

INFRARED REFLECTIVE SAMPLING DEVICE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is being filed on February 5, 2024, as a PCT International Patent Application and claims the benefit of and priority to U.S. Provisional Patent Application No. 63/483,117 filed on February 3, 2023, the disclosure of which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Cancer continues to be a major health challenge globally, ranking as the second most common cause of death in the United States. Each year, in the United States, over 1.9 million people are diagnosed with cancer. Of those, roughly 600,000 will eventually die of their disease. It is widely known that early-stage cancers are treated with a much higher success rate than later stages of cancer. After decades of intense research and recent progress in treatment strategies, virtually all patients with stage IV cancer still eventually succumb to their disease. It is for this reason that countries with advanced health systems have invested in national cancer screening programs with the goal of catching cancer early at a more treatable stage. Evidence has shown when designed properly, these programs lead to lower mortality rates.

[0003] Among the most effective strategies against cancer is early detection, which can lead to improved treatment options and better patient outcomes. However, existing methods for early cancer detection present challenges: they are often invasive, expensive, and not without risk, thereby complicating the battle against cancer. Typically, cancer screening is advised primarily for individuals considered at high risk due to factors such as age, smoking, alcohol use, and environmental exposures. Nonetheless, cancer rates are climbing even among those who don't fall into these risk categories, for instance, in cases of breast cancer, human papillomavirus-related head and neck cancer, and colorectal cancer among younger adults. Common diagnostic tools like CT scans, X-rays, and PET scans can expose individuals to radiation, which paradoxically has the potential to induce cancer. Procedures such as endoscopies and biopsies are invasive and can be uncomfortable, which may deter people from undergoing timely screening. Moreover, traditional methods such as histopathological analysis have a significant chance of producing false negatives or false positives, leading to misdiagnosis. This is

often due to the histological resemblance between different tumor types and the challenge of identifying poorly differentiated cells, making it hard to pinpoint the tissue's origin.

[0004] Optical spectroscopy is emerging as a method for early cancer diagnosis, with Fourier transform infrared (FTIR) spectroscopy showing potential despite the low sensitivity and specificity of many cancer biomarkers in the infrared (IR) or near-infrared (NIR) bands (ranging from 900-3080 nm). FTIR stands out for its simplicity, speed, accuracy, cost-effectiveness, non-destructiveness, and compatibility with automated processes, offering improvements over traditional cancer screening, diagnosis, and management methods. Infrared spectroscopy can serve as a valuable tool for early disease detection, aiding in timely decision-making and enhancing patient outcomes.

[0005] While NIR spectroscopy is not novel in cancer screening, recent studies promote the use of higher wavelengths and a multifaceted approach for analyzing various sample types, traditionally between 600-1100nm. However, longer wavelengths often encounter absorption by water, obscuring the detection of other substances. Despite water's optical clarity, it is highly absorptive at wavelengths below 20 nm and within some parts of the NIR and even more in the mid-IR to far-IR range. The optical IR (OIR) sampling device described herein provides a novel approach to quickly produce dried microlayers of samples ready for a high-throughput reflective IR spectroscopic analysis such as those of liquid biopsies to determine one or more optical properties of the sample.

[0006] Liquid biopsy is another non-invasive technique being explored for cancer detection, such as the method described in U.S. Patent No. 10,288,615 involving blood analysis. Differentiating water content in normal versus cancerous cells has also been attempted, as detailed in U.S. Patent No. 7,706,862, which utilizes NIR spectral optical imaging at key water absorption wavelengths to discern water content variations between cancerous and normal tissue. Key "fingerprint" wavelengths include 980 nm, 1195 nm, 1456 nm, 1944 nm, 2880 nm, 3360 nm, and 4720 nm, with reference wavelengths like 4500 nm, 2230 nm, 1700 nm, 1300 nm, 1000 nm, and 800 nm aiding in image comparison.

[0007] Despite NIR's capability to measure water absorption in cells, its reliability and clarity are inconsistent due to water's extensive absorption of many wavelengths. Molecular fingerprinting, which seeks specific biomarker differences that constitute disease fingerprints, calls for a comprehensive method to identify not only the presence of cancer but its type, based on spectrometric signatures from broad-spectrum spectroscopy. Previous studies endorse this approach, where IR spectroscopy can discern

variations in serum components to create distinct spectrometric signatures for various health conditions. Nonetheless, earlier research has largely concentrated on longer wavelength IR absorption to analyze serum biomolecules, indicating a pressing need for a non-invasive, affordable, and safe method for early cancer detection in the field.

SUMMARY

[0008] Examples presented herein relate to a reflective sampling device for FTIR-based analysis. The reflective sample device includes a base plate defining an array of wells on a surface thereof, wherein each of one or more wells arranged on the surface of the base plate is configured as a concave mirror that is reflective in a predetermined wavelength range.

[0009] In other examples presented herein, the concave mirrors have an IR reflective coating made of a material with high infrared reflectivity. In further examples presented herein, the material is selected from a group including aluminum, copper, nickel, chrome, silver, and gold. In yet other examples presented herein, the wells are optimized in depth and radius to produce an optimal focal point for minimizing reflection interferences and maximizing signal amplitude. In further examples presented herein, the depth of each well is selected from a range of 0.2 to 2 mm and the radius of each well is selected from a range of 1.5 to 5 mm. In other further examples presented herein, the base plate is a 96-well plate.

