Title: BIOASSAY DEVICE FOR DETECTING MOLECULAR EVENTS

Abstract: A bioassay device operable to detect the presence or absence of molecular events within a test sample is presented in four embodiments: a coplanar waveguide detector, a stripline detector, a magic-\( t \) detector, and a microstrip detector. The coplanar waveguide detector includes a center gap operable to contact the sample and to electromagnetically couple an incident test signal between the sample and transmission line. The stripline detector includes a sample chamber operable to contain at least a portion of the supplied sample and to electromagnetically couple an incident at least a portion of the supplied sample and to electromagnetically couple an incident test signal between the contained sample and the transmission line. The magic-\( t \) detector includes a magic-\( t \) coupler and first and second test sample loads electromagnetically coupled thereto. The microstrip detector includes a transmission line, ground plane metallization and a flow channel formed between, and vertically aligned with, the transmission line and ground plane.
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.
BIOASSAY DEVICE FOR DETECTING MOLECULAR EVENTS

BACKGROUND OF THE INVENTION

[0001] Virtually every area of biological science is in need of a system to determine the ability of molecules of interest to interact with other molecules. Likewise, the ability to detect the presence and/or physical and functional properties of biological molecules on a small scale is highly desirable.

[0002] The need to detect molecules and binding events ranges from the basic science research lab, where chemical messenger pathways are being mapped out and their functions correlated to disease processes, to pre-clinical investigations, where candidate drugs are being evaluated for potential \textit{in vivo} effectiveness. The need to detect physical and functional properties is also present in these research areas, such as for functional analysis of a newly discovered protein or of a genetic (or synthetic) variant of a molecule of known biological importance. Other areas that benefit from a better understanding of molecular events include pharmaceutical research, military applications, veterinary, food, and environmental applications. In all of these cases, knowledge of the ability of a particular analyte to bind a target molecule is highly useful, as is information relating to the quality of that binding (\textit{e.g.}, affinity and on-off rate), and other functional information about new molecules, particularly when information can be obtained from a small amount of sample.

[0003] Numerous methodologies have been developed over the years in attempts to meet the demands of these fields, such as Enzyme-Linked Immunosorbent Assays (ELISA), Radio-Imunoassays (RIA), numerous fluorescence assays, nuclear magnetic resonance (NMR) spectroscopy, and colorimetric assays, as well as a host of more specialized assays. Most of these assay techniques require specialized preparation, purification, or amplification of the sample to be tested. To detect a binding event between a ligand and an antiligand, for example, a detectable signal is required that signals the existence or extension of binding. Usually the signal has been provided by a label that is attached to either the ligand or antiligand of interest. Physical or chemical effects which produce detectable signals, and for which suitable labels exist, include radioactivity, fluorescence, chemiluminescence, phosphorescence and enzymatic activity, to name a few. The label can then be detected by spectrophotometric, radiometric, or optical tracking methods.
Unfortunately, in many cases it is difficult or even impossible to label one or all of the molecules needed for a particular assay. The presence of a label also can make the molecular recognition between two molecules not function in its normal manner for many reasons, including steric effects. In addition, none of these labeling approaches determines the exact nature of the binding event, so that, for example, active-site binding to a receptor is indistinguishable from non-active-site binding, such as allosteric binding, and thus no functional information is obtained via the present detection methodologies. In general, limitations also exist in the areas of specificity and sensitivity of most assay systems. Cellular debris and non-specific binding often cause an assay to be noisy and make it difficult or impossible to extract useful information. As mentioned above, some systems are too complicated to allow the attachment of labels to all analytes of interest or to allow an accurate optical measurement to be performed. Therefore, a practical, economic, and universal detection technique that can directly monitor without a label, in real time, the presence of molecular events and/or the extent, function and type of structures or binding events that are actually taking place in a given system would represent a significant breakthrough.

