(19) World Intellectual Property Organization International Bureau



(43) International Publication Date 29 November 2001 (29.11.2001)

PCT

(10) International Publication Number WO 01/90729 A2

(51) International Patent Classification⁷: G01N 21/76, 33/52

(21) International Application Number: PCT/US01/10911

(22) International Filing Date: 4 April 2001 (04.04.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data: 09/575,411 19 May 2000 (19.05.2000) U

(71) Applicant (for all designated States except US): VERIFI-CATION TECHNOLOGIES, INC. [US/US]; Veritec, 85 Westbrook Road, Centerbrook, CT 06409 (US).

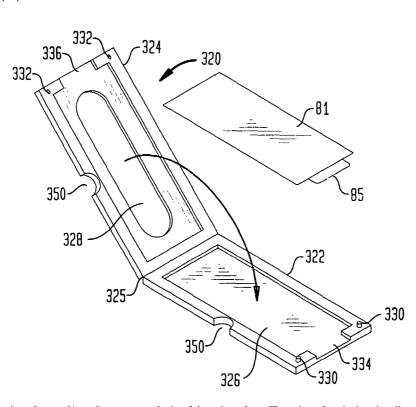
(72) Inventors; and

(75) Inventors/Applicants (for US only): BEHRINGER, Friedrich [—/US]; 11-1 Stonewood Drive, Old Lyme, CT 06371 (US). AUBRECHT, Sarka [—/US]; 10 Mystic Hill Road, Mystic, CT 06355 (US). SELINFREUND, Richard, H. [—/US]; 1285 Moose Hill Road, Guilford, CT 06437 (US). VIG, Rakesh [—/US]; 15 Park Place, Durham, CT 06422 (US).

- (74) Agent: FERRARO, Neil, P.; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210 (US).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: METHOD AND APPARATUS FOR PORTABLE PRODUCT AUTHENTICATION



(57) Abstract: A method and apparatus for on-site verification of product authentication and quality includes a microplate having a substrate with a light-sensitive compound thereon. The substrate provides immobilization of the light-sensitive compounds and provides a three-dimensional environment similar to free solution for reactions with the product sample to occur. The microplate may include any material having desired light reflective properties and a surface to retain the light-sensitive compounds therein. A metered amount of light-sensitive compound is placed on the microplate. Once the light-sensitive compound is applied to the substrate, the microplate may be sent to the test site where product testing is to be performed. A sample product is placed on the microplate and the light-sensitive compound thereon is free to react with key ingredients in the sample product. A holder may be used to facilitate application of the sample on the

microplate and/or subsequent analysis of the microplate. The microplate is then irradiated with a light source and light emission or absorption due to the interaction of the light-sensitive compound and the key ingredient is compared to a fingerprint.



WO 01/90729 A2

WO 01/90729 A2



Published:

 without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

-1-

METHOD AND APPARATUS FOR PORTABLE PRODUCT AUTHENTICATION

Field of the Invention

5

10

15

20

25

30

This invention relates generally to methods and apparatuses for authenticating a sample product, and more specifically, to methods and apparatuses for providing light-sensitive compounds for use with product authentication equipment.

Background of the Invention

Authenticating and monitoring products to discriminate between very similar complex mixtures is useful for various reasons. For example, the use of counterfeit substances (e.g., misbranded product from a competitor or misformulated product from a licensee/franchisee) should be detected to preserve the integrity of a brand. Also, low quality substances (e.g., diluted or misformulated product) should be quickly and conveniently detected for appropriate correction.

One particular industry that could benefit from such authenticity testing and monitoring is the beverage industry. With respect to monitoring production of the beverage, a simple, quick and product specific at-line test to determine whether the beverages being produced are within specification is desirable. Typically, the beverages are being bottled at a rate of 2000 bottles/minute. Therefore, standard off-line analytical techniques for monitoring product quality, such as GC/MS or HPLC, are complex and time consuming in that the beverages that are being tested have already been introduced to the market. A desirable monitoring procedure should provide relatively instant response time, be usable by non-scientific personnel, be accurate (e.g., having an error rate of less that 2.5%) and survive harsh plant environments.

With respect to product authentication, an example of an industry that could benefit is the beer industry. For example, at-line testing allows a determination as to whether a particular tap at a pub was actually serving authentic beer, without being sensitive to batch-to-batch variability of the a particular brand. Similarly, detection of dilution of a product may be important to the distilled spirits industry.

Commonly assigned U.S. Patent No. 5,753,511, which is herein incorporated by reference in its entirety, discloses an automated method of developing a database to store

5

10

15

20

25

30

information for "fingerprint"-type analysis of products (even as to product lot numbers and batch). The automated analysis is a method of evaluating and discriminating products, even within a narrow field or industry, competing and otherwise, to establish authenticity or point of origin of the product. The invention therein relates to an automated method for identifying key ingredients and/or the relative amounts of key ingredients in products mixed with light-emissive compounds. Scanning for light emission of a predetermined wavelength when the sample product is mixed with the light-emissive compound is used when comparing the sample product to a fingerprint.

The laboratory equipment used to authenticate the sample referred to in '511 is not easily and cost effectively transported. Thus, determining product authenticity on site, either at manufacturing points, at distribution points, or at consumption points is impractical.

Co-pending U.S. patent application serial no. 09/232,324, assigned to the present assignee and herein incorporated by reference in its entirety, discloses a portable product authentication device and a method of authenticating products. One embodiment disclosed therein requires a proper mixing of both the light-emissive compound and sample product prior to testing the sample for product authenticity. Although effective, mixing of the sample product and the light-emissive compound on-site can be cumbersome and time-consuming and may require a certain skill level.

In another embodiment disclosed in application '324, the light-emissive compound may be formed on a chip and the chip, together with a small amount of sample, is placed in the authentication device to determine the authenticity of the product. As discussed therein, the light-emissive compound may be attached to the chip through any physical or chemical means including covalent and non-covalent bonding. For example, the light-emissive may be dissolved in a solvent, then applied at a preselected concentration to the surface of the chip. The solvent is then evaporated away, leaving the light-emissive compound non-covalently attached to the surface of the chip. Although this results in a simple solution to providing light-emissive compounds without requiring mixing, the resulting chip may be costly and susceptible to damage.

To overcome this particular disadvantage, also disclosed in '324, the light-emissive compound may be covalently attached to the surface of the chip. In this instance, the light-emissive compound may have groups reactive under appropriate conditions with groups on the surface of the chip, which may be reactive groups of the chip per se, or may be linker molecules attached to the surface of the chip. Such cross-linking, however, often requires a

- 3 -

labor intensive process, resulting in a costly product. In addition, the cross-linking molecules may interfere with a proper reading of the light emission. For example, current microarray technology teaches the art of immobilizing chemistry for the detection of DNA specific or protein specific sequences. The amino-silane surface chemistry allows a fixed molecule to bind products for applied genomic gene expression studies and medical diagnostic information. The inventors of the present invention have found that adopting such technology for use in binding light-emissive compounds met with limited success.

Another example of providing light-emissive compounds is disclosed in co-pending U.S. patent application serial no. 09/173,814, assigned to the present assignee and herein incorporated by reference in its entirety, wherein a microplate may be used in place of the above-mentioned chip. As disclosed therein, the microplate includes a plurality of wells formed in the surface of the microplate. Light-emissive compounds are placed in the wells and attached thereto by directly bonding to the surface or through the use of a linker molecule or incorporated into the matrix created by the base material of the microplate itself. In addition, the invention therein describes the use of a dried light-emissive compound on the microplate or the microplate is packaged.

10

15

20

25

30

What is therefore needed is a simple, low cost method and apparatus that provides light-sensitive compounds for reaction with a sample product in an environment that is or simulates a liquid solution and that provides the ability for authenticity testing and monitoring of sample product at-line.

Summary of the Invention

The present invention features a method and apparatus for on-site verification of product authentication and quality. A film or a microplate having a substrate each includes a light-sensitive compound thereon. The substrate provides immobilization of the light-sensitive compounds and provides a three-dimensional environment similar to free solution for reactions with the product sample to occur. The microplate may include any material having desired light reflective properties and a surface to retain the light-sensitive compounds therein. A metered amount of light-sensitive compound is placed on the microplate or film by any desired metering method, such as hand-metering by skilled technicians, automatic metering using robotic equipment, or printing using for example, piezoelectric dispensing technology. Once the light-sensitive compound is applied, the microplate or film may be sent

- 4 -

to the test site where product testing is to be performed. A sample product is placed on the microplate or film and the light-sensitive compound thereon is free to react with key ingredients in the sample product. A holder may be used to facilitate application of the sample on the microplate or film and/or subsequent analysis of the microplate or film. The microplate or film may be irradiated with a suitable light source and light emission or absorption due to the interaction of the light-sensitive compound and the key ingredient is compared to a fingerprint.

