of human prostate tissue using PS-MS. Spectra recorded using an LTQ ion trap mass spectrometer.

needle from the gray-matter region and the white-matter region, and two distinctive MS peak patterns were observed using the Exactive Orbitrap in the negative ion mode (Figure S-1, Supporting Information). The peaks at m/z 834.53 and 888.62 were identified as corresponding to PS (40:6) and ST (24:1), which are characteristic lipids in the gray-matter region and in the white-matter region, respectively. It can be seen that PS (40:6) was more abundant in the gray-matter region and ST (24:1) was more abundant in the white-matter region, which is in accordance with the results of a previous study.[25] When the ultrahigh resolution setting (100 000) of the Orbitrap was used, more lipids were identified from different organs of rat (kidney, liver, spinal cord, and brain), as shown in Figure S-2 (Supporting Information). The possible lipids which gave signals with the relative abundance higher than 5% in the brain gray-matter region were listed in Table S-1 (Supporting Information).

Therapeutic Drugs. Most therapeutic drug testing is done with blood samples because it is less invasive than collecting a tissue biopsy. However, due to differences in efficiency of drug delivery, the blood concentration of the drug may not correlate with the concentration of the drug in different organs.[40] It is, therefore, highly desirable to find out the local effective concentration of drugs at the disease site and to evaluate the side effect of drugs at the organs where they are metabolized. Different imaging methods have been utilized to identify the distribution of the drugs and their metabolites in tissue, including magnetic resonance imaging,[41] positron emission tomography,[42] autoradiography,[43] and near-infrared fluorescence.[44] The spatial resolution of these methods ranges from micrometers to millimeters, and the time required for analysis ranges from minutes to hours. Chemical reagents are usually needed to enhance imaging contrast and spatial resolution.

In a preliminary experiment, therapeutic drugs of interest were deposited onto the tissue section or spiked into the tissue homogenate and the samples were analyzed using the LTQ ion trap mass spectrometer with paper spray. Atenolol (0.36 ng, on 1 mm^2 mouse adrenal gland section) was identified as shown in

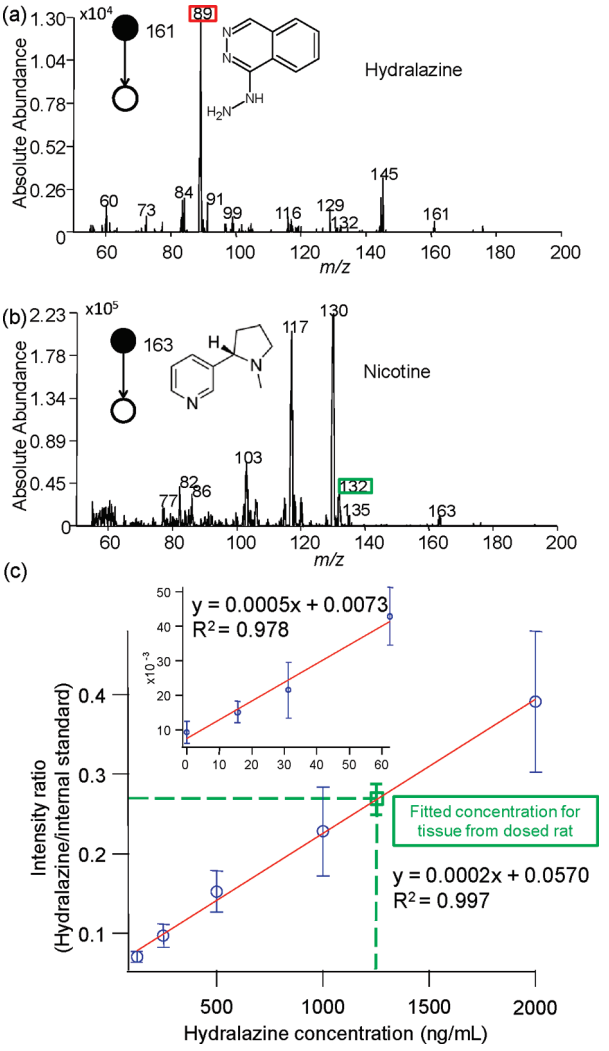


Figure 4. Detection and quantitation of hydralazine in the rat kidney tissue. (a) MS/MS spectrum of hydralazine from the tissue homogenate of rat kidney. (b) MS/MS spectrum of nicotine spiked as internal standard. (c) Calibration curve obtained for quantitative analysis of hydralazine in kidney tissue homogenate. Analysis done by single reaction monitoring (SRM) using a TSQ triple quadrupole mass spectrometer.