In particular, the biomedical industry needs an improved general platform technology that has very broad applicability to a variety of water-based or other fluid-based physiological systems, such as nucleic acid binding, protein-protein interactions, and small molecule binding, as well as other compounds of interest. Ideally, the assay should not require highly specific probes, such as specific antibodies or exactly complementary nucleic acid probes. It should be able to work in native environments, such as whole blood or cytosolic mixtures, as well as other naturally occurring systems. It should operate by measuring the native properties of the molecules and not require additional labels or tracers to actually monitor the binding event. For some uses it should be able to provide information on the nature of the molecular structure or binding event, such as whether or not a given compound binds to the active site as an agonist or an antagonist on a particular drug receptor or if the given compound binds to an allosteric site, and not function simply as a marker to indicate whether or not the binding event has taken place. For many applications, it should be highly miniaturizable and highly parallel, so that complex biochemical pathways can be mapped out, or so that extremely small and numerous quantities of combinatorial compounds can be used in drug screening protocols. In many applications, it should further be able to monitor in real time a complex series of reactions, so that accurate kinetics and affinity information can be
obtained almost immediately. Perhaps most importantly, for most commercial applications it should be inexpensive and easy to use, with few sample preparation steps, affordable electronics and disposable components, such as surface chips for bioassays that can be used for an assay and then thrown away, and it should be highly adaptable to a wide range of assay applications.

**SUMMARY OF THE INVENTION**

[0006] The present inventions provide miniturizable bioassay devices capable of detecting the presence or absence of molecular events within a test sample without the use of labels. The bioassay devices operate using high frequency test signals which are electromagnetically coupled to a portion of the test sample retained on the bioassay device. The molecular event within the test sample modulates the test signal. The modulated signal is recoverable from the bioassay device, the modulation being indicative of the presence of the molecular event within the test sample.

[0007] Four embodiments of bioassay devices are presented: a coplanar waveguide detector, a stripline detector, a magic-t detector, and a microstrip detector. The coplanar waveguide detector includes a dielectric substrate, first and second transmission line sections formed on the dielectric substrate, and a center gap located between the first and second transmission lines. Two ground strips are formed on the dielectric substrate, each spaced apart from and extending parallel to the first and second transmission line sections. The center gap is operable to contact the sample and the sample is signal electromagnetically coupled to the first and second transmission line sections.

[0008] The stripline detector includes first and second dielectric layers, a transmission line interposed therebetween, and a sample chamber formed within the first dielectric layer. The sample chamber is operable to contain at least a portion of the supplied sample, the sample chamber located proximate to the transmission line to enable signal coupling between the sample and the transmission line.

[0009] The magic-t detector includes a magic-t coupler and first and second loads electromagnetically coupled thereto. A test signal is provided to the input/sum port of the coupler and is equally split to the first and second loads. An output signal is produced at the output/delta port of the magic-t coupler and represents the difference in the signals reflected from the first and second loads.
[0010] The microstrip detector includes a transmission line, ground plane metallization, dielectric material positioned between the transmission line and ground plane metallization, and a channel which is generally vertically aligned between the transmission line and the ground plane metallization. The channel permits the passage of the test sample therethrough where a incident test signal is electromagnetically coupled to it.

[0011] The nature and advantages of the present invention will be better understood with reference to the following drawings and detailed description.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[0012] Fig. 1A illustrates a top view of four coplanar waveguide bioassay devices in accordance with the present invention.

Fig. 1B illustrates a detailed view of a coplanar waveguide bioassay device shown in Fig. 1A.

Fig. 1C illustrates a fluidics fixture for mounting with the coplanar waveguide bioassay device shown in Figs. 1A and 1B.

Fig. 2A illustrates a top view of a stripline bioassay device in accordance with the present invention.

Fig. 2B illustrates a side view of the stripline bioassay device shown in Fig. 2.

Fig. 3A illustrates a top view of a test fixture operable to house the stripline bioassay device shown in Figs. 2A and 2B.

Fig. 3B illustrates the stripline bioassay device of Figs. 2A and 2B located within the test fixture illustrated in Fig. 3A.

Fig. 3C illustrates side and bottom views of a top plate assembly for use with the test fixture shown in Fig. 3A.

Fig. 3D illustrates a side view of an assembled test fixture in accordance with the present invention.

Fig. 4A illustrates a schematic block diagram of a magic-t bioassay device in accordance with the present invention.

Fig. 4B illustrates a mask layout (negative) of a coplanar waveguide magic-t coupler.