5

10

15

20

25

30

In one illustrative embodiment of the present invention, a holder for holding a microplate or film is disclosed. The microplate or film has at least one light-sensitive compound disposed thereon for use in verifying a sample liquid product. The holder includes a first section; and a second section securable with the first section. The first and second sections are constructed and arranged to envelope the microplate or film when the first section is secured to the second section and when the microplate or film, having the sample liquid product disposed thereon, is placed therein.

In another illustrative embodiment of the present invention, a kit of parts for use in verifying a sample liquid product is disclosed. The kit includes a microplate or film having at least one light-sensitive compound disposed thereon for reaction with the sample product, a holder constructed and arranged to hold the microplate or film therein, and a package for packaging the microplate or film and the holder.

In another illustrative embodiment of the present invention, a method of verifying a sample liquid product includes applying the sample liquid product to a microplate having at least one light-sensitive compound disposed thereon; placing the microplate into a microplate holder; irradiating the microplate with an irradiating wavelength of light; and comparing light emission or absorption from the reaction of the light-sensitive compound with the sample liquid product.

In another illustrative embodiment of the present invention, a method of verifying a sample liquid product includes applying the sample liquid product to a film having at least one light-sensitive compound disposed thereon; irradiating the film with an irradiating wavelength of light; recording an image of light emission or absorption on the film; and comparing the image to a standard.

Further features and advantages of the present invention, as well as the structure and operation of various embodiments of the present invention are described in detail below with reference to the accompanying drawings.

5

10

15

20

25

30

Brief Description of the Drawings

The invention will now be described, by way of example, with reference to the accompanying drawings, in which:

- FIG. 1 is a perspective view of one embodiment of an authenticating compound disposed on a substrate;
 - FIG. 1a is enlarged view of the area encircled by line 1a of FIG. 1;
- FIG. 2 is a side view of an alternative embodiment of an authenticating compound disposed on a substrate;
 - FIG. 3 is an enlarged view of the area encircled by line 3 of FIG. 2;
- FIG. 4 is a perspective view of an authenticating compound with substrate interacting with a product sample;
- FIG. 5 is a perspective view of an apparatus used to determine product sample authenticity in combination with the authenticating compound on the substrate;
- FIGS. 6a-6c are perspective views of an embodiment of the invention for holding the substrate;
 - FIG. 7 is a perspective view of a container for use with the substrate;
- FIG. 7a is a perspective view of an alternative embodiment of a portion of the container encircled by line 7a of FIG. 7;
- FIGS. 8a-8c are perspective views of another embodiment of the invention for holding the substrate;
- FIGS. 9a-9d are perspective views of still another embodiment of the invention for holding the substrate;
- FIG. 10 is a perspective view of yet another embodiment of the invention for holding the substrate;
 - FIG. 11 is a perspective view of a packaged substrate with a holder;
 - FIG. 12 is a representation of a light source for use in irradiating the microplate.

Detailed Description

The invention features a microplate for use with a portable product authentication device. The microplate is used in conjunction with a product sample to be tested, analyzing key ingredients or analytes in the product. Authenticating compounds, such as light-sensitive compounds, can be used to identify the product sample. In one aspect, the light-sensitive

compound is provided on a microplate in a manner that allows the light-sensitive compound to freely react with a product sample when a product sample is placed thereon. In this respect, the light-sensitive compound is placed on a microplate, with the microplate causing immobilization of the light-sensitive compounds and providing a three-dimensional environment similar to free solution for reactions with the product sample to occur. A holder may be used in conjunction with the microplate to facilitate application of the sample on the microplate and/or facilitate subsequent analysis of the microplate. The light-sensitive compound together with the product sample is then irradiated using a light source to cause the light-sensitive compounds to emit or absorb light. The emitted or absorbed light is then read by an optical detector and then is compared to a stored fingerprint to determine whether the product sample is authentic. Specifically, the emitted or absorbed properties are compared to a standard fingerprint to determine authenticity. It is to be appreciated that the term "authentic", or any derivative thereof, means an identification as being genuine or without adulteration or identification of point of origin or other desired information.

5

10

15

20

25

30

Light-emissive compounds emit light in response to irradiation with light. Light-emission can be a result of phosphorescence, chemiluminescence or more preferably fluorescence. Specifically, the term "light-emissive compounds", as used herein, means compounds that have one or more of the following properties: 1) they are fluorescent, phosphorescence or luminescent; 2) react, or interact, with components of the sample or the standard or both to yield at least one fluorescent, phosphorescence, or luminescent compound; or 3) react, interact, with at least one fluorescent, phosphorescence, or luminescent compound in the sample product, the standard, or both to alter emission at the emission wavelength.

Light-absorbing compounds absorb light in response to irradiation with light. Light absorption can be the result of any chemical reaction known to those of skill in the art. Thus, the present invention may be discussed below with reference to emission of light in response to irradiation with light, however, the present invention is not limited in this respect and light absorbing compounds may be used. Thus, as used herein, the term "light-sensitive compounds" refers to both light emissive compounds as well as light absorbing compounds.

The term "fingerprint," as used herein, means light emission or absorption intensity and/or intensity decay at a particular wavelength or range of wavelengths, from one or more light-sensitive compounds in combination with a standard (e.g., authentic) product.

Accordingly, each product can have a particular fingerprint.

-7-

The term "fingerprint emission profile" as used herein, means an assembly of fingerprints of a standard in combination with a series (or profile) of different light-sensitive compounds.

The term "sample characteristic" as used herein refers to light emission or absorption quantity or intensity and/or intensity decay or change in quantity from one or more light-sensitive compounds in combination with the sample product.

5

10

15

20

25

30

As shown in Fig. 1, a microplate 10 includes a substrate 12 layered thereon for receiving at least one light-sensitive compound 13 in one or more areas, such as, for example, areas located at 14, 16. The light-sensitive compound may be applied to the substrate in a suitably metered amount. One (or up to 100 or more) light-sensitive compound(s) may be applied to the microplate in a manner that allows interaction or a combination of interactions with one or more analyte(s) (key ingredient(s)) in a sample.

Preferably, the microplate also includes a solid base 18 for supporting the substrate 12. The base may be any suitable material having suitable properties such that the combination of the base and the substrate, when the microplate is used in connection with a device having a light source and an optical detector, as will be further described hereinafter, does not interfere with the measurements taken by the device. Preferably, the base is glass. Also, preferably the base is flat.

Substrate 12 is preferably a porous material that may have 500 or more micropores such that the light-sensitive compound may be absorbed in the substrate in a manner to allow the sample, when placed on the substrate, to react with the light-sensitive compound. Porous substrates permit immobilization of the light-sensitive compounds, and can provide a three-dimensional environment similar to free solution for reactions with the product sample to occur. Furthermore, capillary forces in these substrates cause wicking of liquid from a contact dispenser, resulting in undesirable spread of light-sensitive compounds in the substrate. To control such spread, and for other advantages, piezoelectric non-contact dispensing may be used. Piezoelectric dispensing delivers a small, precisely controlled volume, which will be absorbed into the matrix in a consistent manner.

Piezoelectric dispensing technology is based on capillary dispensers which are able to aspirate solutions, such as light-sensitive compounds, from source wells and dispense many droplets to destinations in microarray formats. The dispenser consists of a glass capillary which has an orifice of approximately 75 µm at one end and a connection to a precision syringe pump at the other end. The syringe pump applies vacuum to aspirate solutions

-8-

through the tip. A piezoelectric transducer around the center of the capillary exerts pressure on the capillary when activated by an electronic pulse to create a pressure wave in the capillary which ejects a droplet of about 350 pL from the orifice, and the end of the capillary refills from the reservoir of system fluid by capillary flow. An example of such a dispenser is the BioChip ArrayerTM, available from Packard Instrument Company, Meriden, CT, USA. Other dispensers may be available from ink jet manufacturers.

5

10

15

20

25

30

In one particular example, the substrate 12 may be formed of silica and preferably a plurality of silica particles. However, it should be appreciate that the present invention is not limited in this respect and that other suitable materials may be used, for example, quartz, etc. Alternatively, if a glass base is used, the glass may be etched such that the etched surface provides a suitable substrate. In this respect, the microplate 10 may be a microplate produced by thin layer chromotography, as is known to those of ordinary skill in the art and which is commercially available from Merck of Darmstadt, Germany. Preferably, the silica particles have a size less than about 25 μ m and can total approximately 500 or more to provide approximately 500 or more micropores, although more or less may be provided.

