SAMPLE PRESENTATION DEVICE WITH DIFFERING WETTABILITY

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to sample presentation devices useful in performing analytical measurements. In addition, the present invention relates to the fabrication and use of sample presentation devices.

Background

Most scientific fields that involve some kind of chemical and biological analysis of a sample require researchers to be able to identify and measure compounds or analytes found in aqueous solutions (e.g., the measurement of proteins in blood plasma or the measurement of pesticides in runoff from streams). Here, analytes generally refer to component(s) of a liquid sample that are of interest to an investigator. Typically, fluid samples containing analytes are presented to an analytical measurement instrument by means of a container (e.g., test tube, multiwell plate, or cuvette) or other presentation device (e.g., slide or biochip). Because of the overriding interest in measuring a large number of samples quickly (so called "high throughput" measurement of samples), much attention has been paid to developing standardized containers and devices that can be used in connection with automated analytical instruments. For example, in the drug discovery field, researchers interested in screening drug candidates frequently screen thousands or even millions of possible drug candidates using various analytical techniques (e.g., fluorescence polarization detection), many of which use standard 384 well plates to contain the sample solutions containing the drug candidates. As such, sample presentation devices constitute a critically important component of a researcher's analytical equipment in a wide range of scientific fields, ranging from genomics and proteomics, drug development, clinical diagnostics, and analysis of environmental or biological toxins or agents (e.g., assessing environmental contamination and screening for possible agents used in bioterrorism).

In genomics and proteomics, for example, the focus is on the identification and study of DNA/RNA and proteins/peptides, respectively. These fields collectively refer to the systemic study of chemical and biological moieties in living organisms, their interactions, and the analytical techniques required to discern them. Understanding

complex living systems, rather than individual cell components, is a major focus of current biological and biomedical research in both fields. Specifically, a principal aim of genomics is to sequence and generate large databases of the gene content of entire organisms. Genomes have been compiled for bacteria, yeast, nematodes, drosophila, and, most recently, humans. Similarly, proteomics is the study of all proteins expressed at a specific time in the cell, a principal aim of which is to obtain partial protein amino acid sequences that can be used with database matching tools to identify an entire protein, as opposed to completely sequencing a protein. The identification of proteins allows for the study of protein expression (important to identify proteins that are differentially expressed under different conditions and biomarkers for disease states) as well as mapping protein interactions (which helps develop a picture of the cell architecture). Understanding the role of proteins is critical to our understanding of living systems, as proteins are the main component of biomatter and perform virtually all critical biological functions, from regulating reactions, to transport of oxygen, to providing cellular and extracellular structure. As with genomics, the burgeoning field of proteomics has resulted in the generation of information about the proteome of humans and other organisms, and, while this information is still incomplete, much of this information is and will be stored in databases. It is expected that much of our future understanding of living systems will be extracted from these genomic and proteomic databases.

In the field of clinical diagnostics, researchers focus on the identification and measurement of a wide range of analytes. The analytes of interest may be the actual drug candidates, such as in the example of bioavailability studies conducted in the course of clinical trials that reveal the extent to which a drug candidate is present throughout the organism. Alternatively, the analyte of interest may reflect a physiological response to a drug candidate, such as in the case of measuring the presence or absence of phosphorylated reaction products of kinase enzyme reactions. Because kinase enzymes are important in the growth and reproduction of cells, a high level of kinase activity is observed in patients suffering from diseases in which growth is abnormal (e.g., cancers). Drugs that result in a reduction of kinase activity are thus possible anti-cancer therapeutics, and analytical methods of detecting the efficacy of such drug candidates often focus on measuring the presence or absence of analytes in the form of kinase enzyme reaction products. These and other kinds of direct and indirect measurements of analytes of importance in clinical

diagnostics and drug development depend on the existence of analytical techniques and sample presentation devices that facilitate their measurement.

The importance of sample presentation devices is by no means limited to the biomedical context. For example, researchers interested in determining the extent of environmental contamination (or remediation) need to be able to screen environmental samples of all kinds, including water, air, and soil samples. Many of the analytical techniques used to analyze such sample involve analysis of liquid samples, as is the case of water quality studies or in the case of soil samples that have been extracted by diluting in organic and/or inorganic solvents so as to remove various components. Sample presentation devices that can present liquid samples for analysis are therefore an important tool in accomplishing these kinds of analytical measurements.

In the post-September 11 world, governments are confronted with the need for platforms and analytical techniques to facilitate the detection of chemical and biological agents in both military and civil scenarios. Challenges for biowarfare detection include sample collection and distinguishing between innocuous versus toxic organisms. The current battlefield technique for bioagents utilizes pyrolysis to convert biological compounds to small molecules that can be more easily detected by mass spectometry (MS). Development of techniques that rely upon protein or peptide biomarkers is anticipated, however, because it would be more specific than currently known methods, and could be used to determine potential exposure to warfare agents in combination with breath tests, urinalysis, or blood drawing techniques. Stand-alone biosensors as alerting devices are also of great interest for use on the battlefield as well as in public places. All of these methods present challenges in sample collection, pre-treatment, and presentation of samples to detectors.

A wide variety of analytical techniques have been developed to identify and measure compounds of interest in liquid samples, such as DNA, RNA, proteins, and peptides in blood sera, environmental toxins and agents in environmental samples. While each of these analytical techniques is useful in its own way, each is at least partially dependent upon the type of sample presentation device that is employed. Thus, limitations inherent to such devices may adversely affect the measurement of compounds of interest using these analytical techniques.

Moreover, many analytical techniques focused on identifying, isolating or measuring analytes in liquid samples require that the sample undergo separate pre-processing steps – i.e., processing of the sample before it is exposed to a particular analytical technique to determine the presence and amounts of analytes of interest. For example, many protein cell extraction techniques yield complex protein mixtures and incorporate detergents and salts that interfere with mass spectral analyses that must be removed prior to analysis of the proteins. Current methods of fractionation and purification are time-consuming. Other purification methods, such as liquid chromatography and gel electrophoresis used to purify proteins, routinely involve sample recovery of volumes greater than 10 μL, necessitating additional concentration prior to analysis by various protein detection techniques (e.g., MALDI-MS). The demands of currently known analytical techniques – and the sample presentation devices used in connection with them – underscore the importance of sample purification, sample preparation, automated data acquisition, and automated data analyses.

For example, the most common and preferred type of mass spectrometry used in the field of proteomics is matrix assisted laser desorption ionization mass spectrometry (MALDI-MS). MALDI-MS is a variation of standard laser desorption time-of-flight mass spectrometry wherein proteins of relatively high molecular mass are deposited on a surface in the presence of a very large molar excess of an acidic, UV absorbing chemical matrix (for example, nicotinic acid). This technique allows for desorption of these high molecular weight labile macromolecules in the intact state. Mass spectrometry has become an important analytical tool in proteomic efforts because it provides mass accuracy, sensitive detection, and rapid analysis of minute quantities of samples at moderate cost.

However, MALDI-MS suffers from various drawbacks, particularly problems associated with sample preparation. Collectively, present day MALDI-MS sample supports suffer from a severe sample volume limitation in that they are incompatible with sample volumes in excess of 2 μL. Volumes of up to 2 μL are routinely utilized and afford dried-droplets having a diameter of from 1 mm to 2 mm. (Karas, M. and Hillenkamp, F. *Anal. Chem.* **1988**, *60*, 2299-2301, incorporated herein by reference). Because the laser irradiates only a small portion of the dried-droplet (from 0.015 mm^2 to 0.030 mm^2) during single-site data acquisition, there is no guarantee that all proteins in a sample will be detected. In addition, the sample volume (up to 2 μL) is significantly smaller than the volume in which samples are routinely recovered after purification necessitating their

further concentration prior to MALDI-MS; for example, peptide and protein samples purified by liquid chromatographic and electrophoretic methods are routinely recovered in volumes greater than 10 μL. As a result, such samples must be further concentrated prior to MALDI-MS. Many samples also contain detergents and salts that interfere with mass spectral analyses, necessitating their removal prior to MALDI-MS.

Another drawback associated with MALDI-MS is lack of sample homogeneity. Even volumes as small as 2 μL can prove problematic owing to sample heterogeneity when the dried-droplet approach to sample application is utilized. Sample volumes in the range 0.5–2.0 μL are routinely utilized and dried, which afford dried-droplets having a diameter of from 1 mm to 2 mm. (Karas, M. and Hillenkamp, F. *Anal. Chem.* **1988**, 60, 2299-2301, incorporated herein by reference). Consequently, only a minute portion of the dried-droplet (from 0.015 mm^2 to 0.030 mm^2) is irradiated by the laser during single-site data acquisition. Unfortunately, even small volumes of 0.5–2.0 μL are known to result in sample heterogeneity (the heterogeneous deposition of analytes), which gives rise to significant variations in peak presence, intensity, resolution and mass accuracy when focusing the laser on different regions of the dried-droplet (Strupat, K.; Karas, M.; Hillenkamp, F. *Int'l. J. Mass Spectrom. Ion Processes* **1991**, 111, 89-102; Cohen, S. L. and Chait, B. T. *Anal. Chem.* **1996**, 68, 31-37; and Amado, F. M. L.; Domingues, P.; Santana-Marques, M. G.; Ferrer-Correia, A. J.; Tomer, K. B. *Rapid Commun. Mass Spectrom.* **1997**, 11, 1347-1352, all of which are incorporated herein by reference). These phenomena render necessary the critical inspection of the mass spectral data as well as the accumulation of a large number of single-site spectra per sample. Therefore, only a few hundred samples can be analyzed per day per instrument, and automatic data acquisition is often precluded.

It has been demonstrated that the problem of sample heterogeneity can be minimized as the spot diameter falls to the order of the laser diameter. In that case, a large portion of the sample can be irradiated simultaneously, improving sensitivity and reproducibility (Little, D. P.; Cornish, T. J.; ODonnell, M. J.; Braun, A.; Cotter, R. J.; Koster, H. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *69*, 4540-4546; and Gobom, J.; Nordhoff, E.; Mirgorodskaya, E.; Ekman, R.; Roepstorff, P. *J. Mass Spectrom.* **1999**, *34*, 105-116, incorporated herein by reference). The sample supports described in United States Patent No. 6,287,872 are further described (Schuerenberg, M.; Lubbert, C.; Eickhoff, H.; Kalkum, M.; Lehrach, H; Nordhoff, E. *Anal. Chem.* **2000**, *72*, 3436-3442, incorporated herein by

reference), wherein it is shown that confining the deposition of analytes to a small spot diameter not only reduces problems associated with sample heterogeneity, but also results in a significant increase in sensitivity of detection. The drawback is that to obtain this desired spot size, sample volumes have to be reduced to less than 2 μL.

To overcome these sample volume and impurity problems, researchers have employed sample supports designed or mini-columns used to pre-process samples. An example of such a sample support is commercially available as the AnchorChip™ from Bruker Daltonics GmbH. The AnchorChip™ products improve MALDI-MS sensitivity by concentrating the sample in a precisely-defined location, and specifically involve a thin layer of nonwettable hydrophobic material that carries an array of wettable hydrophilic spots. A principal limitation associated with the use of the AnchorChip™ is the requirement that the volume of liquid sample applied to each anchor be limited to from 0.50 μL to 3.0 μL (No. 1 of Eleven General Rules for Sample Preparation on AnchorChip™ Targets, see AnchorChip™ Technology, Reivsion 1.6, Bruker Daltonics GmbH, November 2000, incorporated herein by reference); the examples provided by the manufacturer in the product's literature further limit the liquid sample drop volume to either 0.5 μL or 1.0 μL. Another limitation is that both analytes and contaminants (salts, detergents) often get concentrated in the laser-irradiating region. Therefore, samples must first be desalted and/or concentrated on a ZipTip® or similar mini-column sample preparation device prior to application onto mass spectrometer sample supports, as described above. (ZipTips®, made by Millipore Corp., are micro-columns for sample concentration and desalting prepared by packing small pipette tips with reverse phase chromatographic media. (Rusconi, F.; Schmitter, J.-M.; Rossier, J.; le Maire, M. *Anal. Chem.* **1998**, *70*, 3046-3052, incorporated herein by reference)). However, the use of home-made micro-columns or commercially available ZipTips® is time consuming, adds considerable cost, has proven difficult to automate and often affords only moderate recoveries of sample material. Therefore, AnchorChips™ suffer many of the same limitations associated with other present day MALDI-MS sample supports.

An alternative technique to MALDI-MS has been developed for protein profiling of serum samples. This technique is called surface enhanced laser desorption ionization mass spectrometry (SELDI-MS), and it has produced results with respect to the discovery of biomarkers for ovarian cancer and for differentiation of prostate cancer and benign prostate hyperplasia. During SELDI-MS, analytes are first selectively retained on a sample support

having a functionalized surface that acts as an affinity capture device. The retained analytes are then ionized by laser desorption at the point of capture to enable their detection without the need to effect their recovery from the retentive surface as is required for other hyphenated liquid chromatography-mass spectrometry approaches. SELDI-MS is described in United States Patent Nos. 5,719,060; 5,894,063; 6,020,208; 6,027,942; 6,124,137; 6,225,047; and 6,579,719, all incorporated herein by reference. Despite the restuls recently reported, the SELDI-MS approach is often problematic in practice as surfaces which are optimum with respect to retention of biological analytes can exhibit less than optimum performance with respect to analyte presentaion during laser desportion ionization.

Still other techniques used to isolate and purify analytes, such as proteins, have been used. For example, fractionation and purification approaches for biological samples via the time consuming techniques of 2D gel electrophoresis and multi dimensional liquid chromatography are well known, as are quicker, low sensitivity techniques such as consumable columns or pipette tips with chromatography beds. Gel electrophoresis, which serves to separate protein mixtures, can be either one or two dimensional. In 1D gel electrophoresis, also known as SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), protein mixtures are separated by their molecular weight only. In 2D gel electrophoresis, also known as 2D-PAGE, mixtures are separated by their isoelectric point followed by their molecular weight. One disadvantage of the technique is that the method has poor resolution, i.e., each resolved spot might contain more than one protein. Another disadvantage is that the dyes used to see the separation do not stain all of the proteins. Liquid chromatography (LC) is known as "high performance liquid chromatography" (HPLC) or "multi-dimensional liquid chromatography," if more than one chromatographic column is used. The advantage of LC in general is the availability of diverse column chemistries. In contrast to gel electrophoresis, which cannot efficiently separate the smaller peptides, LC can be used to separate peptide mixtures from enzymatic digests. Solid phase extraction (SPE) provides a fast way of purification and it is used in many areas, from organic synthesis to environmental sample collection. It is faster than liquid-liquid extraction or HPLC, it consumes less solvent and can be used to extract analytes from gas or liquid samples. The technique of SPE is offered in a variety of devices, such as pipette tips, columns, membranes, and 384-well plates, to mention a few.

In drug discovery, still other sample presentation devices have been developed for use in known analytical methods. For example, ADMET (Absorption Distribution Metabolism Excretion Toxicology) studies using the Empore card (http://www.3m.com/empore), a C18 RP (reverse phase) sorbent embedded in a membrane, are touted as capable of reducing the number of steps in sample purification and the potential for archiving and concentrating because the loaded samples are kept dry. Sample purification requires three steps: loading of samples on to the card, transferring the card to the eluter, and eluting 100% of the sample directly into a mass spectrometer. The Empore card could be used to load peptide digest samples on a MS if the elution volumes are kept as low as possible, otherwise low concentration peptides are below the limit of detection.

Therefore, a need exists for sample presentation devices that can be used in connection with various analytical methods to detect with high sensitivity biological and chemical moieties. Moreover, there is a need for sample presentation devices that are compatible with the sample volumes routinely recovered from liquid chromatographic and electrophoretic separations and other kinds of separation/purification techniques, that direct a liquid sample containing analytes to a confined area so as to minimize the problems associated with sample heterogeneity, that result in an increase in sensitivity of detection. The availability of such sample presentation devices would enable automated sample processing, such as, for example, on the life science industry's standard multi-well plate processors and liquid handling robots. More importantly, they also enable the direct collection and subsequent MALDI-MS analysis of chromatographic eluates. Furthermore, these capabilities would collectively enhance the throughput of the detection and measurement of biological and chemical moieties using the various analytical techniques known to those of skill in the art. These and other benefits of the present invention are described in more detail below.

SUMMARY OF THE INVENTION

The sample presentation devices of the present invention provide attractive alternatives to known sample presentation devices used in various analytical methods used for the identification of chemical and biological entities. In addition, the present invention provides methods of making the sample presentation devices as well as methods of using them to perform a wide range of analytical measurements of analytes contained in liquid

samples. The unique properties of the sample presentation devices of the present invention address many of the shortcomings (described above) associated with known analytical techniques and the sample presentation devices or containers used in connection with them.

In fields such as genomics, proteomics, drug discovery, clinical diagnostics, biosensors, and detection of environmental toxins and agents, mass spectrometry is a technique used to identify chemical and biological moieties, wherein often only very small quantities of the samples are available, and wherein rapid throughput of large numbers of samples is desirable. Other analyte detection methods, such as fluorescence polarization, immunofluorescence spectroscopy, gel chromatography, ion exchange chromatography, affinity chromatography, can also be used for high throughput detection of biological and chemical moieties, and can thus also be used in combination with the sample presentation devices of the present invention.

The sample presentation devices of the present invention provide attractive alternatives to known sample presentation devices used in various analytical methods. For example, the present invention allows for analytes to be selectively retained and concentrated on the surface of the biochip in volumes up to 100 μL. In addition, because analytes are detected from a portion of the sample presentation device that is designed to be substantially non-binding or binding resistant, they may be detected at high sensitivity as compared to direct detection on the surface of a biochip-based affinity capture device, or other sample presentation devices in which the surfaces of the devices have significant affinity for the analytes.

The present invention further minimizes the potential losses associated with the transfer of analytes from one surface to another because the present sample presentation devices, in a preferred embodiment, require only a single liquid manipulation. This, coupled with the analyte-resistant properties of the sample presentation device surfaces, results in a reduction in the loss of the analytes of interest as is the case in known methods. In contrast to SELDI-MS, the present invention does not involve desorption of bound analytes from the point of capture by an affinity capture device, but rather uses sample presentation devices wherein the desorption of analytes from a surface having no appreciable affinity or binding of the analytes to that surface.

In addition, the liquid samples can be manipulated and moved on the surfaces of the sample presentation devices of the present invention in a controlled fashion. This allows for the samples to be concentrated to an analysis zone where there is no substantial binding of analyte to the surface of the sample presentation device. Moreover, this allows the analyte-containing samples to be moved to different zones on the surfaces, each zone having different properties with respect to an analyte, which allows for purification, isolation and/or modification of the analytes prior to detection. In addition, the present invention involves sample presentation devices in which the properties of various portions of the surfaces may change in response to various chemical or physical stimuli (e.g., heat, UV radiation), such that the properties of such surfaces with respect to analytes can be manipulated during sample handling. Such changes in surface properties may be designed to be reversible or non-reversible.

These and other features of the sample presentation devices of the present invention are described in more detail below. The present invention comprises sample presentation devices, methods of making sample presentation devices, and methods of using sample presentation devices.

Sample Presentation Devices

The present invention relates to sample presentation devices that are useful in performing analytical measurements. One aspect of the present invention provides a sample presentation device for detecting analytes in a sample comprising a substrate having a surface, wherein the surface is comprised of a plurality of zones of differing wettability, and wherein the zone from which analytes in the sample are detected is substantially analyte binding resistant.

An alternate aspect of the present invention provides a sample presentation device for storing analytes from a sample comprising a substrate having a surface, wherein the surface is comprised of a plurality of zones of differing wettability, and wherein the zone in which the analytes from the sample are stored is substantially analyte binding resistant.