[0010] In still other examples presented herein, the IR reflective well surfaces are ion-treated to ensure a consistent and smooth thin layer of liquid samples is produced. In still other examples presented herein, the reflective surface is created by a process selected from a group consisting of vacuum metallization, use of reflective embossed metallized films, and aluminum foil laminates.

[0011] Other examples presented herein relate to a reflective sampling device for FTIR-based analysis. The reflective sampling device includes a base plate with an array of wells optimized in depth and radius to produce an optimal focal point for minimizing reflection interferences and maximizing signal amplitude.

[0012] In other examples presented herein, the depth of each well is selected from a range of 0.2 to 2 mm and the radius of each well is selected from a range of 1.5 to 5 mm. In further examples presented herein, the base plate is a 96-well plate.

[0013] In yet other examples presented herein, the array of wells is configured as an array of concave mirrors. In further examples presented herein, the concave mirrors have

an IR reflective coating made of a material with high infrared reflectivity. In other further examples presented herein, the IR reflective coating is applied by a process selected from a group consisting of vacuum metallization, use of reflective embossed metallized films, and aluminum foil laminates.

[0014] Other examples presented herein relate to a method of preparing an infrared (IR) reflective array plate for high-throughput optical cancer fingerprint analysis. The method includes obtaining a base plate including an array of concave wells; and applying an IR reflective coating to one or more wells of the array of concave wells such that each of the one or more wells is a concave mirror.

[0015] In other examples presented herein, the IR reflective coating is selected from a group including aluminum, copper, nickel, silver, and gold. In further examples presented herein, applying the IR reflective coating comprises vacuum metallizing. In yet other examples presented herein, the method further includes ion-treating the concave mirror surface of the one or more wells. In still other examples presented herein, each well of the array of concave wells is optimized in depth and radius to produce an optimal focal point for minimizing reflection interferences and maximizing signal amplitude.

[0016] In other examples presented herein, the method further includes loading on or more samples into the one or more wells; conducting an analysis of the one or more samples; and separating the IR reflective layer from the base plate. In further examples presented herein, the method further includes applying another IR reflective coating to one or more well of the array of concave wells.

[0017] Yet other examples presented herein relate to a method for enhancing signal-to-noise ratio in FTIR-based analysis using diffuse reflectivity from the reflective sampling device described above.

[0018] A variety of additional inventive aspects will be set forth in the description that follows. The inventive aspects can relate to individual features and to combinations of features. It is to be understood that both the forgoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the broad inventive concepts upon which the embodiments disclosed herein are based.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] The accompanying drawings, which are incorporated in and constitute a part of the description, illustrate several aspects of the present disclosure. A brief description of the drawings is as follows:

[0020] FIG. 1A is an example of assembly of an infrared (IR) reflective sample plate, according to embodiments of the present disclosure.

[0021] FIG. 1B is an example of the assembly 105 of an IR reflective sample plate 156 by vacuum metallization and embodying the subject matter of the present disclosure is shown.

[0022] FIG. 2A is a cross-sectional view of a portion of an IR reflective sample plate, according to embodiments of the present disclosure.

[0023] FIG. 2B is a cross-sectional view of the layers that make up an individual reflective well as made by any of the methods of FIG. 2A to include a base layer and an upper coat.

[0024] FIG. 3 is a flowchart of an example method of assembly of an IR reflective sample plate, according to embodiments of the present disclosure.

[0025] FIGS. 4A and 4B are diagrams depicting examples of changes in a target focal point placement based on an angle of incidence.

DETAILED DESCRIPTION

[0026] Embodiments of the present disclosure relate to biomedical analysis devices, particularly those used for detecting cancer and other diseases through Fourier Transform Infrared (FTIR) spectroscopy.

[0027] For purposes of this specification, the following terms are specifically defined as follows:

[0028] “Infrared absorption spectrum” refers to a spectrum that is proportional to the wavelength dependence of the infrared absorption coefficient, absorbance, or similar indication of IR absorption properties of a sample. An example of an infrared absorption spectrum is the absorption measurement produced by a Fourier Transform Infrared (FTIR) spectrometer, i.e. an FTIR absorption spectrum. In general, infrared light will either be absorbed (i.e., a part of the infrared absorption spectrum), transmitted (i.e., a part of the infrared transmission spectrum), or reflected. Reflected or transmitted spectra of a collected probe light can have a different intensity at each wavelength as compared to the intensity at that wavelength in the probe light source. It is noted that IR measurements are often plotted showing the amount of transmitted light as an alternative to showing the amount of light absorbed. For the purposes of this definition, IR transmission spectra and IR absorption spectra are considered equivalent as the two data sets as there is a simple relationship between the two measurements.

[0029] “Infrared source” and “source of infrared radiation” refer to one or more optical sources that generates or emits radiation in the infrared wavelength range, generally between 2-25 microns. The radiation source may be one of a large number of sources, including thermal or Globar sources, supercontinuum laser sources, frequency combs, difference frequency generators, sum frequency generators, harmonic generators, optical parametric oscillators (OPOs), optical parametric generators (OPGs), quantum cascade lasers (QCLs), interband cavity lasers (ICLs), synchrotron infrared radiation sources, nanosecond, picosecond, femtosecond and attosecond laser systems, CO₂ lasers, microscopic heaters, electrically or chemically generated sparks, and/or any other source that produces emission of infrared radiation. The source emits infrared radiation in a preferred embodiment, but it can also emit in other wavelength ranges, for example from ultraviolet to THz. The source may be narrowband, for example with a spectral width of $<10\text{ cm}^{-1}$ or $<1\text{ cm}^{-1}$ less, or may be broadband, for example with a spectral width of $>10\text{ cm}^{-1}$, $>100\text{ cm}^{-1}$ or greater than 500 cm^{-1} . Broadband sources can be made narrow band with filters, monochromators and other devices. The infrared source can also be made up of one of discrete emission lines, e.g. tuned to specific absorption bands of target species.