Figure S-3a, b (Supporting Information). Imatinib, an FDA approved anticancer drug, was spiked into mouse liver tissue homogenate, and then, 0.5 μL of the sample was applied to the paper surface to form a dried spot. Spray solvent was applied subsequently for paper spray, and an MS spectrum for 2.5 ng of imatinib and an MS/MS spectrum for 250 pg of imatinib in 0.5 μL tissue homogenate were recorded as shown in Figure S-3c,d (Supporting Information), respectively.

To validate the fact that paper spray mass spectrometry (PS-MS) could be used for quantitative analysis of drugs in the tissue, a rat was dosed with hydralazine, an FDA approved antihypertensive. Tissue homogenates from several organs were spotted onto the paper for analysis. Hydralazine could be directly identified from the tandem mass spectra for kidney (Figure 4a), liver, and spinal cord tissues. To estimate the concentration of hydralazine in the kidney tissue, a calibration curve was acquired using the kidney tissue homogenate samples from another rat not treated with hydralazine. The samples were prepared with hydralazine spiked

dx.doi.org/10.1021/ac103150a |*Anal. Chem.* 2011, 83, 1197–1201

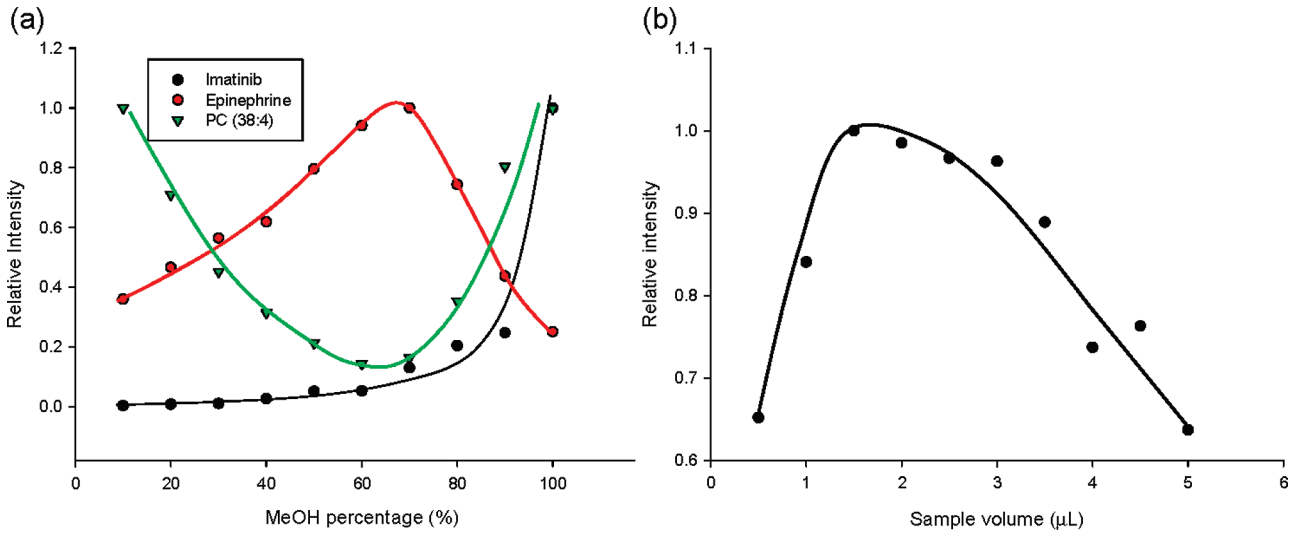


Figure 5. (a) Effect of spray solvent on analysis of imatinib, EP, and PC (38:4) in porcine adrenal gland and (b) effect of sample amount on the detection of imatinib in mouse liver tissue homogenate samples.

at different concentrations (from 16 to 2000 ng/mL) and with nicotine spiked as internal standard at a constant concentration (250 ng/mL). Quantitative analysis was performed using the intensity ratio of the characteristic fragment at m/z 89 for hydralazine and m/z 132 for nicotine in single the reaction monitoring (SRM) mode using TSQ triple quadrupole mass spectrometer. The tissue homogenates from the drug-dosed rat were prepared in the same procedure but only with the nicotine spiked as internal standard. The concentration of dosed hydralazine in the tissue homogenate was measured to be 1.26 μg/mL by fitting to the calibration curve. Since 1 mL of homogenate was made from 0.3 g of tissue, the concentration of the dosed hydralazine in the bulk kidney tissue was calculated to be 4.2 μg per gram.