Another aspect of the present invention provides a sample presentation device for storing analytes from a sample comprising a substrate having a surface, wherein the surface is comprised of a plurality of zones of differing wettability, and wherein the zone in which the analytes from the sample are stored substantially binds analytes.

The sample presentation devices of the present invention may be comprised of distinct zones, one of which is optimal with respect to the retention of a liquid sample. The sample presentation devices of the present invention may further comprise distinct zones of wettabilty, one of which is optimal with respect to high sensitivity detection of analytes.

The sample presentation devices of the present invention may comprise two-dimensional or three-dimensional surfaces, each of which having two or more zones of differing wettability.

The sample presentation devices of the present invention comprise a substrate, which can be made from a variety of materials, including but not limited to, for example, glasses, , semiconductors, metals, polymers (e.g., plastics), and other hydroxylated materials, e.g., SiO_2 on silicon, Al_2O_3 on aluminum, etc. Preferably, the substrate is a metal, such as gold, or semiconductor, such as silicon.

The sample presentation devices of the present invention further comprise a substrate that has been surface-modified by methods known to those of ordinary skill in the art in order to create various zones on the surface of the substrate, which zones have differing properties with respect to wettability. Such surface modifications include but are not limited to the addition of self-assembled monolayers (SAMs), polymers (linear and branched), and Langmuir-Blodgett assemblies to the substrate. Using SAMs as an example, when added to the substrate, the SAMs create a surface of the sample presentation devices to which liquid samples may be exposed. Depending on the composition of the particular SAMs used, the surfaces of the sample presentation devices of the present invention may have different properties in terms of wettability, and in terms of affinity (or lack thereof) for analytes in liquid samples. The SAMs may be added to the sample presentation devices of the present invention in a manner that creates distinct zones whose properties reflect the SAMs used in a particular zone. Other surface modification techniques known to those of skill in the art are also included in the present invention.

With respect to the kinds of zones that the surfaces of the sample presentation devices may include, they are characterized primarily by virtue of their differing wettability with respect to the sample to be analyzed, which in turn results in zones that have differing abilities to retain or bind analytes in liquid samples. These zones are broadly termed "boundary zones," "liquid retention zones," and "analysis zones." The present invention only requires the presence of two types of zones, although inclusion of more than two types of zones is also contemplated. The present invention may also include more than one zone of each kind – e.g., the sample presentation devices may comprise multiple liquid retention zones, each of which may have different properties with respect to a liquid sample and/or the analytes contained therein.

A first type of zone is termed a "boundary zone" and involves a substantially non-wettable zone with respect to the sample to be analyzed. The boundary zone is the zone with the highest contact angle with respect to the sample in comparison to the other zones.

A second type of zone, termed the "liquid retention zone," is relatively more wettable in comparison to the boundary zone with respect to the sample to be analyzed (and is relatively less wettable than the analysis zone, described below). The liquid retention zone has a contact angle relatively lower than the contact angle of the boundary zone (and a contact angle relatively higher than the contact angle of the analysis zone, described below). The liquid retention zone can also have equal or lower contact angle than the analysis zone initially, but because of chemical or physical stimuli, the liquid retention zone may assume a higher contact angle than the analysis zone prior to the chemical or physical stimuli, which results in the liquid sample being directed to one zone preferentially over another.

The liquid retention zone can be of two subtypes. In one subtype, the liquid retention zone is designed to operate for liquid sample retention purposes, while being substantially analyte binding resistant. In a second subtype, the liquid retention zone is designed to retain a liquid sample, but also to substantially bind analytes within a liquid sample, and can thus be termed a "capture zone" in that it captures the analytes. This second subtype may also include a surface that is substantially analyte binding but that becomes substantially non-binding upon being subjected to chemical or physical stimuli, such as, for example, UV radiation, electricity, or heat.

A third type of zone is termed the "analysis zone" and is the zone that is the most wettable (and has the lowest contact angle) with respect to the sample in comparison to the other zones. The analysis zone is designed to be analyte binding resistant. The analysis zone may be optimized in terms of size, shape, and surface properties to enhance the sensitivity of the analysis of the desired analytes.

The liquid capacity of the sample presentation devices of the present invention is dependent on the sizes of the zones. For a 3 mm diameter circular zone, the liquid capacity can be up to about 100 µl. The sample presentation devices can contain this amount of liquid sample without the need for physical boundaries, reservoirs, or wells. The various zones can be precisely positioned in order to facilitate or be compatible with high throughput automation on various analytical instruments, such as, for example, mass spectrometry instruments.

In another embodiment of the sample presentation devices of the present invention, the sample presentation devices can be termed "target chips," and abbreviated Tn, where

"n" is a numerical designation referring to the number of distinct zones on the surface of the sample presentation device, where "n" can be any number from 2 to infinity. Thus, for example, a T2 target chip has two zones, a T3 target chip has three zones, etc. The present invention contemplates sample presentation devices containing many more than 2 or 3 zones and is not limited in any way to a specific number of zones. As the number of zones increases, the overall effect approaches a gradient. Target chips are sample presentation devices comprised of one or more zones that are designed to be resistant to analyte binding.

With respect to a T2 target chip, for example, the sample presentation device comprises two zones – i.e., a boundary zone and an analysis zone. The surfaces of the zone that contacts the liquid sample are designed to be analyte binding resistant – i.e., the analysis zone is analyte binding resistant. The surfaces of the zone that contacts the liquid sample effectively confine the analytes during the drying step before analysis.

With respect to a T3 target chip, the sample presentation device comprises three zones – i.e., a boundary zone, a liquid retention zone, and an analysis zone. The surfaces of the zones that contact the liquid sample are designed to be analyte binding resistant – i.e., the liquid retention zone and the analysis zone are analyte binding resistant. The surfaces of the zones that contact the liquid sample effectively concentrate the analytes to the analysis zone during the drying step.

The sample presentation devices of the present invention may thus comprise distinct zones, each of which exhibits a minimum of adsorption with respect to analytes.

In another embodiment of the sample presentation devices of the present invention, the sample presentation devices can be termed "capture chips" or "capture/concentrate chips," and abbreviated Xn, where "n" is a numerical designation referring to the number of zones on the surface of the sample presentation device, where "n" can be any number from 2 to infinity. Thus, for example, an X2 capture chip has two zones, an X3 capture chip has three zones, etc. The present invention contemplates sample presentation devices containing many more than 2 or 3 zones and is not limited in any way to a specific number of zones. As the number of zones increases , the overall effect approaches a gradient. Capture chips and capture/concentrate chips are sample presentation devices comprised of one or more zones that are designed to bind analytes.

With respect to an X2 capture chip, for example, the sample presentation device comprises two zones – i.e., a boundary zone and a capture zone. The surfaces of the zones that contact the liquid sample are designed to capture the analytes – i.e., the capture zone binds the analytes – based on the chemical or biological properties of the surfaces of the capture zone. The surfaces of the zones that contact the liquid sample effectively confine the analytes during the drying step before analysis.

With respect to an X3 capture/concentrate chip, the sample presentation device comprises three zones – i.e., a boundary zone, a capture zone, and an analysis zone. The boundary zone is designed to be substantially non-wettable. The capture zone is designed to capture and bind analytes. The analysis zone is designed to be analyte binding resistant. Analytes are transferred between the capture and analysis zones, which is done prior to analysis by one of the various known analytical detection methods. The surface of the analysis zone that contains the liquid sample effectively confines the analytes during the drying step before analysis. The transfer of the liquid sample from the capture zone to the analysis zone may be accomplished by virtue of the properties of the surface of the capture zone – i.e., if the capture zone has a lower degree of wettability than the analysis zone, the liquid sample will move from the capture zone to the analysis zone without physical intervention. Alternatively, the capture zone may be designed such that its properties may be changed in response to chemical or physical stimuli (e.g., heat, UV radiation), causing the capture zone to have a lower degree of wettability than the analysis zone, and thus causing the liquid sample to move from the capture zone to the analysis zone.

In yet another embodiment of the sample presentation devices of the present invention, the sample presentation devices can be combinations of the above-described target and capture chips. In this embodiment, the sample presentation devices are comprised of surfaces having different functionality. These kinds of sample presentation devices may involve the transfer of a liquid sample from one zone to another by mechanical means (e.g., via pipetting)or otherwise (e.g., via the differences in wettability between zones). As an example, a "capture-transfer-concentrate chip," abbreviated X2-transfer-T3, is a sample presentation device comprised of both an X2 chip comprised of two zones (i.e., a boundary zone and a capture zone), as well as a T3 chip comprised of three zones (i.e., boundary zone, liquid retention zone, and analysis zone). A transfer (mechanical or otherwise) of the analyte occurs between the capture zone of the X2 chip and the liquid retention zone of the T3 chip. In addition, the embodiments of the sample

presentation devices that involve combinations of capture zones and liquid retention zones may further be used in a combinatorial manner to isolate, concentrate, purify, and modify analytes in liquid samples prior to their detection. So, for example, a liquid sample may be placed onto a T2 chip such that the analytes in the sample are confined in the analysis zone. That sample may then be transferred to an X3 chip that contains a boundary zone, a capture zone, and an analysis zone. In this example, the capture zone may be designed to bind (and thus remove) lipid moieties from the liquid sample, such that when the sample is applied to the X3 chip, it moves from the boundary zone to the capture zone (which has a higher degree of wettability), the lipid moieties in the sample bind to the surface of the capture zone, and the remaining sample moves to the analysis zone (because it has the highest degree of wettability). In this example, the liquid sample is confined on the T2 chip, and then the lipids are moved on the X3 chip, such that the final sample that is analyzed from the analysis zone is concentrated and purified of lipids. Because the capture zones can be designed to bind a multitude of different analytes, and because various combinations of any of these zones may be used, sample presentation devices having a vast range of purification, concentration, isolation, and modification capabilities (vis-à-vis one or more analytes) can be created.

The mechanism of transfer of liquid samples from one sample presentation device to another may vary. Using the above example, the concentrated sample from T2 may be removed mechanically (e.g., by pipetting) and placed on a separate X3 sample presentation device. Alternatively, the T2 and X3 sample presentation devices may be connected by a zone, the wettability of which may be changed in response to chemical or physical stimuli (e.g., UV radiation), such that the concentrated sample in the analysis zone of the T2 sample presentation device is transferred to the capture zone of the X3 device when the exposure of a zone between them to UV radiation results in a wettability that is higher than the analysis zone of the T2 device but lower than that of the capture zone of the X3 device, such that the sample moves from T2 to X3. Again, with a vast number of surfaces (having different wettability and analyte binding properties) and configurations thereof, sample presentation devices having a vast range of purification, concentration, isolation, and modification capabilities (vis-à-vis one or more analytes) can be created.

The sample presentation devices of the present invention further provide zones of different wettability having different shapes or patterns. For example, in one embodiment, a sample presentation device may have zones in the form of concentric circles, with the

center zone being the analysis zone, surrounded by the liquid retention zone, surrounded by the boundary zone. Because the zones can be created using a various photo-patterning techniques, and because known photo-patterning techniques provide for tremendous variation in the resulting patterns, there is a vast range of possible shapes, patterns, and configurations of the various zones. Moreover, the various properties of the different zones of wettability allow for the creation of sample presentation devices capable of directing analytes to single or multiple specified or pre-determined locations on the surfaces (e.g., addressable sites, lanes, or fields).

The sample presentation devices of the present invention are suitable for the handling of both biological and non-biological liquid samples. They are also suitable for application in a wide range of analyte detection methods, for example, including but not limited to, mass spectrometry, various chromatographic methods, immunofluorescence spectroscopy, and other known analytical methods of detecting and measuring analytes in liquid samples.

Each of the above-described variations is designed to allow for maximum flexibility in design and use of sample presentation devices having enhanced capability to present analytes for detection and analysis over known methods. Thus, the sample presentation devices of the present invention have the capability of directing analytes to an analysis zone designed to enhance high sensitivity detection of analytes. The sample presentation devices of the present invention thus afford improved deposition of analytes.

Fabrication of Sample Presentation Devices

Still other embodiments of the invention include methods for creating or fabricating the sample presentation devices described above.

In an embodiment in which the surfaces are comprised of self-assembled monolayers (SAMs) which form distinct zones depending on differences between the SAMs used, the sample presentation devices of the present invention may comprise various SAM zones that are created by known photo-patterning techniques. Accordingly, the present invention further includes methods of creating sample presentation devices comprised of SAMs using, as one preferred method, photo-patterning techniques.

The surface of the substrate of the sample presentation device of the present invention is typically modified or patterned by methods known to those of skill in the art. As an example, the substrate's surface can be modified or patterned by means of applying

self-assembled monolayers (SAMs), which modify the surface of the substrate of the sample presentation device and whose exposed surfaces may impart particular chemistries to the substrate. Selection of various SAMs, including 1°, 2°, 3°, or 4° compositions, for a particular substrate provides the surface of the substrate with unique surface characteristics and properties. In particular, application of multiple SAMs results in the patterning of the substrate so that it contains a plurality of zones, each zone having different surface characteristics and properties. Methods of patterning the SAMs are known in the art, and include UV photo-patterning, photolithographic patterning, microstamping electron-beam patterning, and reactive-ion etching.

The zones that are created on the surface of the substrate can be in any shape, with circular shapes being preferred. In addition, the zones can be either continuous or discontinuous with respect to other zones - i.e., the zones can all be contiguous with each other or one or more zones can be discontiguous with one or more other zones. The zones that are created on the surface of the substrate of the sample presentation devices preferably have a plurality of zones of differing wettability with respect to the sample to be analyzed.

As another embodiment of the invention, methods of fabricating a sample presentation devices that are capable of precisely positioning analytes so as to facilitate automated data acquisition are provided.

Uses arid Applications of Sample Presentation Devices

In another embodiment, the sample presentation devices of the present invention find many uses in combination with various analytical techniques and procedures. Thus, the present invention includes methods for using the aforementioned sample presentation devices.

Virtually any analytical method that permits the detection, identification, or measurement of analytes in a liquid sample can be used in combination with the sample presentation devices of the present invention. Examples of such analytical methods include but are not limited to, MALDI-MS or electrospray ionization MS. The sample presentation devices are particularly well suited to us in combination with high throughput

Another aspect of the present invention provides a method of making a sample presentation device for detecting analytes in a sample, wherein the sample presentation device comprises a substrate having a surface, comprising modifying the surface of the substrate to create a plurality of zones of differing wettability, and wherein the zone from which the analytes in the sample are detected is substantially analyte binding resistant.

A further aspect of the present invention provides a method of storing analytes from a sample, comprising storing the analytes on a sample presentation device, wherein the sample presentation device comprises a substrate having a surface, wherein the surface is comprised of a plurality of zones of differing wettability and wherein the zone in which the analytes from the sample are stored is substantially analyte binding resistant.

A further aspect of the present invention provides the method as defined above, further comprising detecting the analytes in the sample concentrated in the zone of highest degree of wettability.

analytical measurement techniques, such as, for example, for use in MALDI-MS in which the sample presentation device analysis zones are configured in such fashion as to promote high throughput data acquisition.

The sample presentation devices of the present invention may also be used to manipulate liquid samples, and the analytes contained therein. Based on the differing wettability properties and capture properties that the surfaces of the sample presentation devices may be designed to have, the sample presentation devices may be designed to manipulate, concentrate, position, store, transfer (with and without mechanical intervention), recover (with or without mechanical intervention), analyze, modify or process (via use of analyte modifying reagents on the sample presentation devices), or fractionate liquid samples or the analytes contained therein. Moreover, because the sample presentation devices of the present invention may be designed to accomplish any of these functions in response to chemical or physical stimuli (e.g., heat, UV radiation, pressure, electromagnetic radiation), the sample presentation devices of the present invention may accomplish these functions reversibly or irreversibly, and may further perform various combinations of these functions in response to external forces.

Any liquid sample (and analytes) can be used in connection with the sample presentation devices of the present invention. For example, the present invention can be used to analyze fractions recovered from liquid chromatography. The present invention can be used to analyze enzymatic digests prepared from either protein spots excised from 2D gel electrophoresis or from fractions collected from affinity chromatography (i.e., ICAT (Isotope-Coded Affinity Tags)). The present invention can also be used to analyze samples recovered from biosensors. The present invention can also be used for 1:1 sample transfer with standard multi-well format robotics and assays. Indeed, the sample presentation devices of the present invention can be used to handle and manipulate liquid samples obtained from virtually any source, whether such samples are the result of laboratory experiment (such as the enzymatic digest and biosensor sample examples identified above), obtained from the environment (such as a water quality sample from a river), or obtained directly from living organisms (such as a human urine sample).

The present invention can also be used for storage of samples for archival purposes or for further analysis. In other words, the detection and analysis of the analytes contained

in liquid samples need not occur immediately following transfer of the liquid sample to the analysis zone.

Thus, various embodiments of the present invention provide for sample presentation devices that serve a variety of liquid-handling functions, including but not limited to sample/analyte handling, as well as liquid deposition, retention, transfer, locating and re-locating, and storage.

Features and Advantages

In addition to the many features and advantages of the present invention described in the summary of the invention section above, additional features and advantages include at least the following:

Analytical methods to detect analytes present in a liquid sample, such as MALDI-MS, can be performed from a single surface that is substantially analyte non-binding, resulting in increased sensitivity of analysis, increased reproducibility of results, and comparable results from different capture zones.

With respect to sample liquid handling, increased sample volumes – up to about 100 µl for a 3 mm diameter zone – can be analyzed, surfaces can be patterned having SBS (Society for Biomolecular Screening) standard well formats (i.e., 96/384/1536 well formats), and thus are able to be interfaced with common robotics and other high throughput analytical methods.

Increased throughput for the various analytical methods (e.g., MALDI-MS) can be achieved, in that zones are precision placed for high throughput data acquisition. With respect to MALDI-MS, the analysis zone is of optimal size (i.e., less than 2 mm^2, and preferably less than 1 mm^2). The sample/matrix has improved crystallization, leading to improved ionization consistency within the analysis zone. The smaller analysis zone as compared to dried spot analysis results in less area to interrogate, resulting in high throughput of analysis.

The sample presentation devices of the present invention enable analysis of diluted samples by means of the concentration of analyte in the analysis zone.

Separation of analytes in a liquid sample is possible without the need for multiple separation steps, such as with binding analytes to an ion exchange chromatography column and then having to isolate the analytes from the column in a subsequent wash step. Indeed,

by using SAMs with different surface chemistries designed to bind to different analytes, highly specific isolation and purification of particular analytes is possible.

A wide array of liquid samples and analytes can be handled by the sample presentation devices of the present invention, which avoid the shortcomings of known presentation devices and analytical methods described above. While the sample presentation devices of the present invention are particularly well suited to use in the proteomics field and laser desorption ionization mass spectroscopy, as is described in detail below the utility of the claimed devices is not in any way limited to only that field.

BRIEF DESCRIPTION OF THE FIGURES

The above and other objects of the present invention will become apparent from consideration of the detailed description presented in connection with the accompanying drawings in which:

FIG. 1*a* depicts a sample presentation device of the present invention, wherein the central analysis zone and the surrounding liquid retention zone are concentric with respect to one another, and wherein the liquid retention zone is surrounded by a boundary zone. FIG. 1*b* depicts a cross-sectional view of the sample presentation device depicted in FIG. 1*a*.

FIG. 2 depicts the surface of a sample presentation device of the present invention, wherein the surface is further comprised of 16 pairs of analysis zones and liquid retention zones, wherein the analysis zones and liquid retention zones are concentric with respect to one another, and wherein pairs of analysis zones and liquid retention zones are surrounded by a common boundary zone. In this instance, the sample presentation device is organized on geometries corresponding to standard 96-well plate.