[0030] “Interacting” in the context of interacting with a sample means that light illuminating a sample is at least one of scattered, refracted, absorbed, aberrated, diverted, diffracted, transmitted, and reflected by, through and/or from the sample.

[0031] “Optical property” refers to an optical property of a sample, including but not limited to index of refraction, absorption coefficient, reflectivity, absorptivity, real and/or imaginary components of the index refraction, real and/or imaginary components of the sample dielectric function and/or any property that is mathematically derivable from one or more of these optical properties.

[0032] “Optical response” refers to the result of interaction of radiation with a sample. The optical response is related to one or more optical properties defined above. The optical response can be an absorption of radiation, a temperature increase, a thermal expansion, a photo-induced force, the reflection and/or scattering of light or other response of a material due to the interaction with illuminating radiation.

[0033] A “probe source,” “probe light source,” or “probe radiation source” refer to a radiation source that can be used for sensing of an optical property of a sample. A probe light source can be used to sense the response of the sample to the incidence of light from the infrared light source. The radiation source may comprise a gas laser, a

laser diode, a superluminescent diode (SLD), a near infrared laser, a UV and/or visible laser beam generated via sum frequency or difference frequency generation, for example. It may also comprise any or other sources of near-infrared, UV, and/or visible light that can be focused to a spot on the scale smaller than 2.5 micrometer, and or even smaller than 1 micrometer, and possibly smaller than 0.5 micrometer. In some embodiments, the probe light source may operate at a wavelength that is outside the tuning or emission range of the infrared light source, but the probe light source can also be a fixed wavelength source at a select wavelength that does in fact overlap with the tuning range of the infrared light source. A “probe light beam” or “sensing light beam” is a beam originally emitted from a probe light source.

[0034] “Probe beam” is a beam of light or radiation that is directed onto a sample to detect a photothermal distortion or other optical change resulting from the interaction of IR radiation with the sample, for example to detect the absorption of IR radiation by the sample.

[0035] “Signal indicative of” refers to a signal that is mathematically related to a property of interest. The signal may be an analog signal, a digital signal, and/or one or more numbers stored in a computer or other digital electronics. The signal may be a voltage, a current, or any other signal that may be readily transduced and recorded. The signal may be mathematically identical to the property being measured, for example explicitly an absolute phase signal or an absorption coefficient. It may also be a signal that is mathematically related to one or more properties of interest, for example including linear or other scaling, offsets, inversion, or even complex mathematical manipulations.

[0036] “Spectrum” refers to a measurement of one or more properties of a sample as a function of wavelength or equivalently (and more commonly) as a function of wavenumber.

[0037] The terms “about” or “approximate” and the like are synonymous and are used to indicate that the value modified by the term has an understood range associated with it, where the range can be $\pm 20\%$, $\pm 15\%$, $\pm 10\%$, $\pm 5\%$, or $\pm 1\%$.

[0038] The term “substantially” is used to indicate that a result (e.g., measurement value) is close to a targeted value, where close can mean, for example, the result is within 80% of the value, within 90% of the value, within 95% of the value, or within 99% of the value.

[0039] In optical systems, such as fiber optics, and in wireless communication systems, reflections can cause interference. This interference occurs when a signal

reflects off surfaces or interfaces within the system, creating echoes or ghost signals. These reflected signals can interfere with the original signal, leading to distortions, reduced clarity, and data loss. In complex systems, multiple reflections can compound these issues, making it difficult to isolate and transmit clear signals. Without clear signals, optical properties of a sample are difficult to assess.

[0040] In many communication and signal processing systems, maintaining optimal signal amplitude is crucial for clear transmission and reception. Issues arise when signal strength is too low, leading to loss of information, or too high, causing signal distortion. Automatic gain control, used in some cases to address this issue, may be inefficient or too slow to adapt to rapidly changing conditions.

[0041] The infrared (IR) reflective device disclosed herein utilizes digital signal processing techniques to identify and cancel out reflected signals and alters the physical design of the system to minimize points of reflection. The invention focuses on more accurately and dynamically adjusting signal amplitude by deploying sophisticated algorithms for automatic gain control that can adapt quickly to changing signal conditions and implementing feedback systems that continuously monitor signal quality and make adjustments accordingly.

[0042] Disclosed herein is an infrared (IR) reflective sampling device for FTIR-based molecular fingerprint analysis for detecting cancer and other diseases. In embodiments, the sampling device includes a reflective high-throughput biopsy plate. In some examples, the reflective element includes a concave mirror, or an $N \times N$ array of concave mirrors, for example with an $N=1$ to 400.

[0043] As used throughout this application, the term “reflective” means substantially more reflective than absorptive or transmissive. For example, a reflective material may be 99% or more reflective, or 95% or more reflective, or 90% or more reflective. Additionally, reflectivity is often a function of wavelength. Water, for example, is highly absorptive in the ultraviolet regime while nearly transparent in the visible regime. The devices described herein are used for testing of infrared absorption by biological samples, and thus this disclosure generally describes materials that are reflective in the infrared regime. However, it may be that the devices described herein can be used for other kinds of tests in which visible or ultraviolet light is of importance. In any case, there may be a predetermined wavelength range of interest (such as, for example, 500nm–1500nm, or 780nm–1000nm, etc.) for which the devices described herein are best suited. Depending upon the range of interest, different materials or

thicknesses of those materials can be used that will provide more reflection than combined transmission and absorption. While not explicitly described herein, those of skill in the art will understand how to substitute materials that are reflective in other wavelengths to accomplish the results described herein for other predetermined wavelength ranges.