In this regard, by applying the light-sensitive compound to such a substrate, the light-sensitive compound may be held thereon without covalently bonding or linking the light-sensitive compound to the substrate. In addition, the light-sensitive compound may be allowed to dry onto the substrate yet the substrate provides a medium to simulate a liquid environment. Accordingly, although not required, the dried light-sensitive compound on the microplate may be suitable for package and transportation to a test site. If the light-sensitive compound is allowed to dry on the substrate, then application of the product sample, when in liquid form, causes the light-sensitive compound to enter a liquid solution.

The substrate 12 may also be formed of a membrane. Examples of such membranes include nylon, nitrocellulose and AnaporeTM. Nylon and nitrocellulose membranes mounted on microscope slides are commercially available from Schleicher and Schuell and AnaporeTM membranes are available from Whatman (Anadisk Cat#6809-6022), United Kingdom. Compared to non-porous surfaces, conventional nitrocellulose or nylon blotting or transfer membranes have a much larger area available for surface interactions per unit of macroscopic area. Furthermore, a liquid, such as the light-sensitive compound and/or the sample product, dispensed onto these membranes will immediately distribute in the membrane by capillary flow, which may result in a relatively uniform distribution.

-9-

AnaporeTM is an inorganic microporous membrane with a highly controlled, uniform capillary pore structure. It is typically 60 µm thick and is available with approximately 200 nm capillary pores which afford the membrane with a great surface area for the immobilization of the light-sensitive compounds. The light-sensitive compounds can more readily be applied to the membrane using piezoelectric dispensing if the membrane is mounted to a base.

5

10

15

20

25

30

Alternatively, the substrate may be a gel, such as a polyacrylamide gel. Polyacrylamide gel provides a substrate for permitting the immobilization of light-sensitive compounds within a three-dimensional matrix. Relatively large amounts of light-sensitive compounds per unit area of the microplate can be achieved while avoiding the crowding which may occur on planar surfaces. Compared to a planar surface, the polyacrylamide gels more closely approximates solution conditions. However, the gel restricts the size of molecules forming the light-sensitive compound as well as the sample product that can diffuse into the gel.

According to one illustrative embodiment, more than one light-sensitive compound may be applied to the substrate. In some instances, it may be desirable to apply at least two light-sensitive compounds in a manner such that the compounds are interleaved with each other. That is, the light-sensitive compounds are placed on the substrate in a manner so as to be adjacent each other. In this respect, the interleaving allows for the protection of more than one wavelength of light in a relatively small space. In addition, the interleaving provides significant advantages by dampening background signals while providing the ability to apply ratiometrics as will be described hereinafter.

As shown in Fig. 1, the light-sensitive compound is disposed over a selected macroarea 14 of the microplate. Within the macroarea 14, the light-sensitive compounds may be placed on the substrate in a format such that a plurality of spaced-apart microareas are provided. This is shown in greater detail in Fig. 1A, wherein the macroarea 14 comprises a plurality of microareas 20.

In addition, the macroarea may be split into two or more macroareas, 14, 16, which may be spaced apart from each other. However, it should be appreciated that the present invention is not limited in this respect and that the two or more macroareas may be disposed adjacent each other. In one embodiment (not shown), each macroarea is shaped as a square having an edge with a length of about 6.67mm. Preferably, four such square macroareas are

5

10

15

20

25

30

WO 01/90729 PCT/US01/10911

placed adjacent each other in a line running parallel to the longitudinal axis of the microplate. Each such macroarea may contain one or more light-sensitive compounds.

Turning now to Figs. 2 and 3, the microplate alternatively may include a base 30 having a top wall 32 and at least one well 34 integral with the top wall 32. The well defines an inner surface 36. A light-sensitive compound 13 may be deposited into the well 36. In this embodiment, a semi-permeable membrane 40 may be attached to the top wall 32 so as to encapsulate the light-sensitive compound 13 within the well 36. Thus, the semi-permeable membrane acts to hold the light-sensitive compound within the well. In this regard, the light-sensitive compounds may be in a liquid form. According to one aspect of the present invention, the semi-permeable membrane is adapted to transfer at least a desired portion of the sample to be placed on the microplate to the light-sensitive compound within the well. Thus by encapsulating the light-sensitive compound within the well, the need to covalently link or bond the light-sensitive compound to the microplate is obviated while retaining the desired result of providing the light-sensitive compound in liquid form.

As discussed with reference to Fig. 1, adjacent light-sensitive compounds may be applied to the microplate. In this regard, more than one well is provided in the microplate. At least one light-sensitive compound is deposited into the first well 42, and at least one light-sensitive compound is deposited into the second well 44. Preferably, although not required, the light-sensitive compounds in each well is different. In addition, although not required, the first and second wells are adjacent each other. Further, the plurality of wells may be disposed over a selected macroarea 42 of the microplate. In addition, the selected macroarea may include first 42 and second 44 spaced apart macroareas.

The inventors have found that incorporating a barrier material onto the microplate allows for ready authentication of carbonated beverages. In this respect, in the embodiment described with respect to Fig. 1, the silica or the gel acts to allow the product sample to penetrate into the substrate and react with the light-sensitive compound without allowing the larger gas bubbles to also penetrate. Otherwise erroneous readings may occur when attempting to detect emitted wavelength from the reaction of the light-sensitive compound with the key ingredient in the sample product when such gas bubbles are present.

Similarly, with respect to Fig. 3, the membrane 40 is sufficient to allow passage of liquid molecules from the sample product to be tested while preventing the gas bubbles from a carbonated beverage to penetrate into the well. The membrane 40 also retains light-sensitive compound within the well.

-11-

In prior attempts, especially when using the process described in the '511 patent, the sample product to be tested must be diluted in a manner so as to reduce the level of carbonation such that the gas bubbles will not interfere with the proper reaction and reading of the light-sensitive compound together with the key ingredient of the sample products. The barrier configurations, described herein, obviates this.

Thus, according to one aspect of the present invention, the sample product in its commercial state may be directly applied to the microplate. In a preferred embodiment, the microplate 10 may be dipped into a container 50 containing the sample product 52, as shown in Fig. 4. As will be discussed in the specific examples below, the microplate is held in the beaker of the sample solution for a period of time to allow the sample solution to penetrate into the substrate or the membrane and therefore allow the mixing of the product sample with the light-sensitive compound.

10

15

20

25

30

Turning now to Fig. 5, a system for authenticating a product sample is described. As shown schematically in Fig. 5, the system includes a product authentication device 58 for reading the microplate. The device includes at least one light source 60 for irradiating the microplate with a predetermined wavelength of light. At least one optical detector 62, which may be coextensive with the light source, is used to detect at least one emitted wavelength of light generated by the light-sensitive compound in response to the irradiated wavelength of light. This emitted wavelength of light is then used to provide a sample characteristic. A controller 74 is coupled to the optical detector 62 for receiving the sample characteristics. The controller may be coupled to a database (not shown) in which to gain access to a fingerprint representative of an authentic sample. Thus the controller may compare the received sample characteristics with the fingerprint to determine the authenticity of the sample product.

Alternatively, the fingerprint may be a previously stored sample characteristic of a known authentic sample. Thus, in one embodiment, in order to detect authenticity of a sample product, a sample of a known product is applied to the microplate containing the light-sensitive compounds as described above and scanned to receive the emitted wavelength of light. This sample characteristic is stored as a fingerprint in the memory of the controller. A second microplate is prepared with a sample of unknown product and is also scanned using the device disclosed herein to obtain a sample characteristic of the unknown product sample. The unknown product sample characteristic and the fingerprint of the known sample is compared to determine whether the unknown sample is authentic.

- 12 -

The authentic fingerprint data or fingerprint profile data may be stored in the controller or stored in a remote host computer and associated database. In this regard, the controller may communicate with the host computer via data cable, for example, a modem. Of course, those skilled in the art will recognize in view of this disclosure that other communication links may be used, such as a direct data link, satellite transmission, coaxial cable transmission, fiber optic transmission or cellular or digital communication. Also, the communication link may be a direct line or through the Internet.

5

10

15

20

25

30

A hard copy of the fingerprint profile data may also be provided. For example, a printout of the reading performed by the controller may be made using a suitable printer. The printout may include a data table, graph or other image of the light emission or absorption. In one embodiment, a photographic or digital image of the light emission or absorption may be provided. For example, color or black-and-white film 81, whether instant or not, may be loaded into the device such that when the microplate emits or absorbs light in response to the irradiating light, an image is captured on the film. The film may be loaded in a suitable cassette 83 and may include one or more exposures. The film may include a pull tab 85 to facilitate removal of the film. Preferably, the image so captured is viewable by a human.

To facilitate holding the microplate, or the substrate itself, relative to the light source and optical detector, a microplate frame 64 which is adapted to receive the microplate may be provided. Specifically, the microplate of Fig. 1 containing the sample product thereon is inverted such that the 14, 16 is facing toward an opening 66 of the frame. The optical detector and light source is able to scan through the opening of the frame to scan the macroareas 14, 16. The frame may have an indexing feature, such as a recess 67.