FIG. 3 depicts the surface of a sample presentation device of the present invention, wherein a portion of the analysis zone and liquid retention zone are contiguous with respect to one another, wherein those portions of the analysis and liquid retention zones that are not contiguous with respect to one another are surrounded by a common boundary zone, and wherein the surface area of the analysis zone is smaller than the surface of the liquid retention zone.

FIG. 4*a* depicts the surface of a sample presentation device of the present invention, wherein the shape of the analysis zone has been designed to facilitate the

automated acquisition of mass spectral data. FIG. **4***b* depicts an enlargement of the analysis zone indicating 36 regions which measure approximately 100 μm^2, and which correspond to the individual regions that may be sampled by the laser during mass spectrometry.

FIG. **5** depicts the surface of a sample presentation device of the present invention, wherein the surface is further comprised of 96 pairs of analysis zones and liquid retention zones, wherein the analysis zones and liquid retention zones are concentric with respect to one another, and wherein pairs of analysis zones and liquid retention zones are surrounded by a common boundary zone. In this instance, the sample presentation device is organized on geometries corresponding to a standard 96-well plate. The liquid retention zone is elongated to maximize liquid-holding capacity and minimize the distance between adjacent zones. A serpentine pattern is overlaid on the first two rows of the sample presentation device to indicate the path described by deposition of a liquid stream of chromatographic eluate during automated fraction collection.

FIGS. **6***a* through **6***h* illustrate the steps involved in fabrication of a sample presentation device of the present invention, when alkylthiols on gold are utilized for surface modification and UV-photopatterning is exploited for surface patterning.

FIGS. **7***a* through **7***l* illustrate the steps involved in fabrication of a sample presentation device of the present invention, when alkylthiols on gold are utilized for surface modification and photolithography is exploited for surface patterning.

FIGS. **8***a* through **8***l* illustrate the steps involved in fabrication of a sample presentation device of the present invention, when alkylsilanes on silicon are utilized for surface modification and photolithography is exploited for surface patterning.

FIGS. **9***a* through **9***f* depict various stages during the process whereby a large volume of aqueous sample deposited on the surface of a sample presentation device of the present invention dries within the area corresponding to the analysis zone.

FIGS. **10***a* through **10***d* depict the surface and drop drying characteristics associated with a sample presentation device having a liquid retention zone and no analysis zone. FIGS. **10***e* through **10***h* depict the surface and drop drying characteristics associated with a sample presentation device having an analysis zone and no liquid retention zone.

FIGS. **11***a* through **11***h* depict images recorded on a video contact angle apparatus during the drying of a drop on the surface of a sample presentation device of the present

invention, wherein the analysis zone measures 0.6 mm diameter and the liquid retention zone measures 1.5 mm diameter.

FIG. **12** is a graph that summarizes the contact angle, drop width and drop height associated with the images depicted in FIGS. **11***a* through **11***h*.

FIG. **13** is a photograph of a sample presentation device of the present invention with liquid volumes of from 5 μL to 70 μL deposited thereupon.

FIG. **14***a* is a photograph of a sample presentation device of the present invention taken immediately after liquid drops of from 5 μL to 40 μL were deposited thereupon. Each of the liquid drops contained an equivalent amount of alpha-cyano-4-hydroxycinnamic acid (HCCA). FIG. **14***b* is a photograph of the HCCA having been concentrated and directed to the analysis zone due to sample drying on the sample presentation device depicted in FIG. **14***a*. A visual reference to the concentric zones is superimposed above the dried HCCA.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

"Analyte(s)" refers to component(s) of a sample which is desirably detected. The term can refer to a single component or to multiple components in the sample.

"Sample(s)" refers to any material derived from a biological or non-biological sources which is presented on the surface of a sample presentation device. The samples may be applied to the sample presentation devices in their original, untreated form and/or after treatments, including but not limited to modification, fractionation, extraction, and concentration. The samples of the present invention can be liquid or non-liquid samples.

"Substrate" refers to a material that is capable of presenting or supporting a surface.

"Surface" refers to the exterior or upper boundary of a body or a substrate .

"Substantially non-binding" or "binding resistant" or "analyte binding resistant" refers to the property of certain surfaces used in connection with the sample presentation

devices of the present invention that do not exhibit appreciable affinity or binding of an analyte to a surface. While some binding may occur, these surfaces are specifically designed to minimize binding to levels below the limit of detection of the analysis method employed.

"Surface tension" refers to a property of liquids in which a liquid drop deposited on a surface tends to contract to the smallest possible contact area because of unequal molecular cohesive forces near the surface.

"Wettability" refers to the degree to which a solid surface is wetted by a liquid sample. Unless otherwise specified, liquid samples are aqueous in nature.

"Contact angle" refers to the angle between the plane of the solid surface and the tangential line to the liquid drop boundary originating at the point of three phase contact (solid/liquid/vapor).

"Matrix" refers to materials used in mass spectroscopy techniques, such as MALDI-MS or SELDI-MS, for absorbing the energy of the laser and transferring that energy to analyte molecules, enabling ionization of labile macromolecules. In SELDI-MS, the matrix is referred to as "EAM" or "energy absorbing molecule." Reagents frequently used as matrices for the detection of biological analytes include but are not limited to *trans*-3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid, SA), α-cyano-4-hydroxycinnamic acid (HCCA) and 2,5-dihydroxybenzoic acid (DHBA). Other suitable matrices are known to those skilled in this art.

"SAM" refers to self-assembled monolayers. SAMs are molecular assemblies that are formed spontaneously by the immersion of an appropriate substrate into a solution of an active surfactant in an organic solvent.

Description of the Sample Presentation Devices of the Invention

The following description of the sample presentation devices of the present invention provides a more detailed understanding than set forth above in the summary of the invention. However, the sample presentation devices of the present invention are further described by reference to the figures, the methods of fabricating the sample presentation devices of the present invention, and the uses and applications of the sample presentation devices of the present invention, each of which is described in detail below.

As noted above, the sample presentation devices of the present invention provide attractive alternatives to known sample presentation devices used in various analytical methods used for the identification of chemical and biological entities. In addition, the present invention provides methods of making the sample presentation devices as well as methods of using them to perform a wide range of analytical measurements of analytes contained in liquid samples. The unique properties of the sample presentation devices of the present invention address many of the shortcomings (described in the background section above) associated with known analytical techniques and the sample presentation devices or containers used in connection with them.

More specifically, the sample presentation devices of the present invention provide attractive alternatives to known sample presentation devices used in a wide range of analytical methods. They have additional benefits, such as, for example, allowing for analytes in a liquid sample to be selectively retained and concentrated on the surface of the biochip in volumes up to 100 μL. In addition, because analytes are detected from a portion of the sample presentation device that is designed to be substantially non-binding or binding resistant, they are detected at high sensitivity as compared to direct detection on the surface of a biochip-based affinity capture device, or other sample presentation devices in which the surfaces of the devices have significant affinity for the analytes.

The present invention further minimizes the potential losses associated with the transfer of analytes from one surface to another because the present sample presentation devices, in a preferred embodiment, require only a single liquid manipulation. This, coupled with the analyte-resistant properties of the sample presentation device surfaces, results in a reduction in the loss of the analytes of interest.

In addition, because the analytes are not bound to affinity capture devices as in, for example, SELDI-MS biochips, the liquid samples can be manipulated and moved on the surfaces of the sample presentation devices of the present invention in a controlled fashion. This allows for the samples to be concentrated to an analysis zone where there is no substantial binding of analyte to the surface of the sample presentation device. Moreover, this allows the analyte-containing samples to be moved to different zones on the surfaces, each zone having different properties with respect to an analyte, which allows for purification, isolation and/or modification of the analytes prior to detection.

The present invention involves sample presentation devices in which the properties of various portions of the surfaces may change in response to various chemical or physical stimuli (e.g., heat, UV radiation), such that the properties of such surfaces with respect to analytes can be manipulated during sample handling. Such changes in surface properties may be designed to be reversible or non-reversible.

The present invention thus relates to sample presentation devices that are useful in performing analytical measurements. In one embodiment, the present invention involves sample presentation devices having surfaces with one or more zones of differing wettability with respect to various samples to be analyzed. These zones of differing wettability result in zones of differing abilities to retain, concentrate, and move analytes in liquid samples. These zones may be of various shapes and sizes, and may be continuous or discontinuous with respect to each other. The sample presentation devices of the present invention may comprise two-dimensional or three-dimensional surfaces, each of which having two or more zones of differing wettability.

The sample presentation devices of the present invention comprise a substrate, which can be made from a variety of materials, including but not limited to, for example, glasses, silicates, semiconductors, , metals, polymers (e.g., plastics), and other hydroxylated materials, e.g., SiO_2 on silicon, Al_2O_3 on aluminum, etc. Preferably, the substrate is a metal, such as gold, or a semiconductor, such as silicon. The sample presentation devices of the present invention further comprise a substrate that has been surface-modified by methods known to those of ordinary skill in the art in order to create various zones on the surface of the substrate, which zones have differing properties with respect to wettability. Such surface modifications include but are not limited to the addition of self-assembled monolayers (SAMs), polymers (linear and branched), and Langmuir-Blodgett assemblies to the substrate. Using SAMs as an example, when added to the substrate, the SAMs create a surface of the sample presentation devices to which liquid samples may be exposed. Depending on the composition of the particular SAMs used, the surfaces of the sample presentation devices of the present invention may have different properties in terms of wettability, and in terms of affinity (or lack thereof) for analytes in liquid samples. The SAMs may be added to the sample presentation devices of the present invention in a manner that creates distinct zones whose properties reflect the SAMs used in a particular zone. Other surface modification techniques known to those of skill in the art are also included in the present invention.

The sample presentation devices of the present invention are comprised of distinct zones, one of which is optimal with respect to the retention of a liquid sample. The sample presentation devices of the present invention may further comprise distinct zones of wettabilty, one of which is optimal with respect to high sensitivity detection of analytes.

With respect to the kinds of zones that the surfaces of the sample presentation devices may include, they are characterized primarily by virtue of their differing wettability with respect to the sample to be analyzed, which in turn results in zones that have differing abilities to retain or bind analytes in liquid samples. These zones are broadly termed "boundary zones," "liquid retention zones," and "analysis zones." The present invention only requires the presence of two types of zones, although inclusion of more than two types of zones is also contemplated. The present invention may also include more than one zone of each kind – e.g., the sample presentation devices may comprise multiple liquid retention zones, each of which may have different properties with respect to a liquid sample and/or the analytes contained therein. The various zones can be precisely positioned in order to facilitate or be compatible with high throughput automation on various analytical instruments, such as, for example, mass spectrometry instruments.

The "boundary zone" involves a substantially non-wettable zone with respect to the sample to be analyzed. The boundary zone is the zone with the highest contact angle with respect to the sample in comparison to the other zones.

The "liquid retention zone" is relatively more wettable in comparison to the boundary zone with respect to the sample to be analyzed (and is relatively less wettable than the analysis zone, described below). The liquid retention zone has a contact angle relatively lower than the contact angle of the boundary zone (and a contact angle relatively higher than the contact angle of the analysis zone, described below). The liquid retention zone can also have equal or lower contact angle than the analysis zone initially, but because of chemical or physical stimuli, the liquid retention zone may assume a higher contact angle than the analysis zone prior to the chemical or physical stimuli, which results in the liquid sample being directed to one zone preferentially over another. Moreover, the liquid retention zone can be of two subtypes. In one subtype, the liquid retention zone is designed to operate for liquid sample retention purposes, while being substantially analyte binding resistant. In a second subtype, the liquid retention zone is designed to retain a liquid sample, but also to substantially bind analytes within a liquid sample, and can thus

be termed a "capture zone" in that it captures the analytes. This second subtype may also include a surface that is substantially analyte binding but that becomes substantially non-binding upon being subjected to chemical or physical stimuli, such as, for example, UV radiation, electricity, or heat.

The "analysis zone" is the zone that is the most wettable (and has the lowest contact angle) with respect to the sample in comparison to the other zones. The analysis zone is designed to be analyte binding resistant. The analysis zone may be optimized in terms of size, shape, and surface properties to enhance the sensitivity of the analysis of the desired analytes.

Among other benefits, the sample presentation devices of the present invention are able to retain and handle liquid sample volumes that are larger than other biochips used in sample handling, due to the differences in wettability between zones. While the liquid capacity of the sample presentation devices of the present invention is dependent on the sizes of the zones; for a 3 mm diameter circular zone, the liquid capacity can be up to about 100 μL, and at least up to about 70 μL. The sample presentation devices can contain this amount of liquid sample without the need for physical boundaries, reservoirs, or wells.

In another embodiment of the sample presentation devices of the present invention, the sample presentation devices can be termed "target chips," and abbreviated Tn, where "n" is a numerical designation referring to the number of distinct zones on the surface of the sample presentation device, where "n" can be any number from 2 to infinity. Thus, for example, a T2 target chip has two zones, a T3 target chip has three zones, etc. The present invention contemplates sample presentation devices containing many more than 2 or 3 zones and is not limited in any way to a specific number of zones. As the number of zones increases , the overall effect approaches a gradient. Target chips are sample presentation devices comprised of one or more zones that are designed to be resistant to analyte binding. With respect to a T2 target chip, for example, the sample presentation device comprises two zones – i.e., a boundary zone and an analysis zone. The surfaces of the zone that contacts the liquid sample are designed to be analyte binding resistant – i.e., the analysis zone is analyte binding resistant. The surfaces of the zone that contacts the liquid sample effectively confine the analytes during the drying step before analysis. With respect to a T3 target chip, the sample presentation device comprises three zones – i.e., a boundary zone, a liquid retention zone, and an analysis zone. The surfaces of the zones

that contact the liquid sample are designed to be analyte binding resistant – i.e., the liquid retention zone and the analysis zone are analyte binding resistant. The surfaces of the zones that contact the liquid sample effectively concentrate the analytes to the analysis zone during the drying step. The sample presentation devices of the present invention may thus comprise distinct zones, each of which exhibits a minimum of adsorption with respect to analytes.

In another embodiment of the sample presentation devices of the present invention, the sample presentation devices can be termed "capture chips" or "capture/concentrate chips," and abbreviated Xn where "n" is a numerical designation referring to the number of zones on the surface of the sample presentation device, where "n" can be any number from 2 to infinity. Thus, for example, an X2 target chip has two zones, an X3 target chip has three zones, etc. The present invention contemplates sample presentation devices containing many more than 2 or 3 zones and is not limited in any way to a specific number of zones. As the number of zones increases , the overall effect approaches a gradient. Capture chips and capture/concentrate chips are sample presentation devices comprised of one or more zones that are designed to bind analytes. The moieties responsible for capturing analytes typically comprise specific surface modifications that are designed as the distinguishing feature of the capture zone. These surface modifications may comprise biological and chemical moieties that bind analytes specifically (such as monoclonal antibodies) or non-specifically (such as charged groups that bind on the basis of electrostatic attraction) or any combination of such attractive forces. In addition to the ability to capture an analyte of interest, these surface modifications may also retain the analytes in a liquid sample to permit subsequent modification. So, for example, a sample presentation device of the present invention that comprises a capture zone in which the surface modification is a monoclonal antibody may bind a complimentary antigen from a liquid sample and retain that antigen while the rest of the liquid sample moves to another part of the surface of the device, through either physical transfer or differences in wettability. The retained antigen may be modified via the addition of other compounds to the capture zone of the sample presentation device (e.g., the addition of an enzyme that cleaves off a part of the antigen). The modified antigen can then be transferred to another portion of the sample presentation device for further handling, or removed from the device for analysis by known techniques.

With respect to an X2 capture chip, for example, the sample presentation device comprises two zones – i.e., a boundary zone and a capture zone. The surfaces of the zones that contact the liquid sample are designed to capture the analytes – i.e., the capture zone binds the analytes – based on the chemical or biological properties of the surfaces of the capture zone. The surfaces of the zones that contact the liquid sample effectively confine the analytes during the drying step before analysis. With respect to an X3 capture/concentrate chip, the sample presentation device comprises three zones – i.e., a boundary zone, a capture zone, and an analysis zone. The boundary zone is designed to be substantially non-wettable. The capture zone is designed to capture and bind analytes. The analysis zone is designed to be analyte binding resistant. Analytes are transferred between the capture and analysis zones, which is done prior to analysis by one of the various known analytical detection methods. The surface of the analysis zone that contains the liquid sample effectively confines the analytes during the drying step before analysis. The transfer of the liquid sample from the capture zone to the analysis zone may be accomplished by the properties of the surface of the capture zone – i.e., if the capture zone has a lower degree of wettability than the analysis zone, the liquid sample will move from the capture zone to the analysis zone without physical intervention. Alternatively, the capture zone may be designed such that its properties may be changed in response to chemical or physical stimuli (e.g., heat, UV radiation), causing the capture zone to have a lower degree of wettability than the analysis zone, and thus causing the liquid sample to move from the capture zone to the analysis zone.

In another embodiment of the sample presentation devices of the present invention, the sample presentation devices can be combinations of the above-described target and capture chips. In this embodiment, the sample presentation devices are comprised of surfaces having different functionality. These kinds of sample presentation devices may involve the transfer of a liquid sample from one zone to another by mechanical means (e.g., via pipetting) or otherwise (e.g., via the differences in wettability between zones). As an example, a "capture-transfer-concentrate chip," abbreviated X2-transfer-T3, is a sample presentation device comprised of both an X2 chip comprised of two zones (i.e., a boundary zone and a capture zone), as well as a T3 chip comprised of three zones (i.e., boundary zone, liquid retention zone, and analysis zone). A transfer (mechanical or otherwise) of the analyte occurs between the capture zone of the X2 chip and the liquid retention zone of the T3 chip.

These sample presentation devices may involve more than one "capture zone," such that the surfaces may exhibit binding affinity to one or more analytes. The ability to bind analytes seriatim as a liquid sample is moved from one zone to another on the surface of the sample presentation devices is a feature of the present invention that facilitates the analysis of many different fractions of a liquid sample without the need to physically separate them using mechanical intervention. Instead, the different wettability properties of the sample presentation devices of the present invention may direct liquid samples to different zones of the devices, in the process leaving behind analytes that bind to different capture zones, and thereby sequentially process a liquid sample.

More specifically, the embodiments of the sample presentation devices that involve combinations of capture zones and liquid retention zones may further be used in a combinatorial manner to isolate, concentrate, purify, and modify analytes in liquid samples prior to their detection. So, for example, a liquid sample may be placed onto a T2 chip such that the analytes in the sample are confined in the analysis zone. That sample may then be transferred to an X3 chip that contains a boundary zone, a capture zone, and an analysis zone. In this example, the capture zone may be designed to bind (and thus remove) lipid moieties from the liquid sample, such that when the sample is applied to the X3 chip, it moves from the boundary zone to the capture zone (which has a higher degree of wettability), the lipid moieties in the sample bind to the surface of the capture zone, and the remaining sample moves to the analysis zone (because it has the highest degree of wettability). In this example, the liquid sample is confined on the T2 chip, and then the lipids are moved on the X3 chip, such that the final sample that is analyzed from the analysis zone is concentrated and purified of lipids. Because the capture zones can be designed to bind a multitude of different analytes, and because various combinations of any of these zones may be used, sample presentation devices having a vast range of purification, concentration, isolation, and modification capabilities (vis-à-vis one or more analytes) can be created.

The mechanism of transfer of liquid samples from one sample presentation device to another may vary. Using the above example, the concentrated sample from T2 may be removed mechanically (e.g., by pipetting) and placed on a separate X3 sample presentation device. Alternatively, the T2 and X3 sample presentation devices may be connected by a zone, the wettability of which may be changed in response to chemical or physical stimuli (e.g., UV radiation), such that the concentrated sample in the analysis zone

of the T2 sample presentation device is transferred to the capture zone of the X3 device when the exposure of a zone between them to UV radiation results in a wettability that is higher than the analysis zone of the T2 device but lower than that of the capture zone of the X3 device, such that the sample moves from T2 to X3. Again, with a vast number of surfaces (having different wettability and analyte binding properties) and configurations thereof, sample presentation devices having a vast range of purification, concentration, isolation, and modification capabilities (vis-à-vis one or more analytes) can be created.