[0044] Referring now to FIG. 1A, an example of the assembly 100 of an infrared (IR) reflective sample plate 116 embodying the subject matter of the present disclosure is shown. Assembly 100 of the IR reflective sample plate 116 may comprise creation or acquisition of a suitable base plate 102, application of an IR reflective layer 104, and ionization of the IR reflective wells 106.

[0045] At 102, assembly 100 begins with a suitable base plate 108. A suitable base plate 108 may be designed or selected according to considerations such as throughput, sample size, and use setting.

[0046] Base plate 108 may be made of any suitable material, with consideration to the plate's use in FTIR analysis. Some nonlimiting examples of suitable materials for base plate 108 include acrylonitrile butadiene styrene (ABS), polystyrene, polypropylene, polycarbonate, polyetherimide, glass, quartz, and other thermally stable materials. In embodiments, base plate 108 may be made of disposable, reusable, or other recyclable material. Base plate 108 may be formed by a number of different processes, such as injection molding or vacuum forming, e.g., vacuum metallization.

[0047] In examples, base plate 108 is designed according to experimental and sample parameters. Some nonlimiting examples of suitable base plate designs include single sample well plates and multi-well plates, such as 1-, 12-, 24-, 48-, 96-, or 384-well formats. In some examples, a standard design that is readily accommodated by a standard plate reader, such as a plate reader for existing designs of FTIR spectroscopy instruments.

[0048] Each well 110 of one or more wells of base plate 108 may be configured to produce a focal point 112 in a placement to eliminate reflection interferences and achieve a desired signal amplitude. In examples, the desired signal amplitude may be an optimal signal amplitude. In embodiments, wells having a shallow depth and a curved cross-section may be preferred. For example, each well may have a parabolic cross-section with a depth (d), which may also be understood as a height of the well, and a radius (r) configured to optimize the location of a focal point 112. In an example referring to a 96-well format, well depth (d) may range from 0.2 to 2 mm and radius (r) may range

from 1.5 to 5 mm, values which are optimized to produce the optimal focal point to eliminate reflection interferences and achieve the optimal signal amplitude.

[0049] At 104, an IR reflective layer 114 is applied to base plate 108. In examples, IR reflective layer 114 may be a coating, a film, or a foil, such as aluminum, copper, nickel, silver, gold, dielectric mirror, multilayer optical films, etc.

[0050] IR reflective layer 114 can be applied to cover an individual well, such as well 110, or across the entire plate, such as base plate 108. In examples, IR reflective layer 114 may be applied over the full surface of the base plate, such that all plate surfaces are covered, or may be applied only to one or more wells, such that non-well surfaces and one or more wells may not be covered. To ensure smooth and consistent IR reflective coating or layer, an underlying base coat can be applied on the base plate prior to IR reflective coating. The undercoat, or base coat, can be materials compatible with or serves as primer to enhance the adhesion of IR reflective upper coat. The base coat can be an ultra-violet (UV)-curable base coat, epoxy base, polyurethane base coat, and others. In one embodiment, primers under the PARYLENE® brand can be used, which are materials providing barrier layers that prevent moisture, corrosion, and solvent transpiration, while also providing smoothing for an adjacent layer.

[0051] Reflective properties of materials such as aluminum, copper, nickel, chromium, silver, and gold in the visible light spectrum are well-known, but their behavior in the infrared (IR) spectrum is particularly important for various applications. These metals have electrons in the conduction band that can oscillate in response to electromagnetic radiation, such as IR light.

[0052] In the IR spectrum, materials like gold and silver reflect IR radiation effectively due to their high reflectance of longer wavelengths. Gold reflects up to 98% of IR radiation, making it extremely efficient for applications requiring insulation from heat. Silver, although it tarnishes, also provides excellent IR reflectivity when clean. Aluminum, while less reflective than gold and silver, still reflects a significant amount of IR radiation and is more cost-effective. Copper and nickel have more moderate reflectivity in the IR range but are still useful due to their other physical properties.

[0053] The use of these materials in the IR spectrum is multifaceted. Gold's excellent reflectivity is utilized in satellite and space telescope components to protect against the sun's heat. Silver's high IR reflectivity is often used in thermal insulation and in coatings to improve energy efficiency. Aluminum, due to its cost-effectiveness and good IR reflectivity, is widely used in thermal rescue blankets and in architectural

designs to reflect IR and reduce heating. Copper finds its use in heat exchangers due to its ability to reflect IR and its excellent thermal conductivity. Nickel, with its moderate IR reflectivity, is often used as a coating on other materials to add protection against IR radiation while providing corrosion resistance.

[0054] Multiple methods of applying IR reflective layer 114 to base plate 108 are envisioned. IR reflective layer 114, which may be, for example, aluminum, may be vacuum metallized to base plate 108. This method may be preferred in some instances, such as those where cost effectiveness is a significant consideration, due to its simplicity and low cost. In vacuum metallization, also referred to as physical vapor deposition, metal is evaporated in a vacuum environment and then allowed to condense on the substrate's surface to form a thin film. This method is widely used for creating reflective surfaces on items like mirrors, automotive parts, and decorative items. It provides a uniform coating and can be used with a variety of metals like aluminum, which is often chosen for its reflective properties. Vacuum metallization of the plates with, for example, aluminum to make the wells and/or plates reflective, and it is an easy and low-cost process, being more cost effective than adding a reflective metallized film or aluminum foil.