Direct analysis of the dosed hydralazine in bulk rat kidney tissue has also been explored (Figure S-4, Supporting Information), and it was found that the observed spectral intensity for the drug was lower than that for the tissue homogenate samples containing the same amount of drug. A comparison of the extraction efficiency was made by applying 10 μL of spray solvent containing nicotine as internal standard to bulk kidney tissue on paper and to a tissue homogenate dried spot on paper, respectively. The intensity ratios of drug and internal standard were obtained for each case, and the extraction efficiencies were compared. It was found that the extraction of the dosed drug from the tissue homogenate dried spot on paper is about 10 times more efficient than that for bulk tissue on paper.

Effects of Solvent Composition and Sample Volume. Biological tissues are complex mixtures, and it is foreseen that different solvents can extract chemicals with different efficiencies. However, for a paper spray experiment, the solvent affects not only the chemical extraction but also the ionization process. Therefore, the MS signal of particular chemicals is a convolution of the extraction efficiency and the ionization efficiency. To characterize the solvent effect, tissue homogenates made from porcine adrenal gland, which contains relatively large amount of hormones and phospholipids,[45] were used. Three chemicals were taken into account: the therapeutic drug imatinib (spiked at 500 ng/mL), the hormone epinephrine, and the phospholipid 38:4 GPCho. Different solvents were tested, including dimethylformamide, acetone, acetonitrile, a chloroform/methanol mixture, and a methanol/water mixture. It was found that only methanol/water mixtures gave relatively stable and strong signal intensities for these three chemicals. The other solvents did not work well, presumably due to the poor solubilities and/or poor spray formation. We further studied the relationship between the relative intensities of the target analytes with different percentages of methanol in the methanol/water mixture. As shown in Figure 5a, the intensity of imatinib increases with the increasing methanol percentage. Epinephrine and 38:4 GPCho gave opposite trends: the strongest epinephrine intensity was around 70% methanol whereas the weakest 38:4 GPCho intensity was around 60% methanol. However, as shown in Figure S-5 (Supporting Information), when each of these analytes was present as a pure chemical in solution and analyzed using paper spray, the observed solvent effects were significantly different from those for tissue homogenate. This suggests the existence of a strong matrix effect in tissue analysis as well as a possible suppression effect on phospholipids by epinephrine during paper spray. The maximum absolute intensity observed for an analyte in pure solution is higher than that for tissue homogenate samples, for instance, by a favor of five for imatinib. The sharper maxima observed for the pure solutions (Figure S-5, Supporting Information) presumably is due to the lack of the matrix effect.

The amount of tissue homogenate sample was also found to affect the signal intensity. A fixed volume, 10 μL, of the solvent was applied onto paper triangles of a fixed size (5 mm base by 10 mm height) with different amounts of mouse liver tissue homogenate spiked with imatinib at a concentration of 500 ng/mL. Maximum spectral intensity of imatinib was observed for the sample amount of 1.5 μL, as shown in Figure 5b. This phenomenon presumably is due to the trade-off among the total amount of analyte present, efficient extraction of the analyte by the solvent, and the matrix effect on ionization.

CONCLUSION

In this study, paper spray is used for direct analysis of tissue samples. Hormones, lipids, and therapeutic drugs can be readily analyzed from untreated tissue or tissue homogenates with minimum sample preparation. The method provides molecular information in a rapid and convenient fashion for the tissue biopsy

diagnosis and has potential application to fast screening in clinics. It differs from ambient ionization methods like DESI[46] in that it requires transfer of the tissue to be analyzed to the paper and, as such, it is not an imaging method; however, larger tissue sample sizes are generally used, making the data of higher quality than that in the imaging methods. It is also useful to compare this methodology with the solvent extraction method of Van Berkel[47] which also addresses the problem of simplified examination of analytes in a complex solid-phase material using solvent. Reliable quantitative analysis directly using untreated tissue will require better control of the sample amount during the biopsy as well as a reproducible means for transferring the sample onto the paper.

■ ASSOCIATED CONTENT

Supporting Information. Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: (765) 494-2214. Fax: (765) 496-1912. E-mail: ouyang@purdue.edu.