Further, it may be desirable in some instances to move the microplate relative to the optical detector and light source. In this regard, the device 58 may include a tray 68 that is adapted to receive the frame 64. The tray also includes an indexing feature, such as tab 70, to cooperate with the recess 67 on the frame 64. The device may also include a driver 72, coupled to the controller to move the tray relative to the optical detector and the light source and subsequently the frame.

It may be desirable to pre-read or pre-scan the microplate in order to obtain a base line of the emitted wavelength of the light-sensitive compound without interaction with the sample product. In this case, before applying the sample product, a microplate is placed in the device and is scanned as described above. Next, the sample product is applied to the same microplate and the microplate, together with the sample product, is scanned. Thus, any

variation of background reflection or fluorescence or absorption may be obviated due to the use of the base line.

The microplate 10 may be packaged in a manner to facilitate convenient handling as well as sufficient preparation prior to insertion of the microplate into the device 58. In this respect, a holder to facilitate placing the microplate into the liquid to be authenticated may be provided. The holder may also be implemented to serve as the microplate frame. The holder may be configured in any suitable manner, examples of which will be described hereinafter, and may be configured to receive the microplate and/or the substrate.

10

15

20

25

30

Turning now to FIGS. 6a-6c, one embodiment of a holder 100 is shown. In this embodiment, the holder 100 includes a portion 102 that is adapted to receive, for example, the microplate 10. Specifically, the portion 102 includes a channel 104 in which to receive an edge of the microplate 10, as shown. The holder 100 further includes a first section 106 that may be attached to the portion 102 with the use of, for example, a hinge, as will be described below. The holder 100 also includes a second section 108 attached to the portion 102. The second section 108 includes an opening 110 formed therein. In the embodiment shown, the holder 100 is formed of a plastic material such that the first and second sections are hinged to the portion 102 with the use of living hinges 112, 114. It is to be appreciated, however, that the present invention is not limited in this respect and that other suitable materials may be used. For example, a non-plastic, such as a metallic holder may be employed. In such a situation, the first and second sections may be attached, whether hinged or not to the portion 102 in any suitable manner.

In the configuration shown in FIG. 6a, a user may hold the holder 100, specifically sections 106, 108, with his or her hands or other suitable implement. The microplate 10 may then be dipped into a suitable container containing the sample product to be authenticated. Once the microplate is removed from the sample, the first 106 and second 108 sections are rotated relative to the portion 102, as shown in FIG. 6b, to enclose the microplate 10, as shown in FIG. 6c. Once the microplate and holder are in the configuration shown in FIG. 6c, the entire assembly may be placed in device 58 for subsequent analysis.

As with the frame 64 shown in FIG. 5, the opening 110 allows light from the light source to irradiate the microplate and the optical detector to detect emitted or absorbed light. Similarly, as with recess 67, the holder 100 may include an indexing feature 120 that cooperates with the corresponding indexing feature 70 on the tray 68 of the device 58.

- 14 -

The holder 100 may further lock the microplate 10 therein when in the configuration shown in FIG. 6c. In one illustrative embodiment, the holder 100 includes one or more locking tabs 122 that cooperate with corresponding one or more mating locking recess 124. Thus, when the first 106 and second 108 sections are rotated in a manner to surround and enclose the microplate 10, the locking tab 122 engages with the locking recess 124.

5

10

15

20

25

30

To aid in preparation of the microplate prior to insertion into the device for subsequent analysis, it may be desirable to remove excess liquid sample from the microplate. In one embodiment, this may be accomplished by including a blotter 130 attached to the first section 106. This blotter 130 is able to absorb any excess liquid remaining on at least one side of the microplate. Similarly, the second section 108 may also include a blotting material 132 that is capable of absorbing excess liquid on the other side of the microplate 10. Examples of materials forming the blotter include paper or cotton-type of materials. Of course, other suitable materials may be employed.

To facilitate analysis, that the holder may be formed of a suitably dark colored material. Thus, in one embodiment, the holder may be formed of a black plastic material. Similarly, the blotter used may also include a dark surface. The dark surface of both the blotter and the microplate holder has been found to prevent or at least reduce the amount of reflected light occurring in the device in order to sufficiently and accurately obtain a proper reading.

It is to be appreciated that the present invention is not limited in this respect and other suitably non-reflecting surfaces may be employed. In addition, the device itself may compensate for reflection such that a dark material for both the blotter and the holder need not be employed.

Although in the embodiment described with reference to FIGS. 6a-6c, the microplate 10 is attached to the holder with the use of channel 104, it is to be appreciated that the present invention is not limited in this respect. In this respect, the holder 100 may be used simply to receive the microplate after the liquid sample has been placed thereon. As such, the microplate 10 may be sandwiched between the first and second sections 106, 108 without also attaching to the holder 100, examples of which will be described below.

The holder 100 may be configured to cooperate with a suitable container, thereby allowing the microplate 10 to be dipped into a solution of the sample product. One embodiment of a container 150 is shown in FIG. 7. The container may include a fill line 152 so that the desired amount of liquid sample may be placed therein. The fill line 152 may be

- 15 -

positioned on the container 150 such that about 35 milliliters of liquid sample may be placed in the container 150. In this respect, a user merely needs to fill the container to the fill line so that the light-sensitive compounds on the microplate (or substrate) reacts with the appropriate amount of liquid sample. The container 150 may also include a cover 154. The cover 154 may be hinged to the container 150 through the use of a living hinge. In this respect, the container 150 may be formed of a suitable plastic material. The cover 154 may also lock to the container 150 so that the liquid sample may be properly disposed after the microplate 10 has been removed therefrom. The cover may further include a blotter 156 that may be used to blot the microplate 10. In one example, the blotter 156 may be used to blot the portion of microplate 10 exposed by the opening 110.

In an alternative embodiment shown in Fig. 7a, the container 150 may include an enlarged opening 158 to facilitate both filling of the container with the sample liquid as well as to facilitate insertion of the microplate 10.

10

15

20

25

30

The container 150 may also include a lower portion 160 that may be adapted to stand on a compatible surface. In addition, the lower portion 160 may be shaped to be inserted into a suitable container holder (not shown). The container holder may include multiple receptacles for receiving multiple containers 150 and may be formed in the housing of the device 58.

Turning now to FIGS. 8a-8c, an alternative embodiment of the microplate holder is shown. In this example, the holder 200 is constructed as a tri-folding holder. Thus, the holder includes a portion 202 that is adapted to receive the microplate 10. Specifically, the portion 202 includes a frame 204 which receives the microplate 10. The holder 200 further includes a first section 206 attached to the portion 202 via, for example, a living hinge 208. The holder also includes a second section 210 attached to the first section 206 via, for example, a living hinge 212. Although the holder 200 includes living hinges 208 and 212, those skilled in the art will recognize that other suitable hinging mechanisms may be employed.

The holder 200 may also include a blotter 214 disposed on the first section 206. The blotter may be attached to the first section 206 using any suitable attaching technique such as gluing and/or press fitting the blotter into a corresponding recess. As described with reference to FIGS. 6a-6c, the blotter 214 is used to absorb any excess liquid sample on at least one side of the microplate 10. In addition, the blotter may include a suitable non-reflective surface so as not to interfere with the analysis provided by the device 58. In

- 16 -

addition, as described with reference to FIGS. 6a-6c, the blotter may be formed of a dark colored paper or dark colored cotton-type of material, although other suitable materials may be employed. The third portion 210 may include an opening 220, which when covering the microplate 10, allows the microplate to be analyzed by the device 58.

5

10

15

20

25

30

As described with reference to FIGS. 6a-6c, the first 206 and second 210 sections of the holder 200 may be used as a handle to allow the microplate 10 to be dipped into the liquid sample. After the proper amount of liquid sample is applied to the microplate 10 for the appropriate duration, the portion 202 may be folded relative to the first section 206 in the manner shown in FIG. 8b. Any excess liquid sample on microplate 10, at least on the surface adjacent the blotter 214, may be absorbed by the blotter 214. Subsequently, as shown in FIG. 8c, the second section 210 may be folded over relative to the portions 202 and the first section 206 so that the microplate may be contained therein.

As shown in FIGS. 8a-8c, the holder may include an indexing feature, as described with reference to FIGS. 6a-6c. The indexing feature 230 may be used with a corresponding indexing feature on the device 58 as described above.

In addition, the holder 200 may be adapted to lock the microplate therein. Thus, the holder 200 may include a locking device. In one embodiment, the locking device is formed as a tab 234 and mating recess 232.

It is to be appreciated that the holder 200 may also be used in conjunction with the container shown in FIGS. 7 and 7a. Thus, the blotter 156 may be used to wipe the portion of the microplate exposed through opening 220. In addition, it is to be appreciated that once the testing is complete, the used microplate and/or the holder may be placed into the container 150 with the cover 154 being secured thereto to readily dispose of the microplate and/or holder.