[0055] FIG. 1B is an example of the assembly 105 of an IR reflective sample plate 156 by vacuum metallization and embodying the subject matter of the present disclosure is shown. As with the example of the assembly 100, one or more wells 110 are configured to produce a focal point 112 in a placement to eliminate reflection interferences and achieve a desired signal amplitude. IR reflective coating material 154 is applied to one or more wells 110, or to base plate 108 generally, by vacuum metallization. IR reflective coating material 154 produces a thin, reflective layer that matches the concave shape and curve of the well 110, resulting in metallized wells 110 becoming concave mirrors. In this way, reflective sample plate 156 is prepared as an array of concave mirrors.

[0056] Similar to vacuum metallization, sputtering involves the coating of a substrate with a thin, reflective metal layer. However, instead of evaporating the metal, sputtering uses energetic particles to eject atoms from the target material, which then deposit on the substrate. This method is used for applications requiring a very controlled film structure.

[0057] In chemical vapor deposition (CVD), a substrate is exposed to one or more volatile precursors, which react and/or decompose on the substrate surface to produce the

desired deposit. For reflective surfaces, materials like silicon or titanium can be deposited to form a reflective layer.

[0058] Electroplating involves the deposition of a metal coating on an object by passing a current through an electrolyte solution in which the object is submerged. This can be used to create reflective surfaces, especially for metals like chrome.

[0059] Spray coating involves spraying a solution containing metal particles onto the substrate. After the solvent evaporates, a reflective metal layer is left behind. This method is less precise than vacuum metallization but is more cost-effective for large items.

[0060] Metallized films are typically made by coating a thin layer of metal onto a plastic film. The metal layer can be aluminum in one embodiment for its reflective properties and cost-effectiveness. These films can be applied to various surfaces to provide a reflective finish and are commonly used in packaging, insulation, and decorative applications.

[0061] A reflective embossed metallized film may be used as a substrate to form one or more wells as foil laminates, such as an aluminum foil laminate, applied to a polymer film or paper that may also be embossed with small wells corresponding to the final shape of the device as shown in FIG. 1A or FIG. 2A to prevent wrinkling, warping, or cracking that could otherwise occur on application to the base. Concave lens film may be used, such as by being metallized and then cut into an appropriate size. In examples, IR reflective layer 114 may be configured to be separated from the base plate for disposal following sample analysis.

[0062] FIG. 2A is a cross-sectional view of a portion of an IR reflective sample plate, showing one of the active sampling wells (i.e., one of the 96 concaved mirrors; 96X, A) according to embodiments of the present disclosure. Other features surrounding and underneath the active sampling wells are designed to form a rigid base plate with the minimum amount of material used. When made with a polymer such as by injection molding, the produced base plate is highly sturdy and inflexible, ready to receive an IR reflective coating with or without an undercoat. The plate is designed for ease of handling, can be covered with standard microtiter or customized lids, and to fit in industry-standard plate readers.

[0063] FIG. 2A shows the IR reflective coating 114 arranged across a top surface of a substrate of a well 112. As shown in FIG. 2A, the base plate 108 underneath the IR reflective coating provides mechanical support for the well 112, including pillars or other

support structures as necessary to maintain the shape and position of the well 112. However, other areas of the well may be substantially hollow. The materials and support structure arrangement of the base plate 108 are selected according to several criteria. First, it may be desirable for base plate 108 to be the same overall size as that of other 96-well plates (or plates having other numbers of wells) so that it will be interoperable with existing testing equipment. Second, the base plate 108 can be made using materials that are environmentally conscious, such as recyclable or recycled materials. Additionally, the design and materials can be selected to prioritize the position and shape of the wells 112. This means that adequate support should be provided directly below or adjacent the wells 112 to ensure they do not move or bend during standard use conditions (e.g., insertion of a sample, or movement to, from, or within the testing apparatus).

[0064] FIG. 2B is a cross-sectional view of a single well 112, such as the well 112 depicted in FIG. 2A. FIG. 2B is a detailed cross-sectional view, and it should be understood that not all of the features shown therein are to scale. Rather, the features of FIG. 2B may be exaggerated for a better conceptual understanding of the disclosure.

[0065] FIG. 2B shows the base 108 providing support for three layers: a smoothing layer 113, the IR reflective layer 114 described above, and an upper coat 115.

[0066] Smoothing layer 113 can be, as described elsewhere herein, a layer to provide superior binding, adhesion, and smoothness between the base 108 and the IR reflective layer 114. In some embodiments, such as when applying a foil layer to act as IR reflective layer 114, there can be wrinkling, stretching, or other deformation of IR reflective layer 114. Even when using other, generally more precise methodologies to provide IR reflective layer 114 (such as sputtering or CVD), if there is inadequate wetting and adhesion to the base 108 then the IR reflective material 154 can bead, pool, or form other discontinuities that either change the focal point of the well 112 or may make the well 112 lose a focal point altogether.

[0067] Smoothing layer 113 can therefore be provided in some embodiments as a primer and adhesion promoter. Smoothing layer 113 can be chosen from materials that exhibit good binding properties to both the material used as the base 108, and to the material chosen as the IR reflective layer 114 (that is, the IR reflective material 154). Thus, smoothing layer 113 may be a different material depending upon whether the base 108 is made of a polar or non-polar polymer, a metal, or some other material. Likewise, a different material may be used for the smoothing layer 113 for aluminum than gold or

copper, which have very different melt temperatures and may respond differently to adhesives, solders, or other materials that could be used as a smoothing layer.