■ ACKNOWLEDGMENT

This work was supported by National Institutes of Health (1R21RR031246-01 and 1R21EB009459-01) and the Alfred Mann Institute at Purdue. The authors thank Indiana University School of Medicine for providing the human prostate tissue samples and Livia S. Eberlin and Allison L. Dill for preparing the tissue sections and for valuable discussions

■ REFERENCES

(1) Hawkridge, A. M.; Muddiman, D. C. *Annu. Rev. Anal. Chem.* **2009**, *2*, 265–277.
(2) Sakai, K.; Okuyama, H.; Yura, J.; Takeyama, H.; Shinagawa, N.; Tsuruga, N.; Kato, K.; Miura, K.; Kawase, K.; Tsujimura, T.; Naruse, T.; Koike, A. *Carcinogenesis* **1992**, *13*, 579–584.
(3) Schafer, K. C.; Denes, J.; Albrecht, K.; Szaniszlo, T.; Balog, J.; Skoumal, R.; Katona, M.; Toth, M.; Balogh, L.; Takats, Z. *Angew. Chem., Int. Ed.* **2009**, *48*, 8240–8242.
(4) Shimma, S.; Sugiura, Y.; Hayasaka, T.; Hoshikawa, Y.; Noda, T.; Setou, M. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2007**, *855*, 98–103.
(5) Gharib, H.; Goellner, J. R. *Ann. Intern. Med.* **1993**, *118*, 282–289.
(6) Lever, J. V.; Trott, P. A.; Webb, A. J. *J. Clin. Pathol.* **1985**, *38*, 1–11.
(7) Monzon, F. A.; Koen, T. J. *Arch. Pathol. Lab. Med.* **2010**, *134*, 216–224.
(8) Conrads, T. P.; Hood, B. L.; Petricoin, E. F.; Liotto, L. A.; Veenstra, T. D. *Expert Rev. Proteomics* **2005**, *2*, 693–703.
(9) Chen, H. W.; Wortmann, A.; Zhang, W. H.; Zenobi, R. *Angew. Chem., Int. Ed.* **2007**, *46*, 580–583.
(10) Bereman, M. S.; Nyadong, L.; Fernandez, F. M.; Muddiman, D. C. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 3409–3411.
(11) Chen, H. W.; Talaty, N. N.; Takats, Z.; Cooks, R. G. *Anal. Chem.* **2005**, *77*, 6915–6927.
(12) Ifa, D. R.; Wu, C. P.; Ouyang, Z.; Cooks, R. G. *Analyst* **2010**, *135*, 669–681.
(13) Weston, D. J.; Bateman, R.; Wilson, I. D.; Wood, T. R.; Creaser, C. S. *Anal. Chem.* **2005**, *77*, 7572–7580.
(14) Williams, J. P.; Scrivens, J. H. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 3643–3650.
(15) Harris, G. A.; Nyadong, L.; Fernandez, F. M. *Analyst* **2008**, *133*, 1297–1301.
(16) Glish, G. L.; Vachet, R. W. *Nat. Rev. Drug Discovery* **2003**, *2*, 140–150.
(17) Bovet, C.; Plet, B.; Ruff, M.; Eiler, S.; Granger, F.; Panagiotidis, A.; Wenzel, R.; Nazabal, A.; Moras, D.; Zenobi, R. *Toxicol. in Vitro* **2009**, *23*, 704–709.
(18) Kasumov, T.; Huang, H.; Chung, Y. M.; Zhang, R. L.; McCullough, A. J.; Kirwan, J. P. *Anal. Biochem.* **2010**, *401*, 154–161.
(19) Stoeckli, M.; Chaurand, P.; Hallahan, D. E.; Caprioli, R. M. *Nat. Med.* **2001**, *7*, 493–496.
(20) Wiseman, J. M.; Puolitaival, S. M.; Takats, Z.; Cooks, R. G.; Caprioli, R. M. *Angew. Chem., Int. Ed.* **2005**, *44*, 7094–7097.
(21) Huang, M. Z.; Hsu, H. J.; Wu, C. I.; Lin, S. Y.; Ma, Y. L.; Cheng, T. L.; Shiea, J. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 1767–1775.
(22) Nemes, P.; Woods, A. S.; Vertes, A. *Anal. Chem.* **2010**, *82*, 982–988.