The sample presentation devices of the present invention – in each of the embodiments described above – may further provide zones of different wettability having different shapes or patterns (a few examples of which are depicted in the Figures). For example, in one embodiment, a sample presentation device may have zones in the form of concentric circles, with the center zone being the analysis zone, surrounded by the liquid retention zone, surrounded by the boundary zone. Because the zones can be created using a various photo-patterning techniques, and because known photo-patterning techniques provide for tremendous variation in the resulting patterns, there is a vast range of possible shapes, patterns, and configurations of the various zones that can be designed by those of skill in the art. Moreover, the various properties of the different zones of wettability allow for the creation of sample presentation devices capable of directing analytes to single or multiple specified or pre-determined locations on the surfaces (e.g., addressable sites, lanes, or fields). Addressable in this context simply means that the pre-determined site, lane or field can be specified by an automated processing device that works in concert with the sample presentation devices of the present invention such that liquid samples or analytes retained at those specified locations can be processed by an analytical device to measure the analytes of interest. In addition, liquid samples or analytes present at these pre-determined locations may be removed from the sample presentation devices for subsequent handling or manipulation (e.g., modification, purification, concentration, etc.) by another sample presentation device.

The sample presentation devices of the present invention are suitable for the handling of both biological and non-biological liquid samples. They are also suitable for application in a wide range of analyte detection methods, for example, including but not limited to, mass spectrometry, various chromatographic methods, immunofluorescence spectroscopy, and other known analytical methods of detecting and measuring analytes in liquid samples.

Each of the above-described variations is designed to allow for maximum flexibility in design and use of sample presentation devices having enhanced capability to present analytes for detection and analysis over known methods. Thus, the sample presentation devices of the present invention have the capability of directing analytes to an analysis zone designed to enhance high sensitivity detection of analytes. The sample presentation devices of the present invention thus afford improved deposition of analytes.

The sample presentation devices of the present invention may further comprise devices capable of receiving and retaining liquid samples in volumes up to about 100 μL, and at least up to about 70 μL. The sample presentation devices of the present invention may also be utilized as sample positioning devices that directs the deposition of analytes to a surface area measuring less than about 2 millimeter squared (2 mm^2) and preferably less than about 1mm^2. Directing the deposition of analytes to a surface area measuring less than about 1 mm^2 may facilitate the improved deposition of analytes with a concomitant increase in both ease of automated data acquisition and sensitivity of detection. Consequently, the sample presentation device of the present invention provides a surface that exhibits substantial utility both with respect to liquid-holding capacity and controlled deposition of analytes. In preferred embodiments, this combination of attributes affords an increase in sensitivity of detection of from about 4-fold to greater than about 100-fold as compared to known sample supports.

In one embodiment, the sample presentation device of the present invention is comprised of a substrate, wherein the surface of the substrate is further comprised of three contiguous zones organized in a concentric arrangement, wherein the central analysis zone is surrounded by a liquid retention zone, and wherein the liquid retention zone is surrounded by a boundary zone. Alternatively, the sample presentation device of the present invention may be comprised of a substrate, wherein the surface is further comprised of three contiguous zones organized in an adjacent arrangement, wherein some portion of the analysis zone and some portion of the liquid retention zone are contiguous with respect to one another, and wherein those portions of the analysis and liquid retention zones that are not contiguous with respect to one another are surrounded by a common boundary zone.

In an embodiment of the sample presentation devices of the present invention, the surface of the analysis zone has a contact angle of preferably less than about 40°, more

preferably less than about 30°, and most preferably less than about 20°. The surface of the analysis zone preferably exhibits minimum affinity or binding with respect to analytes. The surface of the liquid retention zone has a contact angle preferably in the range of about 40° to about 95°, more preferably in the range of about 60° to about 95°, most preferably in the range of about 80° to about 95°, and further preferably exhibits minimum affinity or binding with respect to analytes. The surface of the boundary zone has a contact angle of preferably greater than about 95°, more preferably greater than about 105°, most preferably greater than about 115°, and further preferably exhibits a minimum of wettability with respect to liquid samples.

In another embodiment of the sample presentation devices of the present invention, the contact angle of the analysis zone is at least about 10°, preferably at least about 20°, more preferably at least about 30°, and most preferably at least about 40° lower then the contact angle of the liquid retention zone, wherein the contact angle of the liquid retention zone is preferably at least about 10°, more preferably at least about 15°, and most preferably at least about 20° lower than the contact angle of the boundary zone. In an embodiment of the sample presentation devices of the present invention, the surface area of the liquid retention zone is preferably at least about 4-fold greater, more preferably at least about 10-fold greater, and most preferably at least about 50-fold greater than the surface area of the analysis zone, and the surface area of the analysis zone is preferably less than about 1 mm^2, is more preferably in the range of from about 0.2 mm^2 to about 0.8 mm^2, and is most preferably in the range of from about 0.4 mm^2 to about 0.6 mm^2.

The sample presentation devices of the present invention may be further comprised of a substrate, wherein the surface of the substrate may be further comprised of, but not limited to, from 1 to 1536 pairs of analysis zones and liquid retention zones, wherein pairs of analysis zones and liquid retention zones are arranged as either concentric or adjacent pairs, and wherein pairs of analysis and liquid retention zones are surrounded by a common boundary zone. The sample presentation devices comprised of multiple pairs of analysis zones and liquid retention zones is preferably configured in a manner analogous to the standard 96-well, 384-well and 1536-well plates so as to be compatible with standardized multi-well plate processors and laboratory liquid handling robots.

Description of the Figures

The descriptions that follow are merely exemplary, supplement the disclosure of the invention set forth elsewhere, and do not limit the scope of the invention.

With reference to FIGS. **1***a* and **1***b*, the sample presentation device of the present invention is illustrated, showing a substrate **1**, wherein the surface of the substrate is further comprised of three contiguous zones organized in a concentric arrangement, wherein the central analysis zone **2** is surrounded by a liquid retention zone **3**, and wherein the liquid retention zone **3** is surrounded by a boundary zone **4**. The surface of the analysis zone **2** exhibits a contact angle of preferably less than about 40°, more preferably less than about 30°, and most preferably less than about 20°, and further preferably exhibits a minimal binding with respect to analytes. The surface of the liquid retention zone **3** exhibits a contact angle preferably in the range of about 40° to about 95°, more preferably in the range of about 60° to about 95°, most preferably in the range of about 80° to about 95°, and further preferably exhibits minimal binding with respect to analytes. The surface of the boundary zone **4** exhibits a contact angle of preferably greater than about 95°, more preferably greater than about 105°, most preferably greater than about 115°, and further preferably exhibits a minimum of wettability with respect to liquid samples.

With further reference to FIGS. **1***a* and **1***b*, a preferred embodiment of the sample presentation device of the present invention is one wherein the contact angle of the analysis zone **2** is preferably at least about 10°, more preferably at least about 20°, more preferably at least about 30°, and most preferably at least about 40° lower than the contact angle of the liquid retention zone **3**, wherein the contact angle of the liquid retention zone **3** is preferably at least about 10°, more preferably at least about 15°, and most preferably at least about 20° lower than the contact angle of the boundary zone **4**, wherein the surface area of the liquid retention zone **3** is preferably at least about 4-fold greater, more preferably at least about 10-fold greater, and most preferably at least about 50-fold greater than the surface area of the analysis zone **2**, and wherein the surface area of the analysis zone **2** is preferably less than about 2 mm^2, is more preferably in the range of from about 0.2 mm^2 to about 1.8 mm^2, and is most preferably in the range of from about 0.4 mm^2 to about 1.6 mm^2.

With reference to FIG. **2**, the sample presentation device of the present invention is comprised of a substrate **5** wherein the surface is further comprised of 16 concentric pairs

of analysis zones **6** and liquid retention zones **7**, all of which are surrounded by a common boundary zone **8**. In this instance, pairs of target and liquid retention zones are arrayed on 9 mm centers that would allow six of these devices to be combined into the format corresponding to a standard 96-well plate.

With further reference to FIG. **2**, a preferred embodiment of the sample presentation device of the present invention is one wherein the contact angle of the analysis zone **6** is preferably at least about 10°, more preferably at least about 20°, more preferably at least about 30°, and most preferably at least about 40° lower then the contact angle of the liquid retention zone **7**, wherein the contact angle of the liquid retention zone **7** is preferably at least about 10°, more preferably at least about 15°, and most preferably at least about 20° lower than the contact angle of the boundary zone **8**, wherein the surface area of the liquid retention zone **7** is preferably at least about 4-fold greater, more preferably at least about 10-fold greater, and most preferably at least about 50-fold greater than the surface area of the analysis zone **6**, and wherein the surface area of the analysis zone **6** is preferably less than about 2 mm^2, is more preferably in the range of from about 0.2 mm^2 to about 1.8 mm^2, and is most preferably in the range of from about 0.4 mm^2 to about 1.6 mm^2.

It is important to note that neither the analysis zone nor the liquid retention zone must be round in shape as illustrated in FIG. **1*a***. Both the analysis zone and the liquid retention zone may assume a variety of shapes as may be required to optimize performance of the sample presentation device with respect to a particular application. Additionally, it is important to note that neither the analysis zone nor the liquid retention zone must be concentric with one another as illustrated in FIGS. **1*a*** and **2**. Both the analysis zone and the liquid retention zone may be positioned accordingly as may be required to optimize performance of the sample presentation device with respect to a particular application.

With reference to FIG. **3**, the sample presentation device of the present invention is comprised of a substrate **9** having a surface further comprised of three contiguous zones organized in an adjacent arrangement, wherein some portion of the analysis zone **10** and some portion of the liquid retention zone **11** are contiguous with respect to one another, wherein those portions of the analysis zone and liquid retention zone that are not contiguous with respect to one another are surrounded by a common boundary zone **12.** The surface of the analysis zone **10** exhibits a contact angle of preferably less than about

40°, more preferably less than about 30°, and most preferably less than about 20°, and further preferably exhibiting minimal binding with respect to analytes. The surface of the liquid retention zone **11** exhibits a contact angle preferably in the range of about 40° to about 95°, more preferably in the range of about 60° to about 95°, most preferably in the range of about 80° to about 95°, and further preferably exhibiting minimal binding with respect to analytes. The surface of the boundary zone **12** exhibits a contact angle of preferably greater than about 95°, more preferably greater than about 105°, most preferably greater than about 115°, and further preferably exhibiting a minimum of wettability with respect to liquid samples.

With further reference to FIG. **3**, a preferred embodiment of the sample presentation device of the present invention is one wherein the contact angle of the analysis zone **10** is preferably at least about 10°, more preferably at least about 20°, more preferably at least about 30°, and most preferably at least about 40° lower then the contact angle of the liquid retention zone **11**, wherein the contact angle of the liquid retention zone **11** is preferably at least about 10°, more preferably at least about 15°, and most preferably at least about 20° lower than the contact angle of the boundary zone **12**, wherein the surface area of the liquid retention zone **11** is preferably at least about 4-fold greater, more preferably at least about 10-fold greater, and most preferably at least about 50-fold greater than the surface area of the analysis zone **10**, and wherein the surface area of the analysis zone **10** is preferably less than about 1 mm^2, is more preferably in the range of from about 0.2 mm^2 to about 0.8 mm^2, and is most preferably in the range of from about 0.4 mm^2 to about 0.6 mm^2.

It is important to note that neither the analysis zone nor the liquid retention zone must be round in shape as illustrated in FIGS. **1*a***, **2** and **3**. Both the analysis zone and the liquid retention zone may assume a variety of shapes as may be required to optimize performance of the sample presentation device with respect to a particular application.

With reference to FIG. **4*a***, the sample presentation device of the present invention is comprised of a substrate **13** having a surface further comprised of three contiguous zones organized in a concentric arrangement, wherein the central analysis zone **14** is surrounded by a liquid retention zone **15**, and wherein the liquid retention zone **15** is surrounded by a boundary zone **16**. With reference to FIG. **4*b***, the shape of the analysis zone **14** (a square)

may facilitate automated acquisition of mass spectral data, in that it corresponds in size to a raster of 36 regions.

With reference to FIG. **5**, the sample presentation device of the present invention is comprised of a substrate **17** comprised of 96 pairs of analysis zones **18** and liquid retention zones **19**, all of which are surrounded by a common boundary zone **20**. In this instance, the concentric pairs of zones are arrayed on 9 mm centers that correspond to a standard 96-well plate. The liquid retention zone **19** was been elongated to maximize liquid-holding capacity and minimize the distance between adjacent zones in each row. A serpentine pattern is overlaid on the first two rows of the sample presentation device to indicate the path described by the deposition of a liquid stream of chromatographic eluate during automated fraction collection.

With further reference to FIG. **5**, a preferred embodiment of the sample presentation device of the present invention is one wherein the contact angle of the analysis zone **18** is preferably at least about 10°, more preferably at least about 20°, more preferably at least about 30°, and most preferably at least about 40° lower then the contact angle of the liquid retention zone **19**, wherein the contact angle of the liquid retention zone **19** is preferably at least about 10°, more preferably at least about 15°, and most preferably at least about 20° lower than the contact angle of the boundary zone **20**, wherein the surface area of the liquid retention zone **19** is preferably at least about 4-fold greater, more preferably at least about 10-fold greater, and most preferably at least about 50-fold greater than the surface area of the analysis zone **18**, and wherein the surface area of the analysis zone **18** is preferably less than about 2 mm^2, is more preferably in the range of from about 0.2 mm^2 to about 1.8 mm^2, and is most preferably in the range of from about 0.4 mm^2 to about 1.6 mm^2.

Fabrication of Sample Presentation Devices

Still other embodiments of the invention include methods for creating or fabricating the sample presentation devices described above. For example, in an embodiment in which the surfaces are comprised of one or more self-assembled monolayers (SAMs) which form distinct zones depending on differences between the SAMs used, the sample presentation devices of the present invention may comprise various SAM zones that are created by known photo-patterning techniques. Accordingly, the present invention further

includes methods of creating sample presentation devices comprised of SAMs using, as one preferred method, photo-patterning techniques.

More generally, the surface of the substrate of the sample presentation device of the present invention is typically modified or patterned by methods known to those of skill in the art. As an example, the substrate's surface can be modified or patterned by means of applying one or more self-assembled monolayers (SAMs), which modify the surface of the substrate of the sample presentation device and whose exposed surfaces may impart particular chemistries to the substrate. Selection of various SAMs, including 1°, 2°, 3°, or 4° compositions, for a particular substrate provides the surface of the substrate with unique surface characteristics and properties. In particular, application of multiple SAMs results in the patterning of the substrate so that it contains a plurality of zones, each zone having different surface characteristics and properties. Methods of patterning the SAMs are known in the art, and include UV photo-patterning, photolithographic patterning, microstamping. electron-beam patterning, and reactive-ion etching.

The zones that are created on the surface of the substrate can be in any shape, with circular shapes being preferred. In addition, the zones can be either continuous or discontinuous with respect to other zones – i.e., the zones can all be contiguous with each other or one or more zones can be discontiguous with one or more other zones. The zones that are created on the surface of the substrate of the sample presentation devices preferably have a plurality of zones of differing wettability with respect to the sample to be analyzed.

As another embodiment of the invention, methods of fabricating a sample presentation device that is capable of precisely positioning analytes so as to facilitate automated data acquisition are provided.

More specifically, approaches to surface patterning, selection of suitable substrates, preparation of self-assembled monolayers as well as other approaches to surface modification are described below. These descriptions are merely exemplary and do not limit the scope of the invention.

The surface of the sample presentation device of the present invention is patterned by one of several approaches which preferably include, but are not limited to: (1) UV-Photopatterning of self-assembled monolayers (SAMs) prepared from alkylthiols on a coinage metal surface; (2) Photolithographic patterning of SAMs prepared from alkylthiols

on a coinage metal surface; (3) Microstamping of SAMs prepared from alkylthiols on a coinage metal surface; and (4) Photolithographic patterning of SAMs prepared from alkylsilanes on either a silicon or glass surface; (5) Electron-beam patterning, and (6) Reactive-ion etching. Preferably, the patterning of the sample presentation device surface is achieved either by application of the UV-photopatterning process described in United States Patent No. 5,514,501, or by the microstamping process described in United States Patent No. 5,512,131, both of which are incorporated herein by reference. Alternatively, the patterning of the sample presentation device surface may be achieved by photolithographic patterning processes described in the literature and understood by those skilled in the art.

With reference to FIGS. *6a* through *6h*, the step-wise process for UV-photopatterning of SAMs comprised of alkylthiols on gold is depicted. Initially, a suitable substrate **21** such as a silicon wafer (750 μm) is appropriately cleaned by a combination of wet process and argon plasma etching. An adhesion layer (25-50 nm) of either chromium or titanium and tungsten (9:1) is first applied to the surface of the wafer followed by a thin film (100-1000 nm) of gold **22**. Metal deposition is accomplished by a sputtering (vapor deposition) process that has been calibrated with respect to metal deposition (thickness) per unit time. The sputtering process may be undertaken with intact wafers or with individual pieces diced from a wafer.

With reference to FIG. *6b*, the first monolayer **23** is assembled on the gold surface by incubation of the substrate in a solution containing from 0.05 to 5 mM alkylthiol in ethanol for a period of from 1 to 24 hours. The surface-modified substrate is then washed with ethanol to remove excess alkylthiol and dried under a stream of nitrogen. The first monolayer **23** is prepared from an alkylthiol which affords a surface that exhibits a contact angle of greater than about 100° and further exhibits a minimum of wettability with respect to liquid samples.

With reference to FIG. *6c*, the surface-modified substrate is photo-patterned by exposure to an ultraviolet light source through a first mask **24** in the presence of oxygen so as to oxidize monomers residing within the exposed zone thereby generating monomer sulfonates that exhibit low affinity with respect to the gold surface. The opening in the mask **25** results in the creation of features of size and shape corresponding to the liquid retention zone.

With respect to FIGS. *6d* and *6e*, subsequent washing of the gold surface removes monomer sulfonates and affords an unmodified region of gold **26**. The second monolayer **27** is assembled on the gold surface by incubation of the substrate in a solution containing from 0.05 to 5 mM alkylthiol in ethanol for a period of from 1 to 24 hours. The surface-modified substrate is then washed with ethanol to remove excess alkylthiol and dried under a stream of nitrogen. The second monolayer **27** is prepared from an alkylthiol that affords a surface that exhibits a contact angle in the range of about 40° to about 95° and further affords a surface that exhibits minimal binding with respect to analytes.

With respect to FIG. *6f*, the patterned substrate is further photo-patterned by exposure to an ultraviolet light source through a second mask **28** in the presence of oxygen so as to oxidize monomers residing within the exposed zone thereby generating monomer sulfonates that exhibit low affinity with respect to the gold surface. The opening in the mask **29** results in the creation of features of size and shape corresponding to the analysis zone.

With respect to FIGS. *6g* and *6h*, subsequent washing of the gold surface removes monomer sulfonates and affords an unmodified region of gold **30**. The third monolayer **31** is assembled on the gold surface by incubation of the substrate in a solution containing from about 0.05 to about 5 mM alkylthiol in ethanol for a period of from 1 to 24 hours. The surface-modified substrate is then washed with ethanol to remove excess alkylthiol and dried under a stream of nitrogen. The third monolayer **31** is prepared from an alkylthiol that affords a surface that exhibits a contact angle of less than about 40° and further exhibits minimal binding with respect to analytes.