[0068] In some embodiments, smoothing layer 113 can be applied to the base 108, then the IR reflective layer 114 can be applied on top of the smoothing layer 113 directly. In embodiments, a further processing step such as heating of the overall construction of these layers can be used to activate the smoothing layer 113 and provide the bonding and smoothing attributes described above. For example, this may be helpful in the instance where a hot-melt adhesive is used as the smoothing layer 113.

[0069] IR reflective layer 114 has been described in detail with respect to other figures, and the discussion therein applies equally to FIG. 2B. The IR reflective layer 114 can be applied as a sheet, via deposition, or by any other method for adding a layer to the top of the base 108.

[0070] Upper coat 115 is an optional layer provided on top of IR reflective layer. Upper coat 115 can be provided, for example, where the IR reflective layer 114 is susceptible to oxidization or other reactions, either with the ambient environment or with the expected samples to be tested. Upper coat 115 can be, for example, a polymer material. Generally speaking, upper coat 115 will be thin enough to prevent distortions or reflections that would otherwise occur at the interface between two layers having different refractive indices. Additionally, upper coat 115 can be either reflective in the wavelengths of interest for testing the samples (e.g., in the infrared regime) or it can be transmissive in these wavelengths. Upper coat 115 will generally not exhibit high levels of absorption in the wavelengths of interest, to avoid overly reducing the focused optical signal after interaction with the sample.

[0071] Although not shown in FIG. 2B, various surfaces between or on top of the layers (108, 113, 114, 115) can be treated. For example, ionization treatment can be used to increase adhesion between any of the layers in various embodiments. Additionally, the top surface of the upper coat 115 can be treated to provide better interaction with an expected sample (e.g., prevention of the formation of meniscus or droplets). One particularly important bond is that between the IR reflective layer 114 and the upper coat 115, in those embodiments where an upper coat 115 is used. As described above, the upper coat 115 should ideally not affect the reflected light, either by distorting or absorbing portions thereof. The better the adhesion between the IR reflective layer 114 and the upper coat 115, the less likely it is that the reflected signal will lose strength or focus.

[0072] Referring now to FIG. 3, a flowchart of an example method 200 of assembly of an IR reflective sample plate, according to embodiments of the present disclosure, is shown.

[0073] At 202, a suitable base plate is prepared or obtained. A suitable base plate may be designed or selected according to considerations such as throughput, sample size, and use setting. In examples, the base plate may be base plate 108 of FIG. 1A or FIG. 1B.

[0074] Of particular interest may be the consideration of the placement of a focal point when a sample in a well of the plate is interrogated. Wells within the plate may be configured to reflect light in a particular direction or toward a particular point to, for example, improve analysis by the reduction of noise. Each well may be configured such that, once the well is made reflective, it reflects light to produce a focal point in a placement to eliminate reflection interferences and achieve a desired signal amplitude.

[0075] In examples, the desired signal amplitude may be an optimal signal amplitude. In embodiments, wells having a shallow depth and a curved cross-section may be preferred. For example, each well may have a parabolic cross-section with a depth (d) and a radius (r) (see FIG. 4B) configured to optimize the location of a focal point. As disclosed here, optics and focal point are configured to optimize interaction between a sample, which may be a thin layer sample, and the interrogation source, such as IR radiation, which is directly correlated to the angle of incidence of the IR beam. The well surface curvature chosen depends on angle of incidence of the source IR beam, or other interrogation beam, and the optimal sample drying time. Therefore, a range for each of a depth of the well and a radius of the well depends on a range of the IR beam angle of incidence and sample drying time. The range also depends on the well array configuration, whether a 96-well, 386-well, or other number of wells per plate. In an example referring to a 96-well format, depth (d) may range from 0.2 to 2 mm and radius (r) may range from 1.5 to 5 mm.

[0076] At 204, an IR reflective layer is applied to the base plate. The IR reflective layer may be applied with or without a base or primer coat, for example, by the base plate being vacuum metallized, laminated an embossed metallized film to a substrate, or by use of metallized concave lens film.

[0077] Multiple methods of applying IR reflective layer 114 to base plate 108 are envisioned. IR reflective layer 114, which may be, for example, aluminum, may be vacuum metallized to base plate 108. This method may be preferred in some instances,

such as those where cost effectiveness is a significant consideration, due to its simplicity and low cost. A reflective embossed metallized film may be used as a substrate to form one or more wells as foil laminates, such as an aluminum foil laminate, applied to a polymer film or paper that may also be embossed with small wells. Concave lens film may be used, such as by being metallized and then cut into an appropriate size.

[0078] In embodiments, vacuum metallization may be a preferred method of applying the IR reflective layer. For example, vacuum metallization may support scalability of the process by supporting large production with relatively low cost material. Vacuum metallization may be accomplished consistently with very thin layers of aluminum. Thin layers may be preferred as they avoid changing the precise curvature of the substrate well. In some example, application of the reflective layer may produce a change in the precise curvature of the well, and produce a plate with wells have different variations in the curve. Application of the reflective layer in a very thin layer, such as with vacuum metallization, may help avoid such variations. Additionally, vacuum metallization is heat compatible, such that when the plate is subsequently heated during a working process, e.g., to dry the sample, the reflective layers remains stable, e.g., it is not prone to air pockets or other distortions.