Fig. 4C illustrates a flow cell for use with the coplanar waveguide magic-t detector shown in Fig. 4A.
Fig. 4D illustrates a waveguide magic-t bioassay device in accordance with the present invention.

Fig. 4E illustrates a flow cell for use with the waveguide magic-t detector shown in Fig. 4D.

Fig. 5A illustrates a perspective view of a microstrip bioassay device in accordance with the present invention.

Fig. 5B illustrates a cross-sectional view of the microstrip detector along the detection length.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Definition of Terms

[0013] As used herein, the term “molecular binding event” (sometimes shortened to “binding event” or “binding”) refers to the interaction of a molecule of interest with another molecule. The term “molecular structure” refers to all structural properties of molecules of interest, including the presence of specific molecular substructures (such as alpha helix regions, beta sheets, immunoglobulin domains, and other types of molecular substructures), as well as how the molecule changes its overall physical structure via interaction with other molecules (such as by bending or folding motions), including the molecule’s interaction with its own solvation shell while in solution. Together, “molecular structures” and “molecular binding events” are referred to as “molecular events.” The simple presence of a molecule of interest in the region where detection/analysis is taking place is not considered to be a “molecular event,” but is referred to as a “presence.”

[0014] Examples of molecular binding events are (1) simple, non-covalent binding, such as occurs between a ligand and its antiligand, and (2) temporary covalent bond formation, such as often occurs when an enzyme is reacting with its substrate. More specific examples of binding events of interest include, but are not limited to, ligand/receptor, antigen/antibody, enzyme/substrate, DNA/DNA, DNA/RNA, RNA/RNA, nucleic acid mismatches, complementary nucleic acids and nucleic acid/proteins. Binding events can occur as primary, secondary, or higher order binding events. A primary binding event is defined as a first molecule binding (specifically or non-specifically) to an entity of any type, whether an independent molecule or a material that is part of a first surface, typically a surface within the detection region, to form a first molecular interaction complex. A secondary binding event is defined as a second molecule binding (specifically or non-specifically) to the first molecular interaction
complex. A tertiary binding event is defined as a third molecule binding (specifically or non-specifically) to the second molecular interaction complex, and so on for higher order binding events.

[0015] Examples of relevant molecular structures are the presence of a physical substructure (e.g., presence of an alpha helix, a beta sheet, a catalytic active site, a binding region, or a seven-trans-membrane protein structure in a molecule) or a structure relating to some functional capability (e.g., ability to function as an antibody, to transport a particular ligand, to function as an ion channel (or component thereof), or to function as a signal transducer).

[0016] Structural properties are typically detected by comparing the signal obtained from a molecule of unknown structure and/or function to the signal obtained from a molecule of known structure and/or function. Molecular binding events are typically detected by comparing the signal obtained from a sample containing one of the potential binding partners (or the signals from two individual samples, each containing one of the potential binding partners) to the signal obtained from a sample containing both potential binding partners. Together, the detection of a "molecular binding event" or "molecular structure" is often referred to as "molecular detection."

[0017] The methodology and apparatus described herein are primarily of interest to detect and predict molecular events of biological and pharmaceutical importance that occur in physiological situations (such as in a cellular or subcellular membrane or in the cytosol of a cell). Accordingly, structural properties of molecules or interactions of molecules with each other under conditions that are not identical or similar to physiological conditions are of less interest. For example, formation of a complex of individual molecules under non-physiological conditions, such as would be present in the vacuum field of an electron microscope, would not be considered to be a preferred "molecular binding event," as this term is used herein. Here preferred molecular events and properties are those that exist under "physiological conditions," such as would be present in a natural cellular or intercellular environment, or in an artificial environment, such as in an aqueous buffer, designed to mimic a physiological condition. It will be recognized that local physiological conditions vary from place to place within cells and organisms and that artificial conditions designed to mimic such conditions can also vary considerably. For example, a binding event may occur between a protein and a ligand in a subcellular compartment in the presence of helper proteins and small molecules that affect binding. Such conditions may differ greatly from the physiological conditions in
serum, exemplified by the artificial medium referred to as "normal phosphate buffered saline" or PBS. Preferred conditions of the invention will typically be aqueous solutions at a minimum, although some amounts of organic solvents, such as DMSO, may be present to assist solubility of some components being tested. An "aqueous solution" contains at least 50 wt.% water, preferably at least 80 wt.% water, more preferably at least 90 wt.% water, even more preferably at least 95 wt.% water. Other conditions, such as osmolality, pH, temperature, and pressure, can and will vary considerably in order to mimic local conditions of the intracellular environment in which, for example, a binding event is taking place. The natural conditions in, for example, the cytosol of a cell and a lysosome of that cell, are quite different, and different artificial media would be used to mimic those conditions. Examples of artificial conditions designed to mimic natural ones for the study of various biological events and structures are replete in the literature. Many such artificial media are sold commercially, as exemplified by various scientific supply catalogues, such as the 2000/2001 issue of the Calbiochem General Catalogue, pages 81-82, which lists 60 commercially available buffers with pH values ranging from 3.73 to 9.24 typically used in biological investigations. Also see general references on the preparation of typical media, such as chapter 7 ("The Culture Environment") of Culture of Animal Cells: A Manual of Basic Techniques, Third Edition, R. Ian Freshney, Wiley-Liss, New York (1994).