In this manner, the step-wise process for UV-photopatterning of self-assembled monolayers prepared from alkylthiols on gold is exploited to prepare the sample presentation device of the present invention. The above-described process of UV-photopatterning of self-assembled monolayers prepared from alkylthiols on gold is exemplary and the invention is not limited to only the process described.

With reference to FIGS. *7a* through *7h*, the step-wise process for photolithographic patterning of SAMs comprised of alkylthiols on gold is depicted. A suitable substrate **32** such as a silicon wafer is appropriately cleaned and an adhesion layer and a thin film of gold **33** (100-1000 nm) is sputtered thereupon.

With reference to FIG. 7*b*, the first monolayer **34** is assembled on the gold surface by incubation of the substrate in a solution containing from 0.05 to 5 mM alkylthiol in ethanol for a period of from 1 to 24 hours. The surface-modified substrate is then washed with ethanol to remove excess alkylthiol and dried under a stream of nitrogen. The first monolayer **34** is prepared from an alkylthiol that affords a surface that exhibits a contact angle of less than 40° and further exhibits minimal binding with respect to analytes.

With reference to FIG. 7*c*, the surface-modified substrate is coated with a photoresist **35** prior to lithography. The resist may be of a negative tone or positive tone. A negative resist results in decreased solubility in the exposed regions of the resist, thus giving a negative image relative to the mask. A positive resist results in increased solubility of the resist in the exposed regions, thus giving a positive image relative to the mask. The use of a positive resist is depicted. The resist may be applied through a dip-type of process, but is preferable applied using a spin-coater. The manufacturers' recommendations with respect to resist thickness and curing time are used as guidelines.

With reference to FIG. 7*d*, the surface-modified substrate is photo-patterned by exposure to an ultraviolet light source as required for use in conjunction with the particular resist employed. The photomask **36** may be prepared from a number of commonly employed materials which include, but are not limited to, chromium-on-quartz, Mylar, acetate, and metallic stencils. The opening in the mask **37** results in the creation of features of size and shape corresponding to the analysis zone.

With respect to FIG. 7*e*, the substrate is initially treated with a commercial solution specific to the resist employed that dissolves the exposed areas of resist while those regions not exposed **38** to the ultraviolet light source remain relatively insoluble. After removal of exposed resist, an oxygen plasma or UV/ozone treatment may be employed to oxidize alkylthiol monomers within the exposed zone thereby generating monomer sulfonates that exhibit low affinity with respect to the gold surface. Subsequent washing of the gold surface removes monomer sulfonates and affords an unmodified region of gold **39**.

With reference to FIG. 7*f*, the second monolayer **40** is assembled on the gold surface by incubation of the substrate in a solution containing from 0.05 to 5 mM alkylthiol in ethanol for a period of from 1 to 24 hours. The substrate is then washed with ethanol to remove excess alkylthiol and dried under a stream of nitrogen. The second monolayer **40** is prepared from an alkylthiol that affords a surface that exhibits a contact angle in the

range 40° to 95° and further affords a surface that exhibits minimal binding with respect to analytes.

With respect to FIGS. 7*g* and 7*h*, the remaining photoresist **38** is removed by further washing the substrate with one of several organic solvents known to dissolve unexposed resist (e.g. acetone, 1-methyl-2-pyrrolidinone, etc.) and the patterned substrate now comprised of two distinctive zones is coated with fresh photoresist **41** prior to lithography as described above.

With respect to FIGS. 7*i* and 7*j*, the patterned substrate is photo-patterned by exposure to an ultraviolet light source through a second photomask **42** as described above. The opening in the mask **43** results in the creation of features of size and shape corresponding to the liquid retention zone. The substrate is initially treated with a commercial solution specific to the resist employed that dissolves the exposed areas of resist while those regions not exposed **44** to the ultraviolet light source remain relatively insoluble. After removal of exposed resist, an oxygen plasma or UV/ozone treatment is employed to oxidize alkylthiol monomers residing within the exposed zone thereby generating monomer sulfonates that exhibit low affinitywith respect to the gold surface. Subsequent washing of the gold surface removes monomer sulfonates and affords an unmodified region of gold **45**.

With reference to FIGS. 7*k* and 7*l*, the third monolayer **46** is assembled on the gold surface by incubation of the substrate in a solution containing from 0.05 to 5 mM alkylthiol in ethanol for a period of from 1 to 24 hours. The substrate is then washed with ethanol to remove excess alkylthiol and dried under a stream of nitrogen. The third monolayer **46** is prepared from an alkylthiol which affords a surface that exhibits a contact angle of greater than 100° and further exhibits a minimum of wettability with respect to liquid samples. Finally, the remaining photoresist **44** is removed by further washing the substrate with one of several organic solvents known to dissolve unexposed resist to afford a patterned surface comprised of three distinctive zones.

In this manner, the step-wise process for photolithographic patterning of SAMs comprised of alkylthiols on gold is exploited to prepare the sample presentation device of the present invention. It should be noted that the sequence of patterning depicted (analysis zone, followed by liquid retention zone, followed by boundary zone) was selected arbitrarily and that the reverse sequence (boundary zone, followed by liquid retention zone,

followed by analysis zone) would also prove as suitable as the sequence illustrated. The above-described process of photolithographic patterning of self-assembled monolayers prepared from alkylthiols on gold is exemplary and the invention is not limited to only the process described.

Numerous alkylthiol monomers are suitable for use in preparation of the sample presentation device of the present invention. The synthesis of alkylthiol monomers, their assembly into monolayers, and their classification with respect to the surface tension of the assembled surfaces has been described (Laibinis, P. E.; Palmer, B. J.; Lee, S.-W.; Jennings, G. K. (1998) "The Synthesis of Organothiols and Their Assembly into Monolayers on Gold" *in* Thin Films, Vol. 24 (Ulman, A., ed.) pp. 1-41, Academic Press, San Diego, CA), incorporated herein by reference.

The aforementioned review article has classified terminal moieties associated with alkylthiol SAMs with respect to the surface energy of the assembled surfaces. Moieties which afford highly wettable surfaces and are thus suitable for the preparation of analysis zone monomers include, but are not limited to: CO_2H, $B(OH)_2$, PO_3H_2, $CONH_2$ and OH. Each of the aforementioned moieties is reported to afford a surface exhibiting a contact angle of less than about 40°. Generally speaking, moieties that afford highly wettable surfaces are comprised of hydrogen bond acceptors, hydrogen bond donors, and combinations thereof. Terminal moieties which afford surfaces of intermediate wettability and are thus suitable for the preparation of liquid retention zone monomers include, but are not limited to: CN (60°, 10), O_2CCH_3 (63°, 11), CO_2CH_3 (67°, 10), $NHCOCH_3$ (68°, 11), $SCOCH_3$ (70°, 11), OCH_3 (74°, 11), $CONHCH_3$ (76°, 11), $NHCOCF_3$ (77°, 11) and $CO_2CH_2CH_3$ (89°, 10). The contact angle associated with the assembled surface and the corresponding alkyl chain length is shown in parenthesis. Generally speaking, moieties which afford intermediately wettable surfaces tend to be comprised of functionalities that participate in dipole-dipole interactions. Terminal moieties which afford minimally wettable surfaces and are thus suitable for the preparation of boundary zone monomers include, but are not limited to: $O(CH_2)_2CH_3$ (104°, 11), $O(CH_2)_3CH_3$ (113°, 16), $NHCO(CF_2)_7CF_3$ (114.5°, 2), $O(CH_2)_4CH_3$ (115°, 16), $O(CH_2)_5CH_3$ (115°, 16), $OCH_2CF_2CF_3$ (118°, 11), and $(CF_2)_5CF_3$ (118°, 2). The contact angle associated with the assembled surface and the corresponding alkyl chain length is shown in parenthesis. Generally speaking, moieties which afford minimally wettable surfaces tend to be comprised of hydrophobic and oleophobic functionalities.

Preferably, both the target and liquid retention zones of the sample presentation device of the present invention are prepared from monomers that confer protein resistance upon the assembled surface. A number of SAMs prepared from alkylthiols on gold have been specifically characterized with respect to the adsorption of proteins. The most protein resistant of the surfaces thus far reported are those derived from monomers which present oligo(ethylene oxide) (OCH_2CH_2) units. The utility of these surfaces was first described by Prime and Whitesides (Prime, K. L. and Whitesides, G. M. *J. Am. Chem. Soc.*, **1993**, *115*, 10714-21, incorporated herein by reference). A survey of structure–property relationships of surfaces that resist protein adsorption has appeared (Ostuni, E.; Chapman, R. G.; Holmlin, R. E.; Takayama, S.; Whitesides, G. M. *Langmuir*, **2001**, *17*, 5605-5620, incorporated herein by reference). Recently, a number of zwitterionic SAMs have been shown to exhibit good resistance to protein adsorption (Holmlin, R. E.; Chen, X.; Chapman, R. G.; Takayama, S.; Whitesides, G. M. *Langmuir*, **2001**, *17*, 2841-50, incorporated herein by reference) and are therefore potentially useful as analysis zones owing to their combination of highly wettable surfaces and good resistance to protein adsorption.

In preferred embodiments, the analysis zone of the sample presentation device of the present invention is prepared from monomers of the General Formula I: $HS(CH_2)_{11}$-$(OCH_2CH_2)_mOH$, wherein m is from 3 to 7. Monomers of this general formula afford surfaces that exhibit contact angles in the range of about 30° to about 38°. Although these surfaces do not exhibit the lowest possible contact angles, they are preferably utilized owing to their superior performance with respect to minimizing the binding of proteins. Furthermore, analysis zone monomers of General Formula I are preferably utilized in conjunction with liquid retention zone monomers that afford surfaces which exhibit contact angles greater than about 60°.

Similarly and preferably, the liquid retention zone of the sample presentation device of the present invention is prepared from monomers of the General Formula II: $HS(CH_2)_{11}$-$(OCH_2CH_2)_mR$, wherein m = 3 to 7, and wherein group R is a terminal moiety which influences surface tension and wettability. Preferably but not exclusively, group R is selected from one of OCH_3, OCH_2CN, CO_2CH_3, $CONHCH_3$, and $CO_2CH_2CH_3$ moieties. Each of the aforementioned terminal moieties affords a surface that exhibits a contact angle in the range of about 62° to about 89°.

Alternatively and preferably, the liquid retention zone of the sample presentation device of the present invention may be prepared from a monomer of the formula $HS(CH_2)_{11}OCH_2C_6H_5$. The terminal benzyl moiety ($CH_2C_6H_5$) exhibits particular utility with respect to samples dissolved in organic solvents and affords a surface that exhibits a contact angle of about 90°.

In preferred embodiments, the boundary zone of the sample presentation device of the present invention is prepared from a monomer which confers a minimum of wettability with respect to liquid samples wherein the analytes are dissolved in aqueous buffers, organic solvents and mixtures thereof. Monomers presenting terminally perfluorinated moieties have been shown to have particular utility in this regard (Naud, C.; Calas, P.; Blancou, H.; Commeyras, A. *J. Fluorine Chem.*, **2000**, *104*, 173-183, incorporated herein by reference).

A preferred embodiment of the present invention is one wherein the analysis zone is prepared from a monomer of the formula $HS(CH_2)_{11}(OCH_2CH_2)_3OH$, wherein the liquid retention zone is prepared from a monomer of the formula $HS(CH_2)_{11}(OCH_2CH_2)_3OCH_3$, and wherein the boundary zone is prepared from a monomer of the formula $HS(CH_2)_{11}OCH_2CH_2(CF_2)_5CF_3$. This combination of monomers affords a surface wherein the contact angle of the analysis zone, liquid retention zone, and boundary zone are about 38°, 62° and 117°, respectively.

Another preferred embodiment of the present invention is one wherein the analysis zone is prepared from a monomer of the formula $HS(CH_2)_{11}(OCH_2CH_2)_3OH$, wherein the liquid retention zone is prepared from a monomer of the formula $HS(CH_2)_{11}OCH_2C_6H_5$, and wherein the boundary zone is prepared from a monomer of the formula $HS(CH_2)_{11}OCH_2CH_2(CF_2)_5CF_3$. This combination of monomers affords a surface wherein the contact angle of the analysis zone, liquid retention zone, and boundary zone are about 38°, 91° and 117°, respectively.

Mixed (binary) self-assembled monolayers prepared from two alkylthiol monomers have been exploited to precisely control surface contact angle and wettability. (Semal, S.; Bauthier, C.; Voué, M.; Vanden Eynde, J. J.; Gouttebaron, R.; De Coninck, J. *J. Phys. Chem. B*, **2000**, *104*, 6225-6232, incorporated herein by reference). Contact angles have been adjusted over a range of greater than 40° by mixing monomers utilized to prepare highly wettable and intermediately wettable surfaces. Preferably, binary SAMs are

exploited to prepare either the analysis zone or the liquid retention zone. Alternatively, ternary and quaternary self-assembled monolayers may be exploited to prepare either the analysis zone or the liquid retention zone. Ternary and quaternary SAMs are prepared from binary mixtures of either substituted alkylthiols and hetero-substituted asymmetric alkyl disulfides (i.e., $HS(CH_2)_{11}R^1$ and $R^2(CH_2)_{11}S\text{-}S(CH_2)_{11}R^3$) or two hetero-substituted asymmetric alkyl disulfides (i.e., $R^1(CH_2)_{11}S\text{-}S(CH_2)_{11}R^2$ and $R^3(CH_2)_{11}S\text{-}S(CH_2)_{11}R^4$), respectively.

With reference to FIGS. **8***a* through **8***l*, the step-wise process for photolithographic patterning of SAMs comprised of alkylsilanes on silicon is depicted. Modification of silicon and glass by reaction with either alkyl dimethylchlorosilanes, alkyl dimethylalkoxysilanes, alkyl trihalosilanes, or alkyl trialkoxysilanes is described in the literature and is understood by those skilled in the art.

With reference to FIG. **8***a*, a suitable substrate **47** such as a silicon wafer, glass wafer, or metallic substrate with silicon dioxide deposed thereupon is appropriately activated for covalent attachment to an alkylsilane by a process involving removal of surface contaminants followed by oxidation of the surface to generate silanol (Si-OH) moieties. Preferably, the substrate is briefly treated with oxygen plasma, washed with an oxidizing solution (Piranha Solution), and then again treated with oxygen plasma to afford an activated surface **48** that presents an average silanol density approaching 4.9 Si-OH/nm^2.

With reference to FIG. **8***b*, following surface activation the first alkylsilane monolayer **49** is assembled on the silicon surface. Silanization may be performed neat, by solution deposition, or by vapor deposition. The first alkylsilane monolayer **49** is preferably prepared from an alkylsilane which affords a surface that exhibits a contact angle of greater than 100° and further exhibits a minimum of wettability with respect to liquid samples.

With reference to FIG. **8***c*, the silanized substrate is coated with a photoresist **50** prior to lithography. The resist may be of either a negative tone or positive tone. A negative resist results in decreased solubility in exposed regions of the resist, thus giving a negative image relative to the mask. A positive resist results in increased solubility in the exposed regions of the resist, thus giving a positive image relative to the mask. The use of a positive resist is depicted throughout FIG. **6**. The resist may be applied through a dip-

type of process, but is preferable applied using a spin-coater. The manufacturers' recommendations with respect to resist thickness and curing times should be used as guidelines.

With reference to FIG. 8*d*, the substrate is photo-patterned by exposure to an ultraviolet light source as required for use in conjunction with the particular resist employed. The photomask **51** may be prepared from a number of commonly employed materials which include, but are not limited to, chromium-on-quartz, Mylar, acetate, and metallic stencils. The opening in the mask **52** results in the creation of features of size and shape corresponding to the liquid retention zone.

With respect to FIGS. 8*e* and 8*f*, the substrate is initially treated with a commercial solution specific to the resist employed that dissolves the exposed areas of resist while those regions not exposed **53** to the ultraviolet light source remain relatively insoluble. After removal of exposed resist, an oxygen plasma treatment is employed to activate the surface **54** in preparation for further silanization. The second alkylsilane monolayer **55** is assembled on the activated silicon surface. Silanization may be performed neat, by solution deposition, or by vapor deposition. The second alkylsilane monolayer **55** is prepared from an alkylsilane that affords a surface that exhibits a contact angle in the range of about 40° to about 95° and further affords a surface that exhibits minimal binding with respect to analytes.

With respect of FIGS. 8*g* and 8*h*, the remaining photoresist **53** is removed by further washing the substrate with one of several organic solvents known to dissolve unexposed resist (e.g., acetone, 1-methyl-2-pyrrolidinone, etc.) The patterned substrate comprised of two distinctive zones is coated with a photoresist **56** prior to lithography as described above.

With respect to FIGS. 8*i* and 8*j*, the patterned substrate is further photo-patterned by exposure to an ultraviolet light source through a photomask **57** as described above. The opening in the mask **58** results in the creation of features of size and shape corresponding to the analysis zone. The substrate is then treated with a commercial solution specific to the resist employed that dissolves the exposed areas of resist while those regions not exposed **59** to the ultraviolet light source remain relatively insoluble. After removal of exposed resist, an oxygen plasma treatment is employed to activate the surface **60** in preparation for further silanization.

With reference to FIGS. 8*k* and 8*l*, the third monolayer **61** is assembled on the activated silicon surface. Silanization may be performed neat, by solution deposition, or by vapor deposition. The third alkylsilane monolayer **61** is prepared from an alkylsilane that affords a surface that exhibits a contact angle of less than about 40° and further affords a surface that exhibits minimal binding with respect to analytes. Finally, the remaining photoresist **59** is removed by further washing the substrate with one of several organic solvents known to dissolve unexposed resist to afford a patterned surface comprised of three distinctive zones.

In this manner, the step-wise process for photolithographic patterning of SAMs prepared from alkylsilanes on silicon is exploited to prepare the sample presentation device of the present invention. It should be noted that the sequence of patterning depicted (boundary zone, followed by liquid retention zone, followed by analysis zone) was selected arbitrarily, and that the reverse sequence (analysis zone, followed by liquid retention zone, followed by boundary zone) would also prove as suitable as the sequence illustrated. The above-described process of photolithographic patterning of self-assembled monolayers prepared from alkylsilanes on silicon is exemplary and the invention is not limited to only the process described.

Numerous alkylsilanes are suitable for use in preparation of sample presentation device of the present invention. Alkylsilanes are mostly commercially available and their synthesis and use in surface modification is understood. (Shriver-Lake, L. C. (1998) "Silane-modified surfaces for biomaterial immobilization" *Immobilized Biomolecules in Analysis: A Practical Approach* (Cass, T. and Ligler, F. S., eds.) Chapter 1, Oxford University Press, Oxford, UK, incorporated herein by reference).

Utilizing an approach that differs somewhat from that outlined above, activated silicon surfaces may be first derivatized with an appropriate alkylsilane having a nucleophilic moiety that is further functionalized by appending a terminal moiety that confers the required wettability. Alternatively, when alkylsilanes with suitable terminal moieties are available, the surface may be modified in a single step. Terminal moieties suitable for use in the preparation of the sample presentation device of the present invention include, but are not limited to, those described above.

In preferred embodiments, the analysis zone of the sample presentation device of the present invention is initially prepared from 3-aminopropyltrimethoxysilane, and then

further functionalized to afford an immobilized silane of General Formula III: $(XO)_3Si$-$CH_2CH_2CH_2NHCOCH_2(OCH_2CH_2)_nOH$, wherein X is linkage to either the silicon surface or an adjacent immobilized silane, and wherein n is from 4 to 8. Monomers of General Formula III afford surfaces that exhibit contact angles in the range from about 30° to about 40°. Although these surfaces do not exhibit the lowest possible contact angles, they are preferably utilized owing to their superior performance with respect to minimizing the binding of proteins. Furthermore, analysis zone monomers of General Formula III are preferably utilized in conjunction with liquid retention zone monomers that afford surfaces which exhibit contact angles greater than 60°.