[0079] Vacuum metallization is a process used to apply a thin metallic layer to various surfaces. The material to be coated (substrate) is cleaned and prepared to ensure that the metallic layer adheres well to the surface. The substrate is placed inside a vacuum chamber. This chamber is then sealed, and the air is removed to create a vacuum. The vacuum helps to eliminate air and other gases that could interfere with the metallization process. A metal (such as aluminum, silver, or copper) is heated to its evaporation temperature. The metal is typically in the form of a solid wire or pellets. The heat causes the metal to vaporize and form a metallic vapor cloud within the vacuum chamber. The vaporized metal condenses onto the substrate, forming a thin, uniform metallic layer. The substrate may be rotated or moved during this process to ensure an even coating. The thickness of the deposited metal layer can be controlled by adjusting parameters such as the temperature of the metal source, the deposition time, and the substrate's movement. After the desired thickness is achieved, the coated substrate is cooled, and the vacuum chamber is opened. The cooling process helps solidify the thin metal layer onto the substrate, creating a durable and adherent coating.

[0080] In embodiments, an upper coating may be applied to the surface of the metallic layer to provide further smoothing of the final surface. Numerous coatings may be

appropriate for the upper coating, provided the coating is transparent and does not interfere with the reflectivity of the concave mirror.

[0081] At 206, the IR reflective plate, resulting from 204, is ionized with an ion treatment. An ion treatment is applied to increase sample adhesion on the well surfaces. This process involves bombarding the surface with ions to create a more reactive surface that can bond more effectively with the sample. There are several different types of ion treatments that can be used, including, for example, a plasma treatment which uses a low-pressure plasma to create a highly reactive surface on the material being treated. The plasma can be generated using a variety of gases, including oxygen, nitrogen, and argon. In another example, a corona treatment uses a high-voltage electrical discharge to create a highly reactive surface on the material being treated. The discharge creates a corona of ions around the material, which can then bond more effectively with the sample. In yet another example, a flame treatment exposes the surface to a flame, which creates a highly reactive surface that can bond more effectively with the sample. In yet another example, a self assembled monolayer can be applied to the reflective layer that promotes sample wetting and adhesion.

[0082] At 208, sample(s) are loaded into the plate. Samples may be applied as a thin layer across the curved ion-treated well surfaces and dried for FTIR analysis of disease fingerprints. A combination of the ion-treatment and the geometry of the well surfaces may provide for improved adhesion of low volume samples to ensure a consistent and smooth thin layer of liquid samples is produced when a sample is added to the well. In embodiments, wells are ion-treated for sterilization purposes.

[0083] At 210, sample(s) are analyzed. Sample plates embodying the subject matter of the present disclosure may be used for a wide array of known and experimental sample analysis methods. In embodiments, sample plates embodying the present disclosure may be optimized particularly for FTIR analysis of lipids, proteins, glycans, DNA, RNA, or other analytes fingerprinting for disease detection.

[0084] In embodiments employing an IR beam, analytes must be infrared active, such as by having a dipole moment that changes during vibration or rotation. The analytes may also have a unique vibrational frequency that can be detected by a detector, for example a detector of a FTIR spectrometer. A “detector” refers to a device that produces a signal indicative of the power, intensity and/or energy of light/radiation incident on the detector surface. The signal will generally be an electrical signal, for example a voltage, current and/or an electrical charge. The detector may be a photodiode, a photo-transistor,

a charge coupled device (CCD). In some cases, a detector may be a semiconducting detector, for example a silicon PIN photodiode. A detector may also be an avalanche photodiode, a photomultiplier tube, or any other device that produce a change in current, voltage, charge, conductivity or similar upon incidence of light. A detector may comprise a single element, multiple detector elements, for example a bi-cell or quad-cell, a linear or two dimensional array of detector elements, including camera based detectors.

[0085] The plate disclosed herein is suitable for use with any assays applied to liquid or thin-layer samples that can be fixed or adhered to the well surface for reflective FTIR spectroscopy. This includes assays for identifying or analyzing the chemical or agent fingerprints, molecular fingerprints, or any related vibrational fingerprints by FTIR. The design allows the greatest IR reflectivity and interaction with the sample by optimizing the IR exposure to the target analytes, particularly the more complex analytes found in the biological specimens.

[0086] At 212, the IR reflective layer may be removed from the base plate. Removal of the IR reflective layer may extend the life of the base plate, as additional IR reflective layers may be applied for reuse.

[0087] FIG. 4A is a diagram depicting examples of changes in a target focal point placement based on an angle of incidence. As noted above and otherwise disclosed herein, optical system and focal point are configured to optimize interaction between a sample, which may be a thin layer sample, and the interrogation source, such as IR radiation, which is directly correlated to the angle of incidence of the IR beam. In example 300 of FIG. 4, three example angles of incidence 302, 304, and 306 are shown originating from an IR source 308. Each angle of incidence 302, 304, and 306 is associated with a specific target focal point 310, 312, and 314. For example, a first angle of incidence 302 is associated with a first focal point 310, while a second angle of incidence 304 is associated with a second focal point 312 and a third angle of incidence 306 is associated with a third focal point 314.

[0088] The curvature of the well design is optimized based on a chosen angle of incidence of the IR beam, and thus, the depth of the curvature or concavity can vary depending on the angle of incidence. Given a fixed angle of incidence, the depth (d) and radius (r) of the mirror (see FIG. 4B) or concavity can change at a constant ratio within a given range while maintaining the same curvature.