[0018] Although most measurements described herein occur to individual molecules or pairs in solution, at some times the method of the invention can be applied to situations in which one of the members of a binding pair is immobilized on a surface at the site of the location receiving electromagnetic radiation while test compounds are allowed to contact the immobilized molecule. As used herein, when one member of a binding pair is immobilized, the term "antiligand" is usually used to refer to the molecule immobilized on the surface. The antiligand, for example, can be an antibody and the ligand can be a molecule such as an antigen that binds specifically to the antibody. In the event that the antigen is bound to the surface and the antibody is the molecule being detected, for the purposes of this document the antibody becomes the ligand and the antigen is the antiligand. Alternatively, once an antiligand has bound to a ligand, the resulting antiligand/ligand complex can be considered an antiligand for the purposes of subsequent binding.

[0019] As used herein, the term "electromagnetically coupled" will generally refer to the transfer of electromagnetic energy of between two or more structures. The
term "directly coupled" will be used to describe the arrangement in which the
components at issue (e.g., the molecular event and the transmission line) come into direct
contact and transfer electromagnetic energy between them. The term "indirectly coupled"
will be used to describe the arrangement in which the components are physically
separated (e.g., through a matrix layer or barrier deposited along the transmission line,
through the material which makes up a microfluidic channel or PTFE flow tube, or
through the aqueous environment of the sample in which the molecular is located) but
remain electromagnetically coupled to each other.

[0020] As used herein, the term "test signal" refers to an ac time varying signal.
In specific embodiments, the test signal is preferably at or above 10 MHz (10x10^6 Hz),
such as 20 MHz, 45 MHz, 100 MHz, 250 MHz, 500 MHz, 750 MHz, 1 GHz (1x10^9 Hz),
2 GHz, 5 GHz, 7.5 GHz, 10 GHz, 12 GHz, 15 GHz, 18 GHz, 20 GHz, 22 GHz, 25 GHz,
28 GHz, 30 GHz, 32 GHz, 40 GHz, 44 GHz, 50 GHz, 60 GHz, 110 GHz, 200 GHz, 500
GHz, 1000 GHz and range anywhere therebetween. A preferred region is from 10 MHz
to 40 GHz, and more particularly from 45 MHz to 20 GHz.