Turning now to Figs. 9A-9D, another alternative embodiment of the microplate holder is shown. In this embodiment, beginning initially with Fig. 9A, the microplate 10 is held within a container 300 which, in turn, is secured with a cap 302. The container 300 may be sufficient to receive the sample product to be authenticated. As with the container 150 described above, container 300 may also include a fill line 304 indicating the amount of liquid sample to placed within the container 300. As best shown in Fig. 9B, the microplate 10 may be removed from the container 300 by removal of the cap 302 from the container 300. In this regard, the cap 302 may include a holder 304, which includes two finger-like projections 306, 308 which securely grasp an edge of the microplate 10, as shown. The

- 17 -

container 300 subsequently may be filled up to, for example, the fill line, with the sample to be authenticated. The microplate 10 then may be dipped in to the sample by replacing the microplate into the container for a suitable amount of time using the cap 302, for example, as a handle. The microplate 10 then may be removed from the liquid sample and placed in a holder 320, as shown in Fig. 9C.

5

10

15

20

25

30

The holder 320 includes a first section 322 attached to a second section 324 via, for example, a living hinge 325. The first section 322 includes a recess 326 to receive the microplate 10. As with the microplate holder examples described above, the holder 320 may further include a blotter (not shown). In this embodiment, the blotter may be positioned within the recess 326. Once the microplate 10 is placed in the holder 320, the second section 324 may be folded relative to the first section 322 about the hinge 325 to enclose the microplate 10 within the holder 320. As with the examples described above, the second section 324 includes an opening 328 to allow the microplate 10 to be analyzed within the device 58. Similarly, to lock the holder 320 about the microplate 10, the holder 320 may include locking features. In this embodiment, the locking feature comprises locking pins 330 formed on the first portion 322 that are adapted to be placed within corresponding locking recesses 332 formed in the second section 324.

The holder 320 may also include a recessed area 334 formed in the first section 322 and another recessed area 336 formed in the second section 324 such that when the holder 320 is enclosed around the microplate 10, the fingers 310 of the cap 302 may be received within the recesses 334, 336, as best shown in Fig. 9D. Once the microplate 10 is enclosed within the holder 320, the cover 302 may be removed from the microplate 10.

As described within the above embodiments, the microplate holder 320 may further include an indexing feature 350 that cooperates with the corresponding indexing feature 70 on the device 58.

Thus, in this example, the holder 304 of cap 302 facilitates application of the sample product onto the microplate while holder 320 facilitates analysis of the sample in device 58.

In yet another illustrative embodiment, a holder 380 may be formed around the microplate 10 using any suitable technique. For example, a polymeric material may be molded around the periphery of the microplate using, for example, injection molding techniques. As described in at least one of the examples above, the holder 380 may be used as a handle to allow the microplate 10 to be dipped into the liquid sample. After the proper amount of liquid sample is applied to the microplate 10 for the appropriate duration, the

- 18 -

microplate 10 and holder 380 is removed from the liquid sample and any excess liquid sample on microplate 10 may be absorbed by a suitable blotter. The holder may include an indexing feature 382 to register the microplate within the device 58. As described in at least some of the examples above, the indexing feature 382 may be used with a corresponding indexing feature on the device 58.

5

10

15

20

25

In any of the illustrative embodiments described above, the holder may include a suitable gasket (now shown) to surround the outside edge of the microplate. The gasket may be useful in sealing in the product and/or sample as well as preventing stray light from irradiating the microplate in undesired locations.

Although in the embodiments described above the microplate is dipped into a sample, it is to be appreciated that the invention is not limited in this respect and that other methods for applying the sample to the microplate may be employed. For example, the sample may be swabbed onto the microplate. Alternatively, the sample may be applied to the microplate using an applicator such as an eye dropper, manual or automatic pipette, or piezoelectric dispensers. Other suitable applicators or dispensers may be employed.

As described above, an image of the light emission or absorption may be recorded on color or black-and-white film 81. Such film may be adapted to be disposed in or positioned adjacent to the microplate holder, as shown in the various embodiments described above. For the sake of clarity, the film is shown removed from the holder. When disposed in the holder, the film may be removed by opening the holder or the film may include pull tab 85, as described above, such that the film may be removed by pulling on at least a portion of the pull tab that is exposed in much the same way instant film is removed from an instant camera.

Turning now to Fig. 11, the microplate and/or any of the holders described above may be suitably packaged as at least part of a kit for handling prior to use. The package may be configured to protect the microplate and/or holder so that the light-sensitive compounds placed on the microplate together with the microplate and/or the holder are protected from the environment. In one illustrative embodiment as shown in Fig. 11, the microplate 10 and/or the holder 320 may be placed within a vacuum sealable bag 400. The bag may be formed of any suitable material including foil or mylar. Once the microplate 10 and/or the holder 320 is placed within the bag 400, the bag 400 may be evacuated of any air contained therein and sealed using, for example, suitable heat sealing techniques. In one embodiment, to further provide an inert environment for the microplate 10, the package is filled with a nitrogen gas to evacuate any air contained therein. The nitrogen gas is then drawn out of the bag 400 and

- 19 -

the bag 400 is subsequently heat sealed. Any of the above or other suitable containers may also be provided in the package or may be separately provided.

The portable authentication device 58 described herein may be a table top device. The controller may be a processor such as a PALM PILOT® or other data logger. Power to the device, of course, may be powered by batteries, such as rechargeable batteries. Although the controller may be a hand-held PALM PILOT®, a dedicated controller or a lap top or desk top computer may be used.

5

10

15

20

25

30

The light source may be provided by light-emitting diodes, such as model no. HLMP CB15, sold by Hewlett Packard, California, USA, which may or may not be infra red light emitting diodes. Alternatively, the light source may be a laser light source. In either case, the light source matches the excitation wavelength of the light-sensitive compounds contained on the microplate or the substrate. Other components of the device 58 may include source filters such as a band pass or cutoff filter to isolate wavelengths of light from the light source. Lenses also may be provided which focus light from the light source onto the microplate. An optical detector, such a charged couple device (CCD) may be used. An example of such a CCD is model no. H53308 sold by Edmonds Scientific, New Jersey, USA. An emission filter, such as a band pass or cutoff filter may be used to isolate excitation wavelengths from emissions spectra due to light emission from the microplate.

In an alternative embodiment, as shown in FIG. 12, the light source may be a vertical cavity surface emitting semiconductor lasers (VCSEL) 500 that emits at a desired wavelength such as in the near-infrared spectrum (750-900 nm). VCSELs are available from Honeywell, Inc. of Morristown, NJ, USA. The VCSEL 500 may be incorporated into the microplate holder, such as holder 320 shown, or may separate from the holder. The VCSEL may be powered through any suitable power source such as separate batteries or may be powered through the controller 74. In one embodiment, the VCSEL 500 may be arranged to be placed over the light-sensitive compound(s) on the microplate. It should be appreciated that the holder 320 shown in FIG. 12 is shown in an open position. Thus, when closed, the VCSEL is disposed over the microplate 10.

Light emission or absorption from the irradiated microplate may be detected by the controller 74 through suitable detectors, as described above. Alternatively, a film 81 may be used, whether disposed within the holder 320 or not, to record an image of the light emission or absorption.

In yet another embodiment, in any of the embodiments described herein, the microplate 10 may be replaced with a film, such as film 81, having one or more light-sensitive compounds disposed thereon. In this embodiment, the sample may be placed directly onto the film using any suitable techniques such as those described herein. The film may be irradiated using a suitable light sources, such as the VCSEL 500. An image of the light emission or absorption may be recorded on the film. The image may then be compared to a standard.

Detection of the light emitted or absorbed from the light-sensitive compound by the controller may be used using any imaging technique, such as infra red, near infra red, far infra red, foyer transformed infra red, ramonspectroscopy, time resolved fluorescence, fluorescence, luminescence, phosfluorescence and visible light imaging.

10

15

20

25

30

A change in spectroscopy, such as light emission, due to the presence of light-sensitive compounds alone can be determined from the formula [(Fd-Fp)/Fd] x 100 where the light emission of the light-sensitive compound and the absence of the sample product is Fd, and the light emission after adding the sample product to the microplate is Fd. The light-emission changes as a result of interaction of the light-sensitive compound with the sample product. The emission filters may be used to filter undesired wavelengths of light emitting from the sample and the light-sensitive compounds such that, for example, only peak wavelengths of light are passed through. The light is then directed to the optical detector, which generates a voltage level indicative of the amount of light emitted.