[0089] FIG. 4B shows one example of an incident IR beam (402) illuminating a well 112 having a depth (d) and a radius (r). Illuminating, as discussed herein, refers to

directing radiation at an object, for example a surface of a sample, the probe tip, and/or the region of probe-sample interaction. Illumination may include radiation in the infrared wavelength range, visible, and other wavelengths from ultraviolet to a millimeter or more. Illumination may include any arbitrary configuration of radiation sources, reflecting elements, focusing elements and any other beam steering or conditioning elements. The incoming IR beam 402 can be, for example, a laser beam that is collimated and collinear such that, upon reflection by a parabolic mirror, it will be focused to a point. Indeed, as shown in FIG. 4B, the light does interact with the parabolic mirror that is the well 112 having radius r and depth d such that it is focused at objective 404.

[0090] In addition to optical considerations, the well 112 is designed to hold samples which are often liquid. Thus the parabolic mirror of the well 112 also has sufficient depth d to act as a bowl that holds this sample. This can be accomplished by sinking the well 112 into the base 108 by the depth d . Alternatively or additionally, and as shown in FIG. 4B, the well 112 can be built up by a height h . In this way the expected sample surface (indicated with the dashed line in FIG. 4B) can be at the same level as the surface of the overall well plate without overflowing. The height h is therefore that of a kind of shoulder used to prevent the sample from flowing out of the well 112.

[0091] As shown in FIG. 4B, incident light 402 passes through the sample (see dashed line) and is reflected to a focal point at the objective 404. The depth d and radius r can be selected based upon the expected angle of incidence θ of the light 402 for a particular apparatus. In some cases, such as where physical space above the sample plate is restricted, a very low angle of incidence θ may be used that would interact with the shoulder, and in such instances the height h can be reduced.

[0092] It should also be understood that while an objective 404 is used in FIG. 4B, other types of detectors, sensors, or cameras could be used that collect the focused light. Generally, the system collects radiation of a probe or interrogation light beam that has interacted with a sample. In some cases, a detector such as a photodiode can have sufficient surface area that the light need not be perfectly focused. Instead, a relatively large spot size for the reflected light can be collected by such a detector or sensor to provide information regarding the reflected signal that has interacted with the sample.

[0093] Having described the preferred aspects and implementations of the present disclosure, modifications and equivalents of the disclosed concepts may readily occur to one skilled in the art. However, it is intended that such modifications and equivalents be included within the scope of the claims which are appended hereto.

What is claimed is:

1. A reflective sampling device for FTIR-based analysis, comprising: a base plate defining an array of wells on a surface thereof, wherein each of one or more wells arranged on the surface of the base plate is configured as a concave mirror that is reflective in a predetermined wavelength range.
2. The device of claim 1, wherein the concave mirrors have an IR reflective coating made of a material with high infrared reflectivity.
3. The device of claim 2, wherein the material is selected from a group including aluminum, copper, nickel, chrome, silver, and gold.
4. The device of claim 1, wherein the wells are optimized in depth and radius to produce an optimal focal point for minimizing reflection interferences and maximizing signal amplitude.
5. The device of claim 4, wherein the depth of each well is selected from a range of 0.2 to 2 mm and the radius of each well is selected from a range of 1.5 to 5 mm.
6. The device of claim 5, wherein the base plate is a 96-well plate.
7. The device of claim 1, wherein the IR reflective well surfaces are ion-treated to ensure a consistent and smooth thin layer of liquid samples is produced.
8. The device of claim 1, wherein the reflective surface is created by a process selected from a group consisting of vacuum metallization, use of reflective embossed metallized films, and aluminum foil laminates.
9. A reflective sampling device for FTIR-based analysis, comprising: a base plate with an array of wells optimized in depth and radius to produce an optimal focal point for minimizing reflection interferences and maximizing signal amplitude.

10. The device of claim 9, wherein the depth of each well is selected from a range of 0.2 to 2 mm and the radius of each well is selected from a range of 1.5 to 5 mm.
11. The device of claim 10, wherein the base plate is a 96-well plate.
12. The device of claim 9, wherein the array of wells is configured as an array of concave mirrors.
13. The device of claim 12, wherein the concave mirrors have an IR reflective coating made of a material with high infrared reflectivity.
14. The device of claim 13, wherein the IR reflective coating is applied by a process selected from a group consisting of vacuum metallization, use of reflective embossed metallized films, and aluminum foil laminates.
15. A method of preparing an infrared (IR) reflective array plate for high-throughput optical cancer fingerprint analysis; the method comprising:
 - obtaining a base plate including an array of concave wells; and
 - applying an IR reflective coating to one or more wells of the array of concave wells such that each of the one or more wells is a concave mirror.
16. The method of claim 15, wherein the IR reflective coating is selected from a group including aluminum, copper, nickel, silver, and gold.
17. The method of claim 16, wherein applying the IR reflective coating comprises vacuum metallizing.
18. The method of claim 15, further comprising ion-treating the concave mirror surface of the one or more wells.

19. The method of claim 15, wherein each well of the array of concave wells is optimized in depth and radius to produce an optimal focal point for minimizing reflection interferences and maximizing signal amplitude.
20. The method of claim 15, further comprising:
 - loading on or more samples into the one or more wells;
 - conducting an analysis of the one or more samples; and
 - separating the IR reflective layer from the base plate.
21. The method of claim 20, further comprising applying another IR reflective coating to one or more well of the array of concave wells.
22. A method for enhancing signal-to-noise ratio in FTIR-based analysis using diffuse reflectivity from the device of claim 1.