[0021] As used herein, the term "test sample" refers to the bulk material in
which the molecular event being detected is located (or is suspected of being located).
The test sample is interrogated by test signal, and the presence or absence of the
molecular event is detected as a result of interaction of the molecular event in the sample
with the test signal. The bulk material can comprise a solid, liquid, or gas, with liquids
(and specifically water) being preferred, as most molecular events of interest occur
naturally in an aqueous environment. In most (but not all) cases, if a gas is present it will
be dissolved in a liquid, while if a solid is present, it will be particulate and serves as a
surface that transports one or more components of a binding reaction into the detection
region after the component or components have become bound or attached to the solid in
another location or that remains in the detection region after other components have been
removed. Gases can also be used as transport media (e.g., bubbles that separate liquid
phases or that move particulate materials) and can be present as the environment that
remains after removal of a liquid (e.g., by filtering or otherwise removing a liquid from a
solid phase transporting material). Solid phase sample components can comprise
naturally occurring materials including carbohydrates, proteins, oligonucleotides, SiO2,
GaAs, and Au or, alternatively, synthetic materials including organic polymers such as
Nylon®, Rayon®, Dacryan®, polypropylene, polystyrene, Teflon®, Neoprene, and Delrin.
Liquid phase sample components can include an aqueous, organic or inorganic primary
component and can exist as simple liquids or be a component of a gel or emulsion. Exemplary sample components include celluloses, dextran derivatives, aqueous solution of d-PBS, Tris and other buffer media, deionized water, DMSO, blood, cerebrospinal fluid, urine, saliva, other physiological fluids, other aqueous solutions containing water, and organic solvents such as ethanol. Pretreatment of a more general sample (by dilution, extraction, etc.) once it is obtained from a source material does not prevent the material from being referred to as a sample.

General Overview

[0022] The present invention makes use of the observation that a vast number of molecules can be distinguished based upon their unique dielectric properties. These unique dielectric properties can be observed by coupling a test signal to a test sample that includes a molecular structure or binding event of interest. When the test signal is electromagnetically coupled to the test sample, the dielectric properties of the molecule or binding event modulate the test signal and produce a unique signal response. The signal response can be recovered and stored and can be used to detect and identify the molecules in other test samples. Additionally, interactions of other molecules with the first molecule (e.g., molecular binding) can be detected, as the test signal is further modified by the interaction of the second molecule with the first.

[0023] The bioassay devices operate using high frequency test signals that are electromagnetically coupled (directly or indirectly, as defined above) to a test sample that contains (or is suspected of containing) a molecular event. In some devices, a test sample is transported to a detection region where a test signal illuminates the sample. In other cases, the detector is moved to the sample and sample manipulation (if any) occurs without movement of either the sample or the detector once they are placed in proximity so that electromagnetic coupling can take place. The dielectric properties of the molecular event within the sample (if present) operate to modulate the test signal, providing a unique signal response. The bioassay device is operable to recover the modulated test signal which is subsequently compared to the incident test signal. The comparison (i.e., of the signal response) provides information as to presence and identity of the molecular event.

[0024] Fig. 1A illustrates a top view of four coplanar waveguide bioassay devices (hereinafter “coplanar detectors”) in accordance with the present invention.
Those of skill in the art of high frequency circuit design will readily appreciate that the detectors may be easily modified to an equivalent, non-planar architecture.

[0025] As illustrated, each coplanar detector 110, 120, 130 and 140 includes longitudinally extending ground plane strips 102, one located on each side of a transmission line 104. A gap (not shown in Fig. 1A) separates the ground plane strips 102 from the transmission line 104.

[0026] The metallization of the transmission line 104 and the ground plane strips 102 (typically .1-10 um gold) is deposited on a dielectric substrate 100. Exemplary dielectric substrates include alumina, glass, polyimide, fused silica, quartz, materials used in the fabrication of semiconductor devices such as silicon dioxide and gallium arsenide, as well as other materials used in the art of high frequency circuit design. Although not shown, a backside ground plane may be formed on the bottom surface of the dielectric substrate 100. Coplanar detectors 110 and 120 are approximately one-half as long (15 mm in one embodiment) as coplanar detectors 130 and 140 (30 mm in one embodiment).

[0027] The dimension of the gaps between the ground plane strips 102 and the transmission line 104, the dielectric constant of the substrate 100, and the separation between the transmission line 104 and the backside ground plane (if one is employed) determine the characteristic impedance of the transmission line 104 as known to those skilled in the art. In a specific embodiment, the width of the transmission line is .170 mm, the separation gap between the transmission line and ground strips is .016 mm and the substrate 100 is fused silica and is 1.5 mm in height (no backside ground plane employed). These dimensions are selected to establish a 50 ohm characteristic impedance for the coplanar line, although other characteristic impedance values can be established by varying one or more of the aforementioned dimensions. Further, the reader will appreciate that the aforementioned dimensions can be scaled down and the detectors fabricated using miniature, monolithic photolithographic or semiconductor processing techniques, thereby enabling the construction of high density detector "chips."