(23) Colliver, T. L.; Brummel, C. L.; Pacholski, M. L.; Swanek, F. D.; Ewing, A. G.; Winograd, N. *Anal. Chem.* **1997**, *69*, 2225–2231.
(24) Ostrowski, S. G.; Van Bell, C. T.; Winograd, N.; Ewing, A. G. *Science* **2004**, *305*, 71–73.
(25) Eberlin, L. S.; Ifa, D. R.; Wu, C. P.; Cooks, R. G. *Angew. Chem., Int. Ed.* **2010**, *49*, 873–876.
(26) Nicola, A. J.; Muddiman, D. C.; Hercules, D. M. *J. Am. Soc. Mass Spectrom.* **1996**, *7*, 467–472.
(27) Wiseman, J. M.; Ifa, D. R.; Zhu, Y. X.; Kissinger, C. B.; Manicke, N. E.; Kissinger, P. T.; Cooks, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 18120–18125.
(28) Kertesz, V.; Van Berkel, G. J.; Vavrek, M.; Koeplinger, K. A.; Schneider, B. B.; Covey, T. R. *Anal. Chem.* **2008**, *80*, 5168–5177.
(29) McDonnell, L. A.; Corthals, G. L.; Willems, S. M.; van Remoortere, A.; van Zeijl, R. J.; Deelder, A. M. *J. Proteomics* **2010**, *73*, 1921–1944.
(30) Chen, L. C.; Nishidate, K.; Saito, Y.; Mori, K.; Asakawa, D.; Takeda, S.; Kubota, T.; Terada, N.; Hashimoto, Y.; Hori, H.; Hiraoka, K. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 2366–2374.
(31) Liu, J. J.; Wang, H.; Manicke, N. E.; Lin, J. M.; Cooks, R. G.; Ouyang, Z. *Anal. Chem.* **2010**, *82*, 2463–2471.
(32) Wang, H.; Liu, J. J.; Cooks, R. G.; Ouyang, Z. *Angew. Chem., Int. Ed.* **2010**, *49*, 877–880.
(33) Manicke, N. E.; Yang, Q.; Wang, H.; Oradu, S.; Ouyang, Z.; Cooks, R. G. *Int. J. Mass Spectrom.* **2010**, in press.
(34) Eberlin, L. S.; Dill, A. L.; Costa, A. B.; Ifa, D. R.; Cheng, L.; Masterson, T.; Koch, M.; Ratliff, T. L.; Cooks, R. G. *Anal. Chem.* **2010**, *82*, 3430–3434.
(35) Xu, Y.; Shen, Z. Z.; Wiper, D. W.; Wu, M. Z.; Morton, R. E.; Elson, P.; Kennedy, A. W.; Belinson, J.; Markman, M.; Casey, G. *JAMA* **1998**, *280*, 719–723.
(36) Wenk, M. R. *Nat. Rev. Drug Discovery* **2005**, *4*, 594–610.
(37) Fernandis, A. Z.; Wenk, M. R. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2009**, *877*, 2830–2835.
(38) Aboagye, E. O.; Bhujwalla, Z. M. *Cancer Res.* **1999**, *59*, 80–84.
(39) Zwaal, R. F.; Comfurius, P.; Bevers, E. M. *Cell. Mol. Life Sci.* **2005**, *62*, 971–988.
(40) Jones, G. R.; Pounder, D. J. *J. Anal. Toxicol.* **1987**, *11*, 186–190.
(41) Viglianti, B. L.; Abraham, S. A.; Michelich, C. R.; Yarmolenko, P. S.; MacFall, J. R.; Bally, M. B.; Dewhirst, M. W. *Magn. Reson. Med.* **2004**, *51*, 1153–1162.
(42) Fischman, A. J.; Alpert, N. M.; Rubin, R. H. *Clin. Pharmacokinet.* **2002**, *41*, 581–602.
(43) Solon, E. G.; Balani, S. K.; Lee, F. W. *Curr. Drug Metab.* **2002**, *3*, 451–462.
(44) Bremer, C.; Tung, C. H.; Weissleder, R. *Nat. Med.* **2001**, *7*, 743–748.
(45) Wu, C. P.; Ifa, D. R.; Manicke, N. E.; Cooks, R. G. *Analyst* **2010**, *135*, 28–32.
(46) Wiseman, J. M.; Li, J. B. *Anal. Chem.* **2002**, *82*, 8866–8874.
(47) Van Berkel, G. J.; Sanchez, A. D.; Quirke, J. M. E. *Anal. Chem.* **2002**, *74*, 6216–6223.