Similarly and preferably, the liquid retention zone of the sample presentation device of the present invention is initially prepared from 3-aminopropyltrimethoxysilane, and then further functionalized to afford an immobilized silane of General Formula IV: $(XO)_3SiCH_2CH_2CH_2NHCOCH_2(OCH_2CH_2)_nR'$, wherein X is linkage to either the substrate or an adjacent monomer, wherein n is from 4 to 8, and wherein group R' is a terminal moiety which influences surface tension and wettability. Preferably but not exclusively, group R' is selected from one of CH_3, CH_2CN, $CH_2CO_2CH_3$, $CH_2CONHCH_3$, and $CH_2CO_2CH_2CH_3$ moieties. Each of the afore-mentioned terminal moieties affords a surface that exhibits contact angles in the range of about 60° to about 90°.

In preferred embodiments, the boundary zone of the sample presentation device of the present invention is prepared in a single step from an alkylsilane which confers a minimum of wettability with respect to aqueous samples of General Formula V: $(CH_3)_2(X')SiCH_2CH_2$-$(CF_2)_7CF_3$, wherein X' is a surface reactive moiety.

A variety of alternative surface-modification chemistries and surface patterning approaches may be exploited to prepare the sample presentation devices of the present invention. Polymeric compositions of matter have recently attracted interest with respect to the patterning of protein resistant surfaces. Patterned surfaces initially prepared from either alkylthiol or alkylsilane SAMs have been further functionalized by either grafting polymeric compositions to the surface or growing polymeric compositions from the surface (e.g., Husemann, M.; Mecerreyes, D.; Hawker, J. L.; Hedrick, R. S.; Abbott, N. L. *Angew. Chem. Int. Ed.* **1999**, *38*, 647-649; Shah, R. R.; Merreceyes, D.; Husemann, M.; Rees, I.; Abbott, N. L.; Hawker, C. J.; Hedrick, J. L. *Macromolecules* **2000**, *33*, 597-605; and Hyun, J. and Chilkoti, A. *Macromolecules* **2001**, *34*, 5644-5652, all incorporated

herein by reference). Recently, the first report of surface patterning by adsorption of block copolymers appeared (Deng, T.; Ha, Y.-H.; Cheng, J, Y.; Ross, C. A.; Thomas, E. L. *Langmuir*, **2002**, *18*, 6719-6722, incorporated herein by reference). Polymeric thin films grafted to SAMs have been shown to resist the adsorption of proteins to an extent comparable to, or better than, SAMs that present tri(ethyleneglycol) groups (Chapman, R. G.; Ostuni, E.; Liang, M. N.; Meluleni, G.; Kim, E.; Yan, L.; Pier, G.; Warren, H. S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 1225-1233, incorporated herein by reference).

It is understood that even the least wettable surfaces may nevertheless retain certain moieties from liquid samples, even if in only a non-specific manner. Such surfaces in fact may contribute to the advantages of the sample presentation devices of the present invention by, for example, enhancing their ability to concentrate analytes by removal of those moieties that are not targets for subsequent analysis. This may be particularly useful in the context of retention of non-biological moieties that might interfere with the analysis of analytes. However, the surfaces of the sample presentation devices are not limited to only this example, but rather may comprise surfaces that bind moieties in regions other than the analysis zone that may be handled or processed separately from the analyte-containing sample. Indeed, any moiety that may be analyzed by analytical biochemical methods may be retained, stored, transported, and subsequently analyzed using the sample presentation devices of the invention. The present invention therefore allows that some retention of moieties in zones other than that having the highest degree of wettability is possible, and that subsequent analysis of those moieties may be desirable. Substantial amounts of the analytes of interest, however, are not typically retained in zones other than those with the highest degree of wettability. Therefore, in the context of the example of analyte analysis by laser desorption spectroscopy, the target analytes retained in the zone of highest wettability are not desorbed from a bound state to the surface of the sample presentation device.

Uses and Applications of Sample Presentation Devices

The descriptions of various uses and applications of the sample presentation devices of the present invention that follow are merely exemplary and do not limit the scope of the invention.

The sample presentation devices of the present invention find many uses in combination with various analytical techniques and procedures. Thus, the present

invention includes methods for using the aforementioned sample presentation devices. More specifically, present invention includes methods of using the sample presentation devices of the present invention to identify the presence of analytes in a sample, and to analyze a plurality of samples, either on a sample presentation device or on a plurality of sample presentation devices.

Virtually any analytical method that permits the detection, identification, or measurement of analytes in a liquid sample can be used in combination with the sample presentation devices of the present invention. Examples of such analytical methods include but are not limited to MALDI-MS or electrospray ionization MS. The sample presentation devices are particularly well suited to us in combination with high throughput analytical measurement techniques, such as, for example, for use in MALDI-MS in which the sample presentation device analysis zones are configured in such fashion as to promote high throughput data acquisition.

The sample presentation devices of the present invention may also be used to manipulate liquid samples, and the analytes contained therein. Based on the differing wettability properties and capture properties that the surfaces of the sample presentation devices may be designed to have, the sample presentation devices may be designed to manipulate, concentrate, position, store, transfer (with and without mechanical intervention), recover (with or without mechanical intervention), analyze, modify or process (via use of analyte modifying reagents on the sample presentation devices), or fractionate liquid samples or the analytes contained therein. Moreover, because the sample presentation devices of the present invention may be designed to accomplish any of these functions in response to chemical or physical stimuli (e.g., heat, UV radiation, pressure, electromagnetic radiation), the sample presentation devices of the present invention may accomplish these functions reversibly or irreversibly, and may further perform various combinations of these functions in response to external forces.

Virtually any liquid sample (and analytes) can be used in connection with the sample presentation devices of the present invention. For example, the present invention can be used to analyze fractions recovered from liquid chromatography. The present invention can be used to analyze enzymatic digests prepared from either protein spots excised from 2D gel electrophoresis or from fractions collected from affinity chromatography (i.e. ICAT). The present invention can also be used to analyze samples

recovered from surface plasmon resonance biosensors. The present invention can also be used for 1:1 sample transfer with standard multi-well format robotics and assays. Indeed, the sample presentation devices of the present invention can be used to handle and manipulate liquid samples obtained from virtually any source, whether such samples are the result of laboratory experiment (such as the enzymatic digest and surface plasmon resonance biosensor sample examples identified above), obtained from the environment (such as a water quality sample from a river), or obtained directly from living organisms (such as a human urine sample).

The present invention can also be used for storage of samples for archival purposes or for further analysis. In other words, the detection and analysis of the analytes contained in liquid samples need not occur immediately following transfer of the liquid sample to the analysis zone.

Thus, various embodiments of the present invention provide for sample presentation devices that serve a variety of liquid-handling functions, including but not limited to sample/analyte handling, as well as liquid deposition, retention, transfer, locating and re-locating, and storage. Some examples of these various uses of the sample presentation devices of the present invention are provided.

With reference to Figs **9***a* through **9***f*, various steps in the process of sample drying are illustrated. A cross-sectional view of the sample presentation device of the present invention shows the surface deposited on the substrate **62** comprised of three distinctive zones, wherein the central analysis zone **63** is surrounded by the liquid retention zone **64**, and wherein the liquid retention zone **64** is further surrounded by the boundary zone **65**.

With reference to FIG. **9***b*, depositing a liquid sample drop **66** on the surface of the sample presentation device initially results in simultaneous confinement of the sample drop volume to the surface of the analysis zone **63** and the liquid retention zone **64**. Sample drop confinement results from the surface tension associated with the limited wettability of the boundary region **65**. Upon deposition, the contact angle of the sample drop is approximately equal to that of a drop residing exclusively on the liquid retention zone.

With reference to FIGS. **9***c* through **9***e*, as the sample drop dries owing to evaporation, both the radius and the contact angle of the drop recede until the radius of the drop corresponds to that of the analysis zone.

With reference to FIG. *9f*, when the radius of the sample drop **67** and that of the analysis zone **63** correspond, the contact angle of the sample drop is found to be approximately equal to that of a drop residing on the analysis zone. As the sample drop continues to dry owing to evaporation, the radius of the sample drop does not further recede, but remains constant as analytes are deposited as a thin film on the surface of the analysis zone. In this manner, aqueous samples of variable volume of up to about 100 µL, deposited on the surface of the sample presentation device, afford upon drying a thin film of analytes confined within an area corresponding to the analysis zone.

For example, the sample presentation device of the present invention with a liquid retention zone having a 3.0 mm diameter (about 7.069 mm^2 surface area) and a analysis zone having a 0.5 mm diameter (about 0.196 mm^2 surface area), confines the deposition of analytes to a analysis zone surface area of about 36-fold smaller than the surface area of the liquid retention zone, with an about 36-fold concomitant increase in average surface analyte concentration. Consequently, in principal the sample drop drying process described above would potentially afford an about 36-fold increase in sensitivity.

With reference to FIGS. **10***a* through **10***d*, in the absence of the analysis zone (only the liquid retention zone **68** and the boundary zone **69** are present) the sample drop **70** dries without a significant reduction in radius resulting in deposition of analytes over much of the surface of the liquid retention zone **71**. With reference to FIGS. **10***e* through **10***h*, in the absence of the liquid retention zone (only the analysis zone **72** and the boundary zone **73** are present) the volume of the sample drop **74** is limited by the liquid-holding capacity of the analysis zone **72**. The sample drop **74** dries without a significant reduction in radius resulting in deposition of analytes over much of the surface of the analysis zone **75**.

A significant increase in the sensitivity of detection results from the process described in FIGS. **9***b* through **9***f*. This phenomenon is best understood with reference to FIGS. **9***a* through **9***d* as well as FIGS. **10***a* through **10***d*. In the absence of the analysis zone (see FIG. **10***a*), the average analyte surface concentration per unit area in the liquid retention zone depicted in Fig **10***a*, **68** is equal to the total analyte concentration divided by the surface area. In the presence of the analysis zone depicted in FIG. **9***a*, however, the deposition of analyte is confined to the analysis zone wherein the average analyte surface concentration per unit area is equal to the total analyte concentration divided by the surface area of the analysis zone. Therefore, the presence of the analysis zone, **63**, depicted in

Figure 9*a*, affords an increase in average surface concentration of analyte which is equal to the ratio of the surface area of the liquid retention zone, **68**, depicted in FIG. **10***a*, to the surface area of the analysis zone, **63**, depicted in FIG. 9*a*. Since the surface area of the analysis zone is significantly smaller than the surface area of the liquid retention zone, confining analyte deposition to the surface area of the analysis zone results in a significant increase in the average surface concentration of analyte presented to the mass spectrometer with a concomitant increase in sensitivity of detection.

For example, the sample presentation device of the present invention with a liquid retention zone having a 3.0 mm diameter (about 7.069 mm^2 surface area) and a analysis zone having a 0.5 mm diameter (about 0.196 mm^2 surface area), confines the deposition of analytes to a analysis zone surface area of about 36-fold smaller than the surface area of the liquid retention zone, with an about 36-fold concomitant increase in average surface analyte concentration. Consequently, in principal the sample drop drying process described above would potentially afford an about 36-fold increase in sensitivity.

Analyte-confining properties of the analysis zone, which afford an increase in sensitivity of detection, are demonstrated in the video contact angle images shown in FIGS. **11***a* through **11***h*. With reference to FIG. **11***a*, the sample presentation device of the present invention was prepared with a liquid retention zone measuring about 1.6 mm OD and an analysis zone measuring about 0.7 mm OD. To facilitate the observation of the focusing effect, the analysis zone was placed off-center. A drop of water was applied to the surface of the biochip and was observed to rapidly confine itself to the surface area corresponding to the liquid retention zone and the analysis zone. The initial left-side and right-side contact angles were recorded and were both found to be 57.1°, a value which corresponds to that exhibited by a surface prepared from exclusively the liquid retention zone monomer. As the drop dried owing to evaporation (see FIGS. **11***b* through **11***h*), both the observed radius and contact angles receded until the radius of the drop corresponded to that of the analysis zone. Furthermore, as the drop dried it was observed that the center of the drop moved to the right so as to allow the drop to center itself over the analysis zone. The left-side and right-side contact angles recorded in FIG. **11***h* were both found to be 35.4°, a value which corresponds to that exhibited by a surface prepared exclusively from the analysis zone monomer. The drop height, width and contact angle data recorded in conjunction with the acquisition of the images depicted in FIGS. **11***a* through **11***h* is summarized graphically in FIG. **12**.

The extraordinary liquid-holding capacity of the liquid retention zone is demonstrated in FIG. 13. A photograph of a 16-site sample presentation device of the present invention shows the retention of sample drop volumes in the range 5 μL to 70 μL. The only factor that appears to significantly limit the sample drop volume is the relative proximity of the adjacent pairs of analysis and liquid retention zones.

Analyte-confining properties of the analysis zone are further demonstrated in FIGS. 14*a* and 14*b*. The first photograph (FIG. 14*a*) is of a 16-site sample presentation device of the present invention with sample drop volumes in the range 5μL to 40 μL deposed on the surface of 8 of the 16 sites. Each of the sample drops contained an equivalent amount of a soluble dye. The second photograph (FIG. 12*b*) is of the same sample presentation device after allowing the sample drops to dry. The dye is now deposed on the surface of the biochip in proximity to the analysis zone. The relative size of the analysis zone and the liquid retention zone is superimposed upon the biochip for comparison purposes. In this instance, an excessive amount of dye was required to afford visible material resulting in the absence of tightly-focused analyte spots.

The sample presentation device of the present invention may be exploited to facilitate high sensitivity mass spectrometric detection of chemical and biological analytes selected from, but not limited to: biological macromolecules such as peptides, proteins, enzymes, enzymes substrates, enzyme substrate analogs, enzyme inhibitors, polynucleotides, oligonucleotides, nucleic acids, carbohydrates, oligosaccharides, polysaccharides, avidin, streptavidin, lectins, pepstatin, protease inhibitors, protein A, agglutinin, heparin, protein G, concanavalin; fragments of biological macromolecules set forth above, such as nucleic acid fragments, peptide fragments, and protein fragments; complexes of biological macromolecules set forth above, such as nucleic acid complexes, protein-DNA complexes, gene transcription complex, gene translation complex, membrane, liposomes, membrane receptors, receptor ligand complexes, signaling pathway complexes, enzyme-substrate, enzyme inhibitors, peptide complexes, protein complexes, carbohydrate complexes, and polysaccharide complexes; and small biological molecules such as amino acids, nucleotides, nucleosides, sugars, steroids, lipids, metal ions, drugs, hormones, amides, amines, carboxylic acids, vitamins and coenzymes, alcohols, aldehydes, ketones, fatty acids, porphyrins, carotenoids, plant growth regulators, phosphate esters and nucleoside diphosphosugars, synthetic small molecules such as pharmaceutically or therapeutically effective agents, monomers, peptide analogs, steroid analogs, inhibitors,

mutagens, carcinogens, antimitotic drugs, antibiotics, ionophores, antimetabolites, amino acid analogs, antibacterial agents, transport inhibitors, surface-active agents (surfactants), amine-containing combinatorial libraries, dyes, toxins, biotin, biotinylated compounds, DNA, RNA, lysine, acetylglucosamine, procion red, glutathione, adenosine monophosphate, mitochondrial and chloroplast function inhibitors, electron donors, carriers and acceptors, synthetic substrates and analogs for proteases, substrates and analogs for phosphatases, substrates and analogs for esterases and lipases and protein modification reagents.. Moreover, analytes that may be handled by the sample presentation devices of the present inventions may be non-biological, and include but are not limited to, synthetic polymers, such as oligomers, and copolymers such as polyalkylenes, polyamides, poly(meth)acrylates, polysulfones, polystyrenes, polyethers, polyvinyl ethers, polyvinyl esters, polycarbonates, polyvinyl halides, polysiloxanes, and copolymers of any two or more of the above, as well as oather non-biological analystes such as pesticides.

Analytes may be dissolved in aqueous buffers, organic solvents or mixtures thereof. Buffers are preferably selected from those prepared from volatile constituents including, but not limited to: ammonium acetate, ammonium bicarbonate, ammonium carbonate, ammonium citrate, triethylammonium acetate and triethylammonium carbonate, triethylammonium formate, trimethylammonium acetate, trimethylammonium carbonate and trimethylammonium formate. Aqueous samples containing high concentrations of non-volatile detergents (>0.1%) should be desalted prior to analysis as the presence of detergent may counteract and analyte-confining properties of the analysis zone. Organic solvents are preferably selected from those know to be miscible in aqueous buffers and to promote the solubility of biological analytes including, but not limited to: acetic acid, acetone, acetonitrile, ethanol, *N,N*-dimethylformamide (DMF), *N,N*-dimethylsulfoxide (DMSO), formic acid, heptafluorobutyric acid, methanol, *N*-methylpyrolidone (NMP), 2,2,2-trifluoroethanol and trifluoroacetic acid.

The sample presentation device may be heated during the sample drying process (either on the surface of a heating block, under an infrared lamp or under a stream of hot air) to facilitate the evaporation of high-boiling organic solvent or simply to reduce the time required for sample drying.

Laser desorption time-of-flight mass spectrometry – a preferred analytical method to measure analytes using the sample presentation devices of the present invention requires a material (matrix) to be applied to the surface of the sample presentation device to absorb energy and thereby assist the ionization of analytes. Reagents frequently used as matrices for detection of biological analytes include *trans*-3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid, SA), α-cyano-4-hydroxycinnamic acid (HCCA) and 2,5-dihydroxybenzoic acid (DHBA). Owing to the limited solubility of the aforementioned matrices in water, stock solutions of these reagents often contain 50% to 100% organic solvent. When utilized in conjunction with the sample presentation devices of the present invention, stock solutions containing matrix are added to aqueous samples prior to applying the sample to the surface of the sample presentation device. Alternatively, stock solutions containing matrix may be applied to the surface of the sample presentation device after sample deposition and drying. In this instance, stock solutions containing a high percentage of organic solvent are preferably utilized to minimize dissolving of the analytes deposited on the surface of the analysis zone into the stock solution.

Numerous applications exist for using the sample presentation devices of the present invention. Examples of the types of samples that could be used in the present invention include, but are not limited to, samples that are to be analyzed directly without any processing done before analysis, as well as samples that are to be analyzed indirectly, in that the samples are to be analyzed after some sort of processing has occurred.

Examples of the types of samples that could be used in the present invention that fall into the category of samples that are to be analyzed directly without any processing done before analysis include, but are not limited to, biofluids; tissue and cell extracts and fractions; cells, bacteria, viruses; culture medium; environmental fluids; environmental air sampling; environmental media extracts (soil extracts, solid waste extracts, elution from wipes, elution from air filters); forensic samples; and libraries (combinatorial chemistry, oligonucleotides, peptides, sugars, lipids, cells and components; chromosomes, and viruses and other large protein and nucleoprotein assemblies).

Examples of types of samples that could be used in the present invention that fall into the category of samples that are to be analyzed indirectly, i.e., after some sort of processing has occurred to the samples include, but are not limited to, liquid chromatography (LC) output; gas chromatography (GC) output; elution from gels; digested

samples from LC output or gel elutions; mass spectrometry output; elutions from surface plasmon resonance (SPR) or other biosensors; desalting column output; solid-phase extraction output; liquid phase fractionated environmental samples; derivatized samples with respect to any of the above; and other chemical or physical processes and any combinations thereof.