[0028] In a specific embodiment of the invention, the transmission line 104 and ground plane strips 102 come into direct contact with the applied test sample 108. For example in a test to detect molecular binding, antiligands may be immobilized along portions of the transmission line 102 and/or ground plane strips 104 to specifically bind target ligands possibly contained within the applied test sample 108. In this embodiment, a test signal propagating along the transmission line 104 will be directly coupled to the
test sample and its constituents. The bound and unbound states of the antiligands produce different signal responses, thereby enabling binding event detection.

[0029] Alternatively, a passivation layer or other intermediate structure (which may include e.g., a matrix, linker, or other molecular layers or structures (for instance, a fluid barrier) such as those described in Applicant’s commonly owned co-pending patent application no. 09/365,558, entitled “Method and Apparatus for Detecting Molecular Binding Events”) may be applied to the transmission line 102 and/or ground plane strips 104. This intermediate layer or structure physically separates the applied test sample 108 from the transmission line 102 and/or ground plane strips 104. In this embodiment, the test signal is “indirectly coupled” to the sample, i.e. the test signal is electromagnetically coupled through the separating layers/structures to the molecular event occurring within the sample 108.

[0030] Fig. 1B illustrates a detailed top view of the sample area 145. The sample area 145 includes two ground strips 102 laterally formed on each side of the first and second transmission line sections 104a and 104b. The first transmission line section 104a includes an input port and a first terminus. The second transmission line section 104b includes a second terminus and an output port. A gap area 106 is formed between the first and second termini and is operable to contact the sample in close proximity to the first and second transmission line sections to enable signal coupling (either direct or electromagnetic) between the sample 108 and the first and/or the second transmission line sections 104a and 104b. The sample 108 may occupy an area between the first and second termini, an area on one or both of the first and second transmission line sections 104a or 104b, an area between the first and/or second transmission line sections 104 and the ground strips 102, or a combination of two or more of these areas.

[0031] Fig. 1C illustrates a top view of a fluidics fixture 150 operable to provide the sample 108 to the coplanar detectors 110, 120, 130 and 140. The fluidics fixture 150 includes two mounting screw holes 151 and four fluidic ports 153, each of which includes a bottom counter-bored portion configured to accept an o-ring 155. When assembled with the dielectric substrate 100, each fluidic port is located to provide a volume of sample 108 to one of the coplanar detectors 110, 120, 130 and 140. The fluid ports are aligned to provide sample 108 to the terminus of coplanar detectors 110 and 120, and to the mid-point of coplanar detectors 130 and 140.

[0032] Prior to measuring a molecular structure or binding event within the supplied sample, measurements may be made to determine baseline responses for the two
types of coplanar detectors 110/120 and 130/140. In one embodiment, the baseline and subsequent measurements are made using the same coplanar detector. In another embodiment, one of the two matching coplanar detectors (e.g., 110) is used to measure the baseline response and the other matching coplanar detector (e.g., 120) is used to measure the test sample. As illustrated, coplanar detectors 110 and 120 can be used to make one port return loss measurements, and coplanar detectors 130 and 140 can be used to make one port and/or two port insertion loss measurements.

[0033] A variety of baseline responses may be made. In one embodiment, a baseline response is made without supplying buffer 108 to the coplanar detector (i.e., the transmission line 104, one or both ground strips, or both). This type of response is useful in calibrating and characterizing the signal response of the coplanar detector itself. In another embodiment, a baseline response is made using a buffer 108 (e.g., d-PBS, de-ionized water, DMSO, etc.) which is supplied to and electromagnetically coupled (either directly or indirectly) to the coplanar detector. This baseline response allows the detection of new molecular event occurring within the buffer-based sample through comparing the new signal response to the baseline response. In still another embodiment, the baseline response is made by using a buffer containing a known molecular binding event at a known concentration. This type of baseline response allows for the detection of changes in the binding event when enzymes or other reagents are introduced into the sample. Those skilled in the art will appreciate that the foregoing represent only a few examples of the numerous baselines responses possible and that others may be made under alternative embodiments.