It is to be appreciated that although a dedicated authentication device 58 is described with reference to Fig. 5, the microplates according to the present invention may be used in conjunction with any suitable imager, such as a Molecular Dynamics FluorImager 575. Of course any microplate reader can be used, (e.g., Cytofluor).

It is also to be appreciated that the intensity or quantity of light-emission from the sample is detected. However, according to one aspect of the invention, intensity, decay or change in the quantity of light-emission or absorption over time may be used to provide the sample characteristic. Alternatively, any such combinations may be used to provide the sample characteristic. Thus, "light-emission" or "light absorption" means intensity or quantity or intensity, decay or change in quantity of light emitted or absorbed from the sample.

Rather than, or in addition to, comparing certain spectral properties, such as light emission and/or absorption from the light-sensitive compound to a stored fingerprint, in some

- 21 -

instances it may be desirable to compare a ratio of light emission or absorption of two different wavelengths of light to a stored ratio fingerprint. In one embodiment, this may be accomplished by providing a light-sensitive compound, for example, a light-emissive compound, that is capable of emitting two different peak wavelengths of light or, alternatively, providing two or more different light-sensitive compounds, each producing a characteristic peak wavelength having a certain light emission. For example, two light-emissive compounds are applied to the substrate. An excitation wavelength is applied such that the first light-emissive compound may have a relative fluorescence unit (RFU) of 98 at a peak wavelength (λ 1) of 575 μ m and the second light-sensitive compound has an RFU of 76 at a peak wavelength (λ 2) of 525 μ m. The ratio of the RFU values at the peak wavelengths 575 to 525 is approximately 1.3. This ratio of 1.3 may then be used in comparison to the stored fingerprint ratio. Although relative fluorescence units are used in this example to indicate the value of the amount of light emitted, other units may be used, such a photon count, for example. A similar analysis may be employed when the selected light-emissive compound is a light-absorbing compound.

5

10

15

20

25

30

It is to be appreciated that the sampling rate of the device may include about 10,000 readings. Thus, a high degree of confidence may be obtained in providing the sample characteristics.

With such a large amount of data generated, although possible, conventional data analysis comparing one or two variables at a time, is not practical. Thus, according to one aspect of the invention, multivariable analysis or multivariable padding recognition may be used. In a preferred embodiment, Tukey's analysis and Principle Component Analysis (PCA) are used. Other multivariable techniques that may be utilized include Hierarchical Cluster Analysis, K Nearest Neighbor, Pineapple Component Regression, Partial Least Squares Regression, and Soft Independent Modeling of Class Analogy (SIMCA). These multivariable techniques reduce the dimensionality of the data to two or three dimensions, allowing the pattern or relationships to be generated.

Analysis of the data may also be performed by developing plots having distinct clusters summarizing the similarity and differences among the samples being analyzed to a stored standard. Such analysis may be performed in addition to or in the alternative to the above-mentioned multivariable or multivariable pattern recognition.

Example #1) Detection of Authentic Guinness:

5

10

15

20

25

30

In this example, the sample products are Guinness, Beamish and Murphy's stout. Lightsensitive compounds are identified in a manner similar to that described in U.S. Patent 5,753,511. For Guinness authentication, light-sensitive compound #29, which is bis-(1,3diethylthiobarbituric acid) trimethine oxonol, and light-sensitive compound #18, which is Fluorescein-6-isothiocyanate, are prepared at 25 and 10 uM respectively, and transferred to a silica substrate which is available affixed to a 25 x 75 mm glass microscope slide. Both light emissive compounds can be obtained from Molecular Probes, Eugene, Oregon, USA. The light-sensitive compounds are dried onto the substrate. Comparison of the authentic and test samples is performed by testing them at the same location at the same time. This reduces any inaccuracies associated with differential temperature, light, or light-sensitive compound concentration. A portable fluorescent reader is programmed for the appropriate emission filters (emission filters set at 570 and 535 nM for dyes 29 and 18, respectively). A first microplate is used to enter reference values for the authentic sample. This is accomplished by placing the a microplate, without any product sample thereon, in the reader. The reader measures the light emission of the light-sensitive compounds (dry read). The microplate is then dipped into authentic Guinness for a period of time. The microplate is then removed and excess product sample is wiped off. The microplate is then placed back into the reader. A second reading is taken (wet read). The dry read value is divided by the wet read value to give the relative change in fluorescence. This is entered into the controller as a reference value for the authentic sample. A second microplate is dry read and wet read for use with the test sample. The relative change in fluorescence is computed. If the test sample value is different from the reference by a given percentage, then the sample fails and is suspected of being non-authentic or of poor quality.

The algorithm for deciding a pass versus fail is as follows:

calculate dry/wet value for reference (R) calculate dry/wet value for test sample (T) from prior testing establish a range for authentic product (typically 5-15%)

- 23 -

test sample fails if either T > R x 1.07 or T < R x .93 (tolerance set at 7%) test sample passes if R x .93 \leq T \leq R x 1.07

5 Results with light-sensitive compound #29:

	Sample	Serial #	dry read divided by
			wet read
	1. Guinness, draft can	21 D 9 G3 01:49	5.12
	2. Beamish, draft can	5 3 9 20 15:49	7.41 (F)
10	3. Murphy's, draft can	L9096E 826 11:49	6.06 (F)
	4. Guinness Stout (repeat 1)	21 D 9 G3 01:49	5.02 (P)

(Tolerance range for Guinness at 7% is 4.76 - 5.47%)

15

Example #2) Detection of Authentic Ballantine's Finest:

20

25

The following provides an example for authenticating Ballantine's Finest using microplates with light-sensitive compound #149, which is available as Newport Green and can be obtained from Molecular Probes, Eugene, Oregon, USA, applied at a concentration of 20 mM and read at an emission wavelength of 550 nM. The testing was performed as described in example 1.

Portable Authentication Device Readings:

	Sample	dry/wet (Pass/Fail)
	Ballantine's Finest (BF)	(authentic reference) 3.76
30	BF cut 20% with water	2.21 (F)
	BF cut 10% with water	3.13 (F)
	BF cut 20% with vodka	2.28 (F)
	BF cut 10% with vodka	2.66 (F)

- 24 -

3.72 (P)

BF (repeat)

10

15

20

25

(Tolerance range or Ballantine's Finest at 10% is 3.38 - 4.14)

5 Example 3: Placing Aspartame specific dyes on a controlled-porosity membrane (non-silica surfaces)

In this example light-sensitive compound samples (light-sensitive compound 120) was placed on AnaporeTM membranes (Whatman Anadisk Cat#6809-6022, United Kingdom) using a BioChip ArrayerTM from Packard Instruments (Meriden, CT). Small spots (less than 1000 spots/cm²) can be placed on this membrane. The spot size can range from 0.1 microliters to 100 microliters. In this case an 8x7 array of spots was placed on the membrane. The spot size was approximately 1.6 mm x 1.6 mm with a 250 micron spacing.

Placing the membrane with Aspartame light-sensitive compound 120 in a solution of Coke Classic, the fluorescence was 5.93. Placing an exact replicate membrane with Aspartame light-sensitive compound 120 in a solution of Diet Coke (containing Aspartame) the fluorescence was 15.4. The measurement was made using FluorImager 575 from Molecular Dynamics (Sunnyvale, CA).

Having thus described certain embodiments of the present invention, various alterations, modifications, and improvements will readily occur to those skilled in the art. Such alterations, modifications, and improvements are intended to be within the scope of the invention. In addition, various embodiments of the present invention provide certain advantages. Not all embodiments of the invention share the same advantages and those that do may not share them under all circumstances. Thus, various features of an above described embodiment may be used with other embodiments. Accordingly, the foregoing description is by way of example only, and not intended to be limiting. The invention is limited only as defined in the following claims and the equivalents thereof.

What is claimed is:

PCT/US01/10911

CLAIMS

- 25 -

- 1. A holder for holding a microplate or film, the microplate or film having at least one light-sensitive compound disposed thereon for use in verifying a sample liquid product, the holder comprising:
- a first section; and

5

25

- a second section securable with the first section, the first and second sections constructed and arranged to envelope the microplate or film when the first section is secured to the second section and when the microplate or film, having the sample liquid product disposed thereon, is placed therein.
- 10 2. The holder according to claim 1, further comprising a holding portion coupled to at least one of the first and second sections, the holding portion constructed and arranged to receive the microplate or film.
 - 3. The holder according to claim 1, further comprising a blotter disposed therewithin.
- 4. The holder according to claim 3, wherein the blotter comprises a non-reflective surface. 15
 - 5. The holder according to claim 3, wherein the blotter comprises a dark color.
 - 6. The holder according to claim 1, wherein the first and second sections comprise a non-reflective surface.
- 7. The holder according to claim 1, wherein the first and second sections comprise a dark color. 20
 - 8. The holder according to claim 3, wherein the first section is constructed and arranged to receive the blotter.
 - 9. The holder according to claim 1, wherein the second section is formed with an opening therein to allow irradiating light to irradiate the light-sensitive compound disposed on the microplate or film when the microplate or film is enveloped within the holder.
 - 10. The holder according to claim 1, further comprising a lock for locking the first and second sections together.