The sample presentation device of the present invention further facilitates the mass spectrometric analysis of biological analytes recovered from fractionation schemes that exploit either column liquid chromatography or electrophoresis. In particular, utility results from the combination of the liquid-holding capacity of the device (which enables direct collection of chromatographic fractions, samples purified by electrophoresis, samples recovered from sample presentation devices and samples recovered from biosensors without prior sample volume reduction) and the precise positioning of the sample and increased sensitivity of detection (which enables automated data acquisition). The liquid-holding capacity afforded by the sample presentation device of the present invention enables direct collection of fractions recovered from, but not limited to, the following techniques: affinity chromatography, hydrophobic interaction chromatography, ion exchange chromatography, immobilized metal ion affinity chromatography and size exclusion chromatography, as well as fractions recovered from orthogonal separations involving sequential utilization of two or more of the chromatographic approaches enumerated. Furthermore, the availability of the sample presentation device in standard 96-well, 384-well and 1536-well formats enables biochip-based sample collection and processing on multi-well plate processing devices and laboratory liquid handling robots. Consequently, the sample presentation device may be exploited to enable high-throughput mass spectrometric platforms as are needed to support the emergence of proteomics and other important fields of chemistry and biotechnology.

Contemporary protein identification often involves enzymatic digestion of proteins purified either by column liquid chromatography or excised from 2-dimensional electrophoreses gels. Protein digests generally require desalting on reverse phase liquid chromatography (RPLC) or solid-phase extraction (SPE) prior to mass spectrometry. The sample presentation devices of the current invention are suitable for direct collection and subsequent analysis of protein digests desalted by high performance RPLC or SPE.

As a specific example, surface plasmon resonance (SPR) biosensors exploit immobilized proteins to study protein-protein and other biological interactions. Unfortunately, a large volume of eluant is required to recover an analyte from a biosensor and the concentration of analyte in the sample is too low for optimum mass spectrometry. The sample presentation device of the present invention is suitable for direct collection of analytes recovered from biosensor systems; it may be configured to a standard 96-well format so as to be compatible with sample collection devices already integrated into biosensor systems and can be exploited to enable automated sample collection for mass spectrometric analysis, and can concentrate liquid samples of large volumes.

The liquid-holding limitations associated with known mass spectrometer sample presentation devices have prompted the development of various micro-column liquid chromatography approaches involving the use of small pipette tips packed with minute quantities of chromatographic media (e.g., ZipTips®). Micro-column approaches enable the desalting of protein digests with a concomitant reduction in sample volume reported to be sufficient to enable the sample to be applied directly to prior art mass spectrometer devices for retaining samples. The sample presentation devices of the present invention are suitable for direct collection and subsequent analysis of protein digests desalted by micro-column RPLC.

In general, the sample presentation devices of the present invention can be used to accomplish the following with respect to the above-described samples: concentrating; diluting; locating; transporting; storing; presenting for analysis; fractionating; washing; and post-application processing (including digesting, derivatizing, and eluting). It should be understood that this list is not exhaustive and merely provides examples in general terms as to the various applications the sample presentation devices of the present invention can be used.

Once the samples have been applied to the sample presentation devices of the present invention, and the samples have undergone any of the above-identified operations with respect to movement of liquid samples thereon, the following applications can be performed either on the sample presentation device itself or after removal from the device: MALDI-MS; other mass spectrometry techniques; surface plasmon resonance (SPR); fluorescence; atomic force microscopy (AFM); optical spectroscopy; bio- and chemiluminescence; x-ray photoelectron spectroscopy; ellipsometry; electrochemical

detection; phosphorescence; and UV, visible and IR spectroscopies. It should be appreciated that this is only a partial list of such applications. It should also be understood that any of the above analyses may be combined and/or serialized, and that where appropriate, these analyses may be performed directly or indirectly upon the analyte(s).

Numerous fields of use are contemplated as being applicable to the sample presentation device of the present invention and include, but are not confined to, such fields as genomics, proteomics, pharmacogenomics, physiomics, toxiomics, metabonomics, drug discovery/drug development/clinical trial monitoring, toxicology, diagnostics, environmental, biosensors, and biological and chemical weapons/bioterrorism. A few specific examples of the applications of the sample presentation devices are described below. The descriptions that follow are merely exemplary and do not limit the scope of the invention.

Genomics: The application of mass spectrometry to genotypic and phenotypic problems has an essential prerequisite of desalting the nucleic acid analyte(s) prior to ionization. Traditionally this desalting is performed before the sample is placed on a MALDI source. In one embodiment, the sample presentation device in an X3 format can accomplish the desalting simultaneously with concentrating the nucleic acid analyte(s). This embodiment is comprised of a reverse phase capture zone and an analyte binding resistant analysis zone. Another embodiment may be comprised of an X4, wherein two capture zones and a single analysis zone would be employed. In a concentric arrangement, the outer capture zone would specifically bind polynucleotide analytes through complementary hybridization with immobilized capture probes; the inner capture zone would perform a desalting function as described above, and the analysis zone presents the analyte for detection. In both of these embodiments, the performance of desalting and presentation for analysis on the same chip increases throughput, minimizes sample loss, and decreases cost.

Drug Discovery/Development/Clinical Trial Monitoring: Many drugs are effective on only a portion of the population. An example of this phenomenon is the drug Herceptin, which is useful for only about 30% of breast cancer patients. In the case of Herceptin, the genetic and protein basis of the sensitivity was integral to the design of the drug, but in most cases the population cannot be divided into likely responders and non-responders prior to expensive and lengthy clinical trials. One of the principal challenges of

interpreting such clinical trial results is to understand the biological and/or chemical basis for response and non-response. That knowledge can then be used both for targeting of populations and for further refinement of the drug itself.

One approach to this problem is to obtain profiles (e.g., protein, carbohydrate, lipid) from the patients before, during, and after treatment, and to correlate these profiles with treatment outcome. Several embodiments of the present device can be applied to such studies. Samples (e.g., blood, urine, tissue) obtained from the patients can be subjected to one or more of the pre-processing methods enumerated in the section described above, such as multi-dimensional liquid chromatography, and the fractionated materials produced by that method applied to the device for concentration and presentation for mass spectrometric analysis. Alternatively, samples subjected to minimal processing can be applied to one or more of the present devices with capture zones of known specificity. The analytes are then transferred either to capture zones of complementary specificity before transfer to analysis zones, or directly to analysis zones. In this manner, surfaces with different specificities can be used both in series and in parallel in an automated manner, with the fractionated analytes presented on identical analysis zones for mass spectrometry.

Mass spectrometry provides both profiles (the full mass spectrum) and the opportunity to unambiguously identify specific molecular entities of interest. The mass spectra can then be collected into a database, and multifactorial analysis tools applied to correlate the profiles with patient response. In this way one can discover patterns within the profiles and/or specific molecular entities that enable: prediction of response to therapy; monitoring of response to therapy; and identification of molecular entities that affect response to therapy, thus allowing increasingly sophisticated drug design.

This area of scientific inquiry, like the others described herein, is dependent in large measure on the ability to measure analytes in liquid solution. The sample presentation devices of the present invention, and their uses described herein, represent an important tool that can be used to conduct further study.

Environmental: Analyzing environmental samples for the presence of contaminants is a worldwide effort. Among the particular problems faced by such studies are the low concentrations of analytes and the diversity of samples that must be studied, as contaminants may be present in gaseous, liquid, and solid materials. In general, such analyses involve collection, extraction, derivatization, fractionation, and detection steps.

The present devices may be applied in a number of ways to the analysis of environmental samples. These examples are representative, but by no means complete. Devices with capture zones can be used for direct collection of analytes from gaseous or liquid media. For example, capture of hydrophobic pesticide residues from aqueous solutions by a hydrophobic surface may replace liquid/liquid extractions, which can be time-consuming and generate hazardous waste. The collected material can then be transferred directly to analysis zones, fractionated by serial or parallel transfer to capture zones of complementary specificity prior to transfer to analysis zones, or transferred from the device to enable analysis by one or more of the techniques enumerated in the sections described above. Mass spectrometry is generally used for identification of pesticide residues, but other techniques such as immunoassay may be applied. The present devices can also be used as previously described to present and/or fractionate materials resulting from any of the steps of environmental analysis listed above. The present devices can be used as a platform to derivatize analytes and present them for analysis in altered form. For example, silyl- and/or acetyl- moieties may be added to pesticides immobilized on the device to enable unambiguous identification of molecular structure.

Biological and Chemical Weapons/Bioterrorism: The United States government is confronted with the need for platforms and analytical techniques to facilitate the detection of chemical and biological agents in both military and civil scenarios. Challenges for biowarfare detection include sample collection and distinguishing between innocuous versus toxic organisms. The current battlefield technique for bio agents utilizes pyrolysis to convert biological compounds to small, easily detectable molecules by MS. A technique relying on peptide biomarkers is largely anticipated, since it would be more specific than current methods. Tests on individuals to determine potential exposure to warfare agents should involve breath tests or blood drawing techniques. Stand-alone biosensors as alerting devices are also of great interest for use in public places or in the battlefield. All these methods present challenges in sample collection, pre-treatment, and presentation of samples to detectors by robotics or other remote means. Techniques that can store, manipulate, concentrate or purify samples or those that can be coupled to aerosol impactors currently used have the potential of attracting the interest of defense agencies. The present devices can be applied to biowarfare/bioterror detection in a manner similar to that described for environmental samples. In addition, devices with custom capture zones can be designed to collect microorganisms of interest from environmental or biofluid samples,

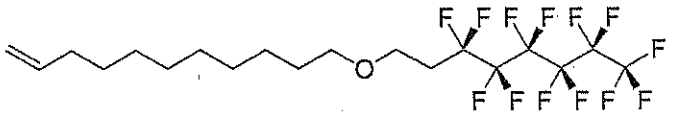
allow processing of the cells (or viruses) to release key markers, and present those markers for detection.

Examples

The following examples provide additional detail about the composition, manufacture, and use of the sample presentation devices of the present invention, but are exemplary only and do not in any way limit the scope of the present invention.

Example I

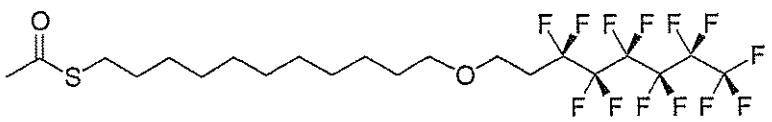
Preparation of 11-(3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyloxy)undec-1-ene (**1**)



An amber shell vial (40 mL) was charged with 3.0 mL of 1H,1H,2H,2H-perfluorooctanol (13.7 mmol) and to this was added 1.4 mL of 50% aqueous potassium hydroxide (13.7 mmol). The solution was warmed to 80 °C, stirred for 30 minutes and 3.3 mL of 11-bromoundec-1-ene (1.5 mmol) added. The reaction was maintained at 80 °C for 52 hours until TLC analysis (hexane) showed the starting material was consumed. The product was allowed to cool to room temperature, added to 100 mL ethyl acetate and extracted with water (2 × 50 mL) and brine (1 × 50 mL). The ethyl acetate extract was dried over magnesium sulfate, filtered and the solvent evaporated *in vacuo* to afford an oily residue. The residue was purified on a silica gel flash column (50 × 300 mm, 0% ethyl acetate/hexane followed by 10% ethyl acetate/hexane). Fractions containing the desired product were combined and the solvent evaporated to afford 4.52 g (64%) of **1** as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): □ 5.80 (m, 1H), 4.95 (m, 2H), 3.69 (t, J = 6.8 Hz, 2H), 3.43 (t, J = 6.8 Hz, 2H), 2.39 (m, 2H), 2.03 (m, 2H), 1.55 (m, 2H), 1.36 (m, 2H), 1.27 (broad m, 10H).

Example II

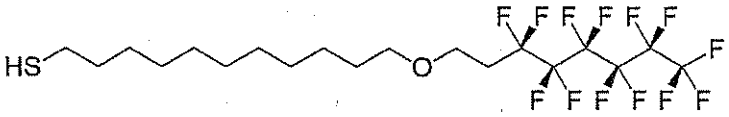
Preparation of Thioacetic Acid *S*-[11-(3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyloxy)undecyl] Ester (**2**)



A dry round bottom flask (100 mL) was charged with 1.0 g of **1** (1.9 mmol) under argon and 10 mL of dry methanol added. To the resulting solution was added 426 □L of thiolacetic acid (6.0 mmol) followed by 52 mg of 2,2'-azobis(2-methylpropionamidine) dihydrochloride (0.2 mmol). The reaction was shrouded in a foil tent and exposed to light from a low pressure mercury lamp. After 4 hours, TLC analysis (5% ethyl acetate/hexane) revealed that the starting material had been consumed. The solvent was evaporated *in vacuo* to give an oily residue. The residue was purified on a silica gel flash column (40 x 300 mm, 0% ethyl acetate/hexane followed by 5% ethyl acetate/hexane). Fractions containing the desired product were combined and the solvent evaporated to afford 856 mg (76%) of **2** as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.69 (t, J = 6.8 Hz, 2H), 3.43 (t, J = 6.8 Hz, 2H), 2.39 (m, 2H), 2.31 (s, 3H), 1.55 (m, 2H), 1.33 (m, 2H), 1.25 (broad m, 10H).

Example III

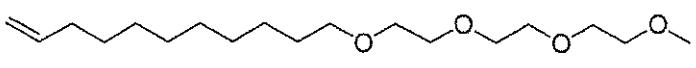
Preparation of 11-(3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyloxy)undecane-1-thiol (**3**)



An amber shell vial (20 mL) was fitted with a Teflon-lined silicon septum, charged with 850 mg of **2** (1.1 mmol) and 5 mL of 3N methanolic hydrogen chloride (15 mmol) added. The resulting solution was warmed to 40 °C for 4 hours. The solvent was removed to afford 782 mg (98%) of **3** as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.69 (t, J = 6.8 Hz, 2H), 3.43 (t, J = 6.6 Hz, 2H), 2.51 (dd, J = 7.3, 7.6 Hz, 2H), 2.39 (m, 2H), 1.58 (m, 4H), 1.32 (t, J = 8.0 Hz, 1H), 1.25 (broad m, 12H).

Example IV

Preparation of 11-{2-[2-(2-Methoxyethoxy)ethoxy]ethoxy}undec-1-ene (**4**)

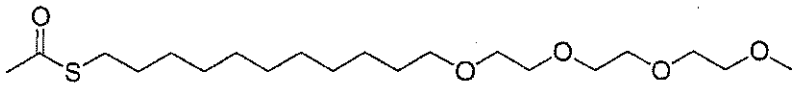


A round bottom flask (200 mL) was charged with 27.4 mL of triethyleneglycol monomethyl ether (171 mmol) and 9.1 mL of 50% aqueous sodium hydroxide (114 mmol) added. The pale yellow solution was warmed to 80 °C, stirred for 30 minutes and 26.6 mL

of 11-bromoundec-1-ene (114 mmol) was added dropwise. The reaction was maintained at 80 °C for 7.5 hours until TLC analysis (100% ethyl acetate) showed the starting material to be consumed. The product was cooled to room temperature, diluted into 50 mL of water and extracted with hexanes (3 × 50 mL). The hexanes extracts were combined, dried over magnesium sulfate, filtered and the solvent evaporated *in vacuo* to afford 20 g (56%) of **4** as a clear, colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.81 (m, 1H), 4.96 (m, 2H), 3.68-3.56 (m, 12H), 3.44 (t, J = 6.8 Hz, 2H), 3.38 (s, 3H), 2.04 (m, 2H), 1.57 (m, 2H), 1.36 (m, 2H), 1.27 (broad s, 10H).

Example V

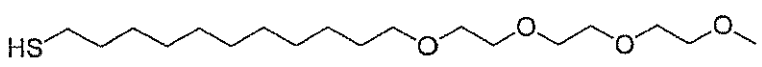
Preparation of Thioacetic acid *S*-(11-{2-[2-(2-methoxyethoxy)ethoxy]ethoxy}undecyl) ester (**5**)



A dry round bottom flask (200 mL) was charged with 5.0 g of **4** (15.8 mmol) under argon and 10 mL of dry methanol was added. To this was added 3.6 mL of thiolacetic acid (50 mmol) followed by 434 mg of 2,2'-azobis(2-methylpropionamidine) dihydrochloride (1.6 mmol). The reaction was shrouded in a foil tent and exposed to light from a low pressure mercury lamp. After 15.5 hours, TLC analysis (ethyl acetate/hexane, 1:3) revealed the starting material had been consumed. The solvent was evaporated *in vacuo* to give a residue with a strong sulfur-like odor. The residue was purified on a silica gel flash column (40 x 300 mm, 30% ethyl acetate/hexane, and 50% ethyl acetate/hexane). Fractions containing the desired product were combined and the solvent was evaporated to afford 5.83 g (94%) of **5** as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.67-3.54 (m, 12H), 3.44 (t, J = 7.2 Hz, 2H), 3.38 (s, 3H), 2.86 (t, J = 7.2 Hz, 2H), 2.32 (s, 3H), 1.57 (m, 4H), 1.36-1.26 (broad m, 14H).

Example VI

Preparation of 11-{2-[2-(2-Methoxyethoxy)ethoxy]ethoxy}undecane-1-thiol (**6**)

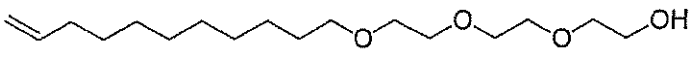


An amber shell vial (20 mL) fitted with a Teflon-lined silicon septum was charged with 5.0 g of **5** (12.7 mmol) and 7 mL of 3N methanolic hydrogen chloride (21 mmol) was

added. The solution was warmed to 40 °C for 6 hours. The solvent was then evaporated *in vacuo* to afford 4.40 g (98%) of **6** as a colorless waxy gel. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.67-3.54 (m, 12H), 3.44 (t, J = 6.8 Hz, 2H), 3.37 (s, 3H), 2.51 (dd, J = 7.3, 8.0 Hz, 2H), 1.57 (m, 4H), 1.32 (t, J = 7.6 Hz, 1H), 1.26 (broad m, 14H).

Example VII

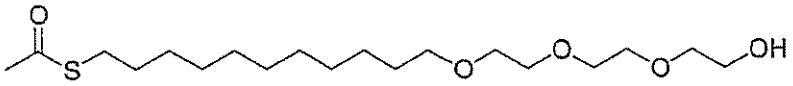
Preparation of 2-[2-(2-Undec-10-enyloxyethoxy)ethoxy]ethanol (7)



A round bottom flask (250 mL) was charged with 67.0 mL of triethyleneglycol (0.5 mol) and 8.0 mL of 50% aqueous sodium hydroxide (8 mL, 0.1 mol) was added. The solution was warmed to 100 °C, stirred for 30 minutes and 22.0 mL of 11-bromoundec-1-ene (0.1 mol) were added dropwise to give a dark yellow solution which produced a precipitate of sodium bromide. The reaction was maintained at 100 °C for 2.5 hours until TLC analysis (methanol/ethyl acetate/hexane, 1:1:8) revealed that the starting material to be consumed. The reaction was cooled to room temperature, diluted into 300 mL of water and extracted with hexanes (3 × 100 mL). The organic extracts were combined, washed with brine (50 mL), dried over magnesium sulfate and filtered. The solvent evaporated *in vacuo* to give an oily residue. The residue was purified on a silica gel flash column (50 x 400 mm, methanol/ethyl acetate/hexane 5:5:90). Fractions containing the desired product were combined and the solvent was evaporated to give 20.8 g (69%) of 7 as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.78 (m, 1H), 4.93 (m, 2H), 3.72-3.55 (m, 12H), 3.42 (t, J = 7.2 Hz, 2H), 2.64 (t, J = 5.6 Hz, 1H), 2.01 (m, 2H), 1.54 (m, 2H), 1.34 (m, 2H), 1.25 (broad s, 10H).

Example VIII

Preparation of Thioacetic acid *S*-(11-{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}undecyl) ester (**8**)

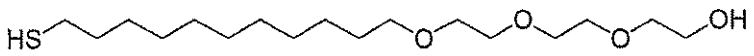


A dry round bottom flask (100 mL) was charged with 2.0 g of 7 (6.6 mmol) under argon and 10 mL of dry methanol added. To this was added 2.85 mL of thiolacetic acid (40 mmol) followed by 271 mg of 2,2'-azobis(2-methylpropionamidine) dihydrochloride (1.0

mmol). The reaction was shrouded in a foil tent and exposed to with light from a low pressure mercury lamp. After 6 hours, TLC analysis (methanol/ethyl acetate/hexane, 1:1:8) revealed that the starting material had been consumed. The solvent was evaporated *in vacuo* to give yellow oil. The oil was purified on a silica gel flash column (50 x 300 mm, methanol/ethyl acetate/hexane, 1:1:8). Fractions containing the desired product were combined and the solvent was evaporated to afford 2.44 g (98%) of **8** as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.71-3.54 (m, 12H), 3.42 (t, J = 6.6 Hz, 2H), 2.83 (t, J = 7.2 Hz, 2H), 2.66 (broad s, 1H), 2.29 (s, 3H), 1.52 (m, 4H), 1.36-1.23 (broad m, 14H).

Example IX

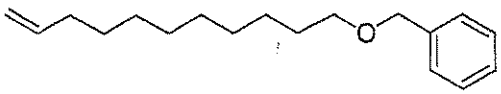
Preparation of 2-{2-[2-(11-Mercaptoundecyloxy)ethoxy]ethoxy}ethanol (**9**)



An amber shell vial (20 mL) was fitted with a Teflon-lined silicon septum, charged with 2.40 g of **8** (6.4 mmol) and 5.0 mL of 3N methanolic hydrogen chloride (15 mmol) added. The resulting solution was warmed to 40 °C for 4 hours. The solvent was then evaporated *in vacuo* to afford 2.05 g (95%) of **9** as a colorless waxy gel. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.72-3.55 (m, 12H), 3.43 (t, J = 6.8 Hz, 2H), 2.71 (broad s, 1H), 2.50 (dd, J = 7.6, 7.4 Hz, 2H), 1.62-1.52 (m, 4H), 1.31 (t, J = 7.6 Hz, 1H), 1.26 (broad m, 14H).

Example X

Preparation of Undec-10-enyl-oxymethylbenzene (**10**)

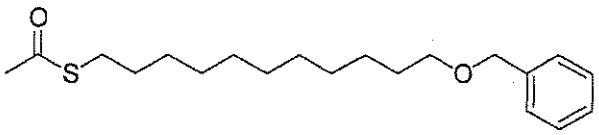


A dry round bottom flask (100 mL) was charged with 5.0 g of undec-10-en-1-ol (29.4 mmol) under argon and 25 mL of dry *N,N*-dimethylformamide was added. The resulting solution was cooled to 0 °C and 2.16 g of 60% sodium hydride in mineral oil (45 mmol) was added in one portion. The frothing mixture was stirred under argon at 0 °C for 30 minutes. To the chilled, stirred solution was added dropwise 7.7 g of bromomethylbenzene (45 mmol) in 5mL of dry *N,N*-dimethylformamide and the reaction was allowed to warm to room temperature while stirring for 3 hours. The reaction was quenched by the slow addition of 100mL of ethyl acetate, extracted with 1N hydrochloric acid (2 × 50 mL) and brine (1 x 50ml). The organic layer was dried over magnesium sulfate, filtered and the solvent evaporated to give an oily residue (9.5 g). The residue was

purified on a silica gel flash column (50 x 300 mm, 94:5:1 hexane/toluene/ethyl acetate) and the fractions containing the desired product were combined. Finally, the solvent was evaporated *in vacuo* to afford 7.1g (93%) of **10** as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32 (d, 4H), 7.28 (m, 1H), 5.81 (m, 1H), 4.95 (m, 2H), 4.49 (s, 2H), 3.46 (t, 2H), 2.03 (m, 2H), 1.61 (m, 2H), 1.35 (broad m, 4H), 1.24 (broad s, 10H).

Example XI

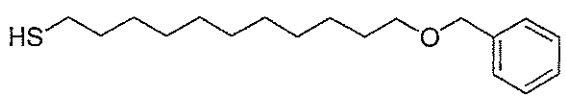
Preparation of Thioacetic Acid *S*-(11-Benzyloxyundecyl)ester (**11**)



A jacketed photo-reaction vessel (250 mL) was first charged with 5.0 g of **10** (19.2mmol) and 0.520 g of 2,2'-azobis(2-methylpropionamidine) dihydrochloride (1.92mmol). The vessel was sealed, evacuated and back-flushed with argon (several cycles). While under argon, 60 mL of anhydrous methanol and 0.520 g of thioacetic acid (92 mmol) were injected into the reaction vessel and the contents of the vessel were stirred. The vessel was again evacuated and back-flushed with argon (several cycles). The UV lamp was activated and the mixture irradiated under argon with constant stirring for 3 hours. The reaction was continually cooled (water jacket) and the temperature maintained below 38 °C during the photo-reaction process. The reaction vessel was allowed to cool to room temperature and the solvent was evaporated to give pale yellow oil (10.8 g). The oil was purified on a silica gel flash column (50 x 300 mm, 98:2 hexane/ethyl acetate) and the fractions containing the desired product were combined. Finally, the solvent was removed *in vacuo* to afford 5.0g (77%) of **11** as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32 (d, 4H), 7.28 (m, 1H), 4.49 (s, 2H), 3.46 (t, 2H), 2.86(t, 2H), 2.31(s, 3H), 1.50-1.66 (m, 4H), 1.20-1.40 (broad m, 14H).

Example XII

Preparation of 11-Benzyloxyundecane-1-thiol (**12**)



An amber shell vial (40 mL) was fitted with a Teflon-lined silicon septum, charged with 3.04 g of **11** (9.03mmol) followed by 2mL of dichloromethane, 1mL of hexane, and 12 mL of 4.9 N ethanolic hydrogen chloride. The resulting solution was warmed to 40° C

for 4.5 hours. The solvent was then evaporated *in vacuo* to afford a colorless oily residue (2.8 g). The residue was purified on a silica gel flash column (25 x 450 mm, 9:1 hexane/chloroform) and the fractions containing the desired product were then combined. The solvent was evaporated *in vacuo* to give 2.5 g (94%) of **12** as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32 (d, 4H), 7.28 (m, 1H), 4.49 (s, 2H), 3.46 (t, 2H), 2.51(q, 2H), 1.55-1.65 (m, 4H), 1.20-1.40 (broad m, broad t, 15H).

Example XIII

Preparation of Self-Assembled Monolayers on Gold-Coated Silicon Substrates

Silicon wafers (200 mm, P-type, Prime Grade Silicon 100) were diced to individual substrates and cleaned to afford a surface having fewer than 10 particles (0.16 μm to 3000 μm) per substrate. Metal deposition was carried out in a CPA 9900 sputtering system with a base pressure of 5×10^{-7} mm. In the sputtering chamber, the substrates were cleaned and etched by argon plasma and an adhesive layer of titanium and tungsten (1:9) was sputtered at a rate of 5 Å/s to a thickness of 250 Å. Gold was then sputtered at a rate of 20 Å/s up to a thickness of 1000 Å. Substrates were cooled under an argon flow prior to removal.

Prior to monolayer assembly, gold-coated substrates were cleaned by treatment with argon plasma at 200 W for 300 s. The substrates were rinsed with ethanol and then transferred to a 0.1 mM solution of **3** (11-(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-octyloxy)undecane-1-thiol) in ethanol and incubated at room temperature for a period ranging from 1 to 24 hours. Finally, surface-modified substrates were removed from the assembly bath, spin washed at 1000 rpm with ethanol and dried under a stream of nitrogen. The advancing contact angles of water drops (0.5 μL) applied to the surface-modified substrates were in the range 114° to 120°. Surface-modified substrates were stored in fitted plastic containers with transparent amber UV resistant covers.

Example XIV

Preparation of Patterned Sample Presentation Devices

Twenty-four (24) surface-modified substrates were prepared as described above, mounted in a custom alignment jig and covered with a pin-registered etched stainless steel shadow mask (0.002 inch) having features corresponding in size and shape to the liquid retention zone. The jig was placed on the moving belt of an air-cooled ultraviolet curing system fitted with a low-pressure mercury light source rated at 120 W/cm^2 and passed under the light source 45 to 75 times over the course of one hour. Following UV exposure,

the substrates were removed from the jig, spin washed at 1000 rpm with ethanol and dried under a stream of nitrogen. The exposed substrates were placed in a 0.1 mM solution of **6** (11-{2-[2-(2-methoxyethoxy)ethoxy]ethoxy} undecane-1-thiol) in ethanol and incubated at room temperature for a period ranging from 1 to 24 hours. Patterned surface-modified substrates were removed from the assembly bath, spin washed at 2400 rpm with ethanol and dried under a stream of nitrogen. The advancing contact angles of water drops applied to the liquid retention zone were in the range 60° to 65°, and when applied to the boundary zone were in the range 110° to 119°.

Patterned surface-modified substrates were mounted in a custom alignment jig and covered with a second pin-registered etched stainless steel shadow mask having features corresponding in size and shape to the analysis zone. The jig was placed on the moving belt of the ultraviolet curing system and passed under the light source 45 to 75 times over the course of one hour. Following UV exposure, the substrates were removed from the jig, spin washed at 1000 rpm with ethanol and dried under a stream of nitrogen. The exposed substrates were placed in a 0.1 mM solution of **9** (2-{2-[2-(11-mercaptoundecyloxy)ethoxy]ethoxy}ethanol) in ethanol and incubated at room temperature for 1-24 hours. Finally, twice-patterned surface-modified substrates were removed from the assembly bath, spin washed at 1000 rpm with ethanol and dried under a stream of nitrogen. The advancing contact angles of water drops applied to the analysis zone were less than 47°. Twice-patterned surface-modified substrates were stored in fitted plastic containers with amber transparent UV resistant covers.

While various embodiments of the present invention have been described above, it should be understood that they have been presented by way of example only, and not limitation. In particular, the physical arrangement of the analysis zone, liquid retention zone, and boundary zone is not limited by the examples described above. Thus, the breadth and scope of the present invention should not be limited by any of the above-described exemplary embodiments.

Example XV

Sample Containment and Positioning

Analyte-confining properties of the analysis zone, which afford an increase in sensitivity of detection, are demonstrated in the video contact angle images shown in FIGS. **11***a* through **11***h*. With reference to FIG. **11***a*, the sample presentation device of the

present invention was prepared with a liquid retention zone measuring about 1.6 mm OD and an analysis zone measuring about 0.7 mm OD. To facilitate the observation of the focusing effect, the analysis zone was placed off-center. A drop of water was applied to the surface of the biochip and was observed to rapidly confine itself to the surface area corresponding to the liquid retention zone and the analysis zone. The initial left-side and right-side contact angles were recorded and were both found to be 57.1°, a value which corresponds to that exhibited by a surface prepared from exclusively the liquid retention zone monomer. As the drop dried owing to evaporation (see FIGS. **11***b* through **11***h*), both the observed radius and contact angles receded until the radius of the drop corresponded to that of the analysis zone. Furthermore, as the drop dried it was observed that the center of the drop moved to the right so as to allow the drop to center itself over the analysis zone. The left-side and right-side contact angles recorded in FIG. **11***h* were both found to be 35.4°, a value which corresponds to that exhibited by a surface prepared exclusively from the analysis zone monomer. The drop height, width and contact angle data recorded in conjunction with the acquisition of the images depicted in FIGS. **11***a* through **11***h* is summarized graphically in FIG. **12**.

Example XVI

Liquid-Holding Capacity of Patterned Sample Presentation Devices

The extraordinary liquid-holding capacity of the liquid retention zone is demonstrated in FIG. **13**. A photograph of a 16-site sample presentation device of the present invention shows the retention of sample drop volumes in the range 5 μL to 70 μL. The only factor that appears to significantly limit the sample drop volume is the relative proximity of the adjacent pairs of target and liquid retention zones.

Example XVII

Analyte Directing and Concentration

Analyte-confining properties of the analysis zone are further demonstrated in FIGS. **14***a* and **14***b*. The first photograph (FIG. **14***a*) is of a 16-site sample presentation device of the present invention with sample drop volumes in the range 5μL to 40 μL deposed on the surface of 8 of the 16 sites. Each of the liquid drops contained an equivalent amount of HCCA. FIG. **14***b* is a photograph of the HCCA having been concentrated and directed to the analysis zone due to sample drying on the sample presentation device depicted in FIG.

14*a*. The relative size of the analysis zone and the liquid retention zone is superimposed above the HCCA for comparison purposes.

While various embodiments of the present invention have been described above, it should be understood that they have been presented by way of example only, and are not limitations. In particular, the physical arrangement of the analysis zone, liquid retention zone, and boundary zone is not limited by the examples described above. Thus, the breadth and scope of the present invention should not be limited by any of the above-described exemplary embodiments.

WHAT IS CLAIMED IS:

1. A sample presentation device for detecting analytes in a sample comprising a substrate having a surface, wherein the surface is comprised of a plurality of zones of differing wettability, and wherein the zone from which analytes in the sample are detected is substantially analyte binding resistant.

2. The sample presentation device of claim 1, wherein one of the zones of differing wettability is optimal with respect to retention of the sample.

3. The sample presentation device of claim 1, wherein one of the zones of differing wettability is optimal with respect to high sensitivity detection of the analytes.

4. The sample presentation device of claim 1, wherein the substrate is selected from one or more of the group consisting of glasses, semiconductors, metals, polymers, plastics, SiO_2 on silicon, and Al_2O_3 on aluminum.

5. The sample presentation device of claim 1, wherein one or more of the zones of differing wettability is comprised of self-assembled monolayers.

6. The sample presentation device of claim 1, further comprising a boundary zone that is substantially nonwettable and one or more additional zones, each of which is more wettable than the boundary zone.

7. The sample presentation device of claim 6, wherein the one or more additional zones comprise a liquid retention zone that is more wettable than the boundary zone, and an analysis zone that is more wettable than the liquid retention zone.

8. The sample presentation device of claim 6, wherein the boundary zone has a higher contact angle than each zone of the one or more additional zones.

9. The sample presentation device of claim 7, wherein the boundary zone has a higher contact angle than the liquid retention zone, and wherein the liquid retention zone has a higher contact angle than the analysis zone.

10. The sample presentation device of claim 1, wherein the plurality of zones of differing wettability comprise a boundary zone that is substantially nonwettable, and at least one wettable zone that substantially binds analytes.

11. A sample presentation device for storing analytes from a sample comprising a substrate having a surface, wherein the surface is comprised of a plurality of zones of differing wettability, and wherein the zone in which the analytes from the sample are stored is substantially analyte binding resistant.

12. A sample presentation device for storing analytes from a sample comprising a substrate having a surface, wherein the surface is comprised of a plurality of zones of differing wettability, and wherein the zone in which the analytes from the sample are stored substantially binds analytes.

13. The sample presentation device of claim 1, wherein the sample is less than or equal to 100 μL in volume.

14. The sample presentation device of claim 1, wherein the sample is less than or equal to 70 μL in volume.

15. A method of making a sample presentation device for detecting analytes in a sample, wherein the sample presentation device comprises a substrate having a surface, comprising modifying the surface of the substrate to create a plurality of zones of differing wettability, and wherein the zone from which the analytes in the sample are detected is substantially analyte binding resistant.

16. The method of claim 15, wherein modifying comprises applying one or more self-assembled monolayers to the surface of the substrate.

17. The method of claim 16, wherein applying the self-assembled monolayers comprises patterning the surface with a patterning technique selected from one or more of the group consisting of UV photo-patterning, photolithographic patterning, microstamping. electron-beam patterning, and reactive-ion etching.

18. A method of detecting analytes in a sample, comprising contacting the sample with the sample presentation device of claim 1, and detecting analytes in the sample.

19. A method of detecting analytes in a plurality of samples, comprising contacting the plurality of samples with the sample presentation device of claim 1, and detecting analytes in the plurality of samples.

20. The method of claim 18, wherein detecting analytes in the sample comprises one of the group consisting of mass spectrometry, surface plasmon resonance, fluorescence, atomic force microscopy, optical spectroscopy, bioluminescence, chemiluminescence, x-ray photoelectron spectroscopy, ellipsometry, electrochemical detection, phosphorescence, ultraviolet spectroscopy, visible spectroscopy, and infrared spectroscopy.

21. The method of claim 20, wherein the mass spectrometry is laser desorption ionization mass spectrometry.

22. A method of concentrating a sample containing analytes using the sample presentation device of claim 1, comprising concentrating the sample in a zone of highest degree of wettability.

23. The method of claim 22, wherein the zone of highest degree of wettability is less than 2 mm^2 in area.

24. The method of claim 22, wherein the zone of highest degree of wettability is less than 1 mm^2 in area.

25. The method of claim 22, further comprising transferring the sample concentrated in the zone of highest degree of wettability to one or more additional sample presentation

devices, each device comprising a plurality of zones of differing wettability with respect to the concentrated sample.

26. A method of detecting analytes in a sample, comprising capturing from the sample analytes that bind substantially to one or more zones of the sample presentation device of claim 10.

27. A method of detecting analytes in a sample, comprising depleting from the sample substances that interfere with subsequent sample handling processes, wherein the substances bind substantially to one or more zones of the sample presentation device of claim 10.

28. A method of storing analytes from a sample, comprising storing the analytes on a sample presentation device, wherein the sample presentation device comprises a substrate having a surface, wherein the surface is comprised of a plurality of zones of differing wettability and wherein the zone in which the analytes from the sample are stored is substantially analyte binding resistant.

29. A method of handling a sample containing analytes compromising, contacting the sample with a sample presentation device comprised of a plurality of zones of differing wettability, concentrating the sample in the zone of highest degree of wettability, and wherein the zone with the highest degree of wettability is substantially analyte binding resistant.

30. The method of claim 29, further comprising detecting the analytes in the sample concentrated in the zone of highest degree of wettability.

31. The method of claim 30, wherein detecting analytes in the sample comprises one of the group consisting of mass spectrometry, surface plasmon resonance, fluorescence, atomic force microscopy, optical spectroscopy., bioluminescence, chemiluminescence, x-ray, photoelectron spectroscopy, ellipsometry, electrochemical detection, phosphorescence, ultraviolet spectroscopy, visible spectroscopy, and infrared spectroscopy.

32. The method of claim 31, wherein the mass spectrometry is laser desorption ionization mass spectrometry.

33. A method of modifying analytes using the sample presentation device of claim 7, comprising modifying the analytes within the liquid retention zone or the analysis zone or both.

34. The method of claim 33, wherein modification of the analytes is reversible.

35. The method of claim 33, wherein modification of the analytes is irreversible.

36. A method of altering the wettability of one or more zones of the sample presentation device of claim 1, comprising modifying the surface of the sample presentation device by physical stimuli or chemical stimuli or both, wherein the relative wettabilities of the zones are altered.

37. The method of claim 36, wherein the modification of the surface of the sample presentation device is reversible.

38. The method of claim 36, wherein the modification of the surface of the sample presentation device is irreversible.

39. A method of positioning one or more samples using the sample presentation device of claim 1, wherein the one or more samples move from a point of initial contact to one or more zones of higher wettability relative to the point of initial contact.

40. A method of positioning one or more samples using the sample presentation device of claim 10, wherein the one or more liquid samples move from a point of initial contact to one or more zones of higher wettability relative to the point of initial contact.