-26-

- 11. The holder according to claim 10, wherein the lock comprises a tab and mating recess.
- 12. The holder according to claim 1, wherein the first and second sections are hingedly connected together.
 - 13. The holder according to claim 1, further comprising an indexing feature.
- 5 14. The holder according to claim 2, wherein the holding portion includes a channel constructed and arranged to receive an edge of the microplate or film.
 - 15. The holder according to claim 2, wherein the holding portion includes a frame constructed and arranged to receive the microplate.
- 16. The holder according to claim 2, wherein the first and second sections are hingedly connected to the holding portion.
 - 17. The holder according to claim 2, wherein the first section is hinged to both the second section and the holding portion and wherein the second section is not hinged to the holding portion.
- 18. The holder according to claim 1, wherein at least one of the first and second sections include a recess constructed and arranged to facilitate placement of the microplate or film into the holder.
 - 19. The holder according to claim 1, in combination with the microplate or film.
 - 20. The holder according to claim 1, in combination with a container for receiving a sample liquid product to be authenticated, the container comprising a blotter disposed thereon constructed and arranged to absorb excess liquid sample on at least a portion of the microplate.
 - 21. The combination according to claim 20, wherein the container includes a funnel-shaped opening.

20

22. The combination according to claim 20, wherein the container comprises a body and a cover securable with the body.

-27-

- 23. The combination according to claim 19, further comprising a package for packaging the holder and the microplate or film.
- 24. The combination according to claim 23, wherein the package comprises a vacuum sealed package.
- 5 25. The holder according to claim 23, further comprising a light source disposed therein.
 - 26. The holder according to claim 25, wherein the light sources is a VCSEL.
 - 27. A kit of parts for use in verifying a sample liquid product, the kit comprising: a microplate or film having at least one light-sensitive compound disposed thereon for reaction with the sample product;
- a holder constructed and arranged to hold the microplate or film therein; and a package for packaging the microplate or film and the holder.
 - 28. The kit according to claim 27, wherein the holder comprises:
 - a first section; and

15

- a second section securable with the first section, the first and second sections constructed and arranged to envelope the microplate or film when the first section is secured to the second section and when the microplate or film is placed therein.
- 29. The kit according to claim 28, wherein the holder comprises a holding portion coupled to at least one of the first and second sections, the holding portion constructed and arranged to receive the microplate or film.
- 30. The kit according to claim 27, further comprising a blotter.
 - 31. The kit according to claim 30, wherein the blotter is disposed within the holder.
 - 32. The kit according to claim 30, wherein the blotter comprises a non-reflective surface.
 - 33. The kit according to claim 30, wherein the blotter comprises a dark color.
- 34. The kit according to claim 28, wherein the first and second sections comprise a nonreflective surface.

WO 01/90729

5

20

35. The kit according to claim 28, wherein the first and second sections comprise a dark color.

-28-

PCT/US01/10911

- 36. The kit according to claim 28, wherein the second section is formed with an opening therein to allow irradiating light to irradiate the light-sensitive compound disposed on the microplate or film when the microplate or film is enveloped within the holder.
- 37. The kit according to claim 28, wherein the holder further comprises a lock for locking the first and second sections together.
 - 38. The kit according to claim 37, wherein the lock comprises a tab and mating recess.
- 39. The kit according to claim 28, wherein the first and second sections are hingedly connected together.
 - 40. The kit according to claim 27, wherein the holder further comprises an indexing feature.
 - 41. The kit according to claim 31, wherein the holding portion includes a channel constructed and arranged to receive an edge of the microplate or film.
- 42. The kit according to claim 31, wherein the holding portion includes a frame constructed and arranged to receive the microplate or film.
 - 43. The kit according to claim 29, wherein the first and second sections are hingedly connected to the holding portion.
 - 44. The kit according to claim 43, wherein the first section is hinged to both the second section and the holding portion and wherein the second section is not hinged to the holding portion.
 - 45. The kit according to claim 28, wherein at least one of the first and second sections include a recess constructed and arranged to facilitate placement of the microplate or film into the holder.

- 46. The kit according to claim 27, further comprising a container for receiving the sample product.
- 47. The kit according to claim 46, wherein the container comprises a blotter disposed thereon constructed and arranged to absorb excess liquid sample on at least a portion of the microplate or film.
 - 48. The kit according to claim 46, wherein the container includes a funnel-shaped opening.
 - 49. The kit according to claim 46, wherein, the container comprises a body and a cover securable with the body.
- 50. The kit according to claim 46, wherein, the container is disposed within the package.
 - 51. The kit according to claim 27, wherein the package comprises a vacuum sealed package.
 - 52. The kit according to claim 27, further comprising a light source.
 - 53. The kit according to claim 52, wherein the light source is a VCSEL.
- 54. A sample verification kit comprising a plurality of kits as claimed in claim 27.
 - 55. The kit according to claim 27, wherein the microplate comprises:
 - a base;

5

- a porous substrate layered on the base; and
- the at least one light-sensitive compound absorbed in the substrate in a manner to allow
 the sample liquid product placed on the microplate to react with the at least one lightsensitive compound.
 - 56. The kit according to claim 27, wherein the microplate comprises:
 - a base having a top wall;
- at least one well integral with and opening into the top wall, the at least one well defining
 25 an inner surface;
 - at least one light-sensitive compound deposited into the at least one well to allow a

sample placed on the microplate to react with the at least one light-sensitive compound in the well; and

- 30 -

a semi-permeable membrane formed over the at least one well, the semi-permeable membrane being adapted to allow the sample to permeate from outside the at lease one well into the at least one well while retaining the at least one light-sensitive compound within the at least one well.

57. The kit according to claim 27, wherein the microplate comprises:

a base;

5

10

15

20

25

at least one light-sensitive compound held to the base to allow a sample placed on the microplate to react with the at least one light-sensitive compound; and

a barrier formed on the base, the barrier being adapted to transfer a desired portion of the sample to at least one light-sensitive compound while holding the at least one light-sensitive compound to the base.

- 58. The kit according to claim 53, wherein the substrate is formed of one of silica and gel.
- 59. The kit according to claim 55, wherein the barrier is formed of one of silica and gel.
 - 60. The kit according to claim 53, wherein the substrate is a membrane.
 - 61. The kit according to claim 55, wherein the barrier is a membrane.
 - 62. A method of verifying a sample liquid product comprising:

applying the sample liquid product to a microplate having at least one light-sensitive compound disposed thereon;

placing the microplate into a microplate holder;

irradiating the microplate with an irradiating wavelength of light; and

comparing light emission or absorption from the reaction of the light-sensitive compound with the sample liquid product.

- 63. The method according to claim 62, wherein irradiating the microplate comprises irradiating the microplate with a VCSEL.
 - 64. A method of verifying a sample liquid product comprising: applying the sample liquid product to a film having at least one light-sensitive compound

- 31 -

disposed thereon;

irradiating the film with an irradiating wavelength of light; recording an image of light emission or absorption on the film; and comparing the image to a standard.

- 5 65. The method according to claim 64, wherein irradiating the film comprises irradiating the film with a VCSEL.
 - 66. The method according to claim 64, further comprising placing the film in a holder.

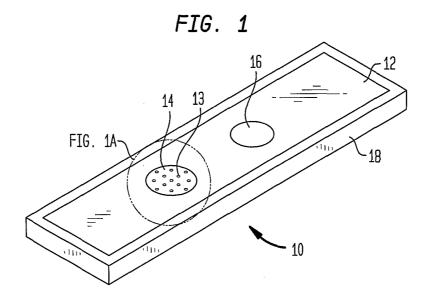
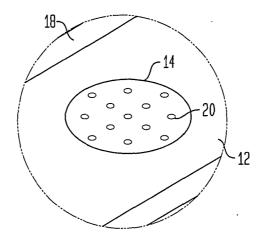


FIG. 1A



2/9

FIG. 2

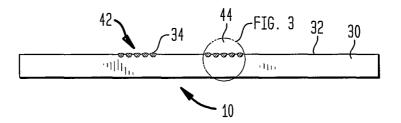


FIG. 3

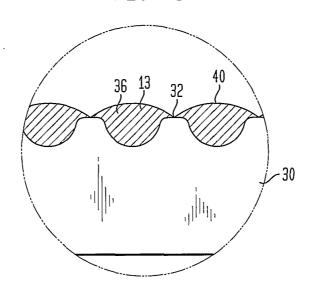
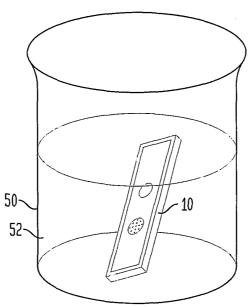
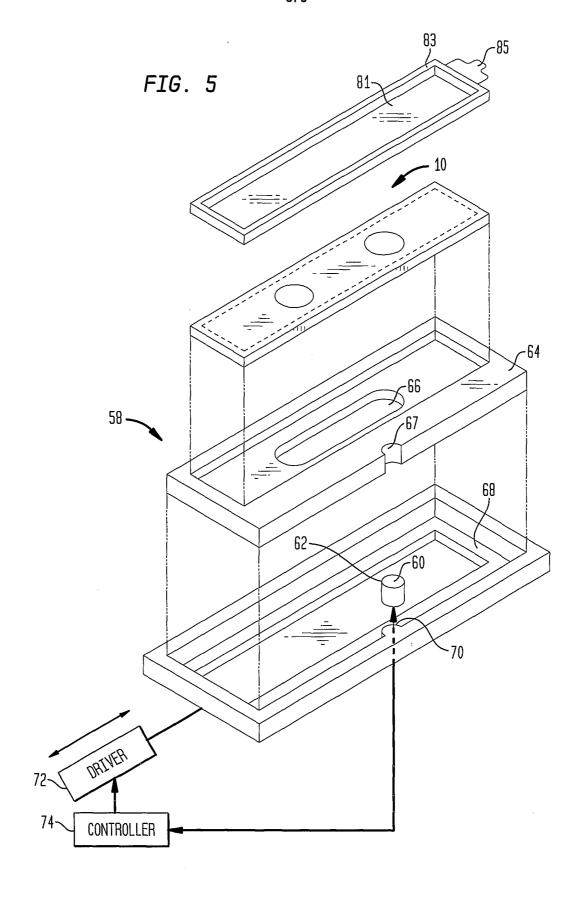


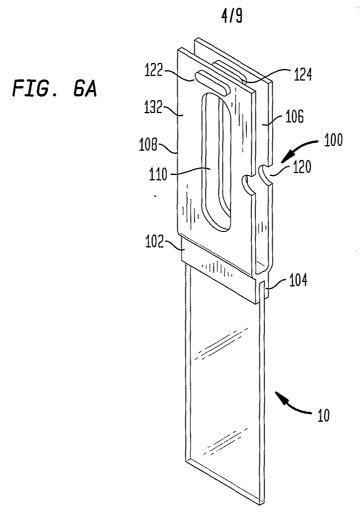
FIG. 4

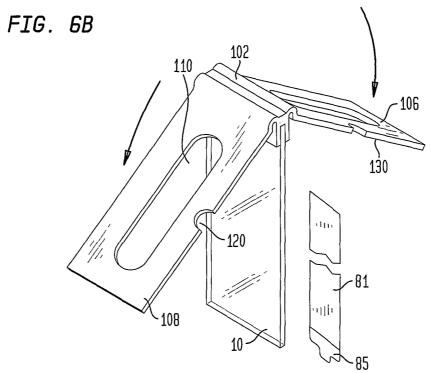


3/9

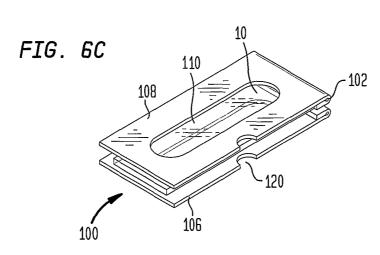
PCT/US01/10911

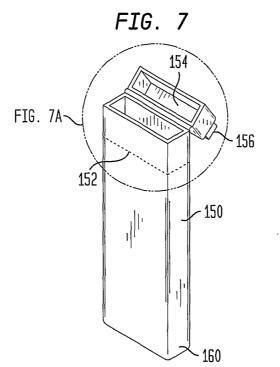


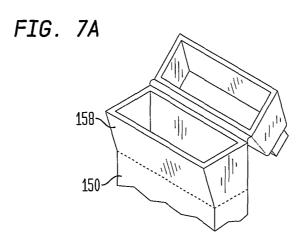


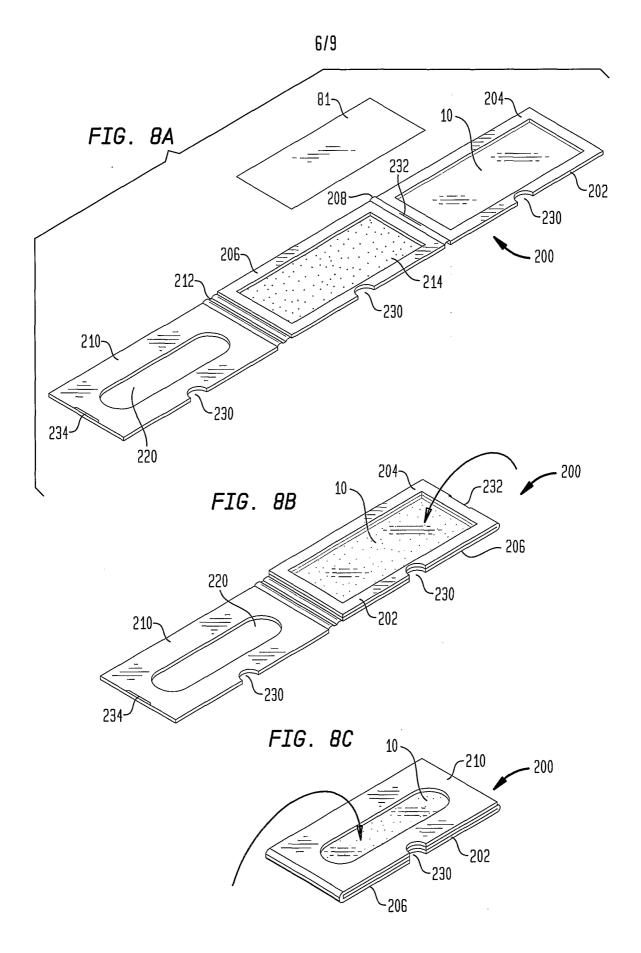


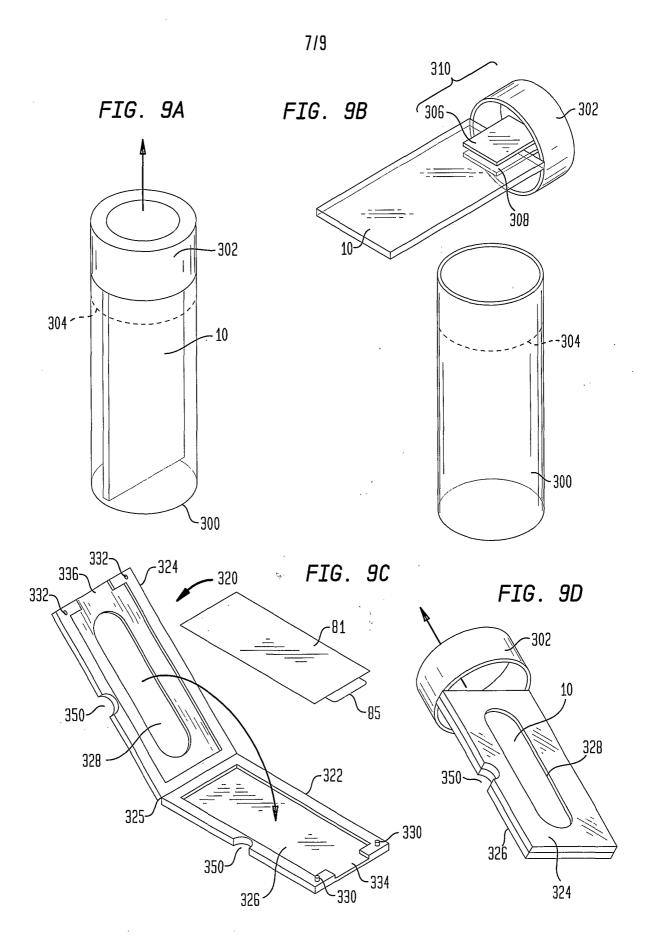


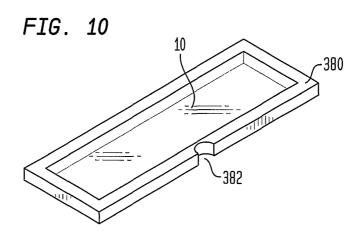












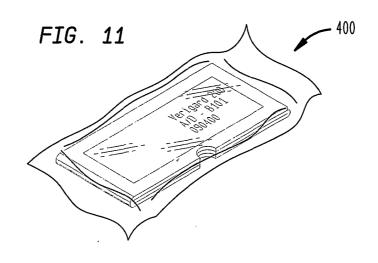


FIG. 12