[0034] Once the baseline response(s) have been measured and stored, a volume of test sample is transported to the terminus of coplanar detector 110 and over the gap area 106 of coplanar detector 140 via fluidic ports 153. The test sample may be transported using positive pressure, negative pressure, or a combination of both. The sample may be transported using a microfluidic system, such as those described in applicant's commonly-owned, co-pending U.S. Patent Application serial no. 09/687,456 entitled “System and Method For Detecting Molecular Events in a Test Sample.”

[0035] Next, a one-port reflection (return loss) measurement is made using coplanar detector 110 and a one or two-port measurement (insertion loss) is made using coplanar detector 140. At the terminus of coplanar detector 110, the permittivity, conductance, susceptibility, as well as other dielectric properties of the sample will operate to modulate the incident signal. The open-end of coplanar detector 110 operates to reflect
at least a portion of the modulated signal back toward the test system where it can be recovered and compared with the baseline response to indicate the presence of a molecular event.

[0036] Referring again to Fig. 1B, the dimensions of the gap 106 is chosen such that a detectable portion of the incident signal is electromagnetically coupled from the first transmission line section 104a to the second transmission line section 104b when the analyte-free buffer occupies the gap area 106. Those of skill in the art of high frequency circuit design will appreciate that the gap dimension will vary depending upon a variety of factors including the amount of coupling desired and the test frequency, however most applications can be meet using a gap dimension of between 0.1 μm to 10 mm. In the illustrated embodiment, the gap 106 extends 0.016mm for coplanar detector operation near 1.0 GHz using a d-PBS buffer solution.

[0037] When the test sample is applied over the gap 106, its dielectric properties will alter the signal coupling across the gap, thereby producing a new signal response. When the baseline response is made using a sample having a known molecular structure or binding event, a new response can then be compared to the baseline response to detect a change in the signal response, thereby indicating the presence or absence of the molecular event. Alternatively, the new response can be compared to one or more previously obtained signal responses in order to determine the correlation with (and accordingly, the identity of) the unknown molecule or binding event. Alternatively or in addition to monitoring the signal response (which is typically rendered in amplitude versus frequency graphs), other signal parameters may be monitored. For example, quantities such as the sample’s change in permittivity, susceptibility, group delay, and impedance may be monitored during testing. Those of skill in the art will appreciate that other signal parameters may be observed as well.

[0038] While the coplanar detectors have been described in terms of their use with periodic sinusoidal test signals, those of skill in the art of high frequency analysis will understand that the coplanar detectors of the present invention can also be used to propagate non-period or non-sinusoidal test signals, such as pulse waveforms used in time domain reflectometry. For example, applicant’s co-pending application no. 09/365,578 describes a time domain test system compatible with the bioassay devices of the present invention.
Test signals may be launched onto the transmission line 104 using a coplanar wafer probe card such as those manufactured by Cascade Microtech Corporation of Beaverton, Oregon. Those of skill in the art will appreciate that other connections such as coaxial connectors, may be alternatively used with the invention.

Figs. 2A and 2B illustrate another embodiment of a two port bioassay device in accordance with the present invention, a grooved stripline bioassay device 200 (hereinafter "stripline detector"). Fig. 2A shows the top view of the stripline detector 200 showing the top metallized ground plane 210, and a grooved cut-out 240 for retaining a volume of sample. The ground plane 210 may be any one of a variety of conductive materials as known in the art, for instance, copper or gold.

Fig. 2B illustrates a side view of the stripline detector 200 which includes two dielectric layers 220 interposed between the top and bottom ground planes 210. The stripline detector 200 further includes a center layer 230, the top or bottom surface of which will include a transmission line 250. In an alternative embodiment, the center layer 230 is omitted and the transmission line 250 is plated on one of the sides of the top or bottom dielectric layers 220.

The top dielectric layer 220 includes a sample chamber 240 which forms a void in the ground plane 210 and extends into the top dielectric layer 220. The sample chamber contains at least a portion of the supplied sample proximate to the transmission line 250, thereby permitting electromagnetically coupling between the molecular events within the contained sample and the transmission line 250. In another embodiment, the sample chamber 240 extends through the dielectric layer 220 and exposes the transmission line 250, such that a supplied sample contacts the transmission line 250. Alternatively, the sample chamber 240 may be located within the bottom dielectric layer 220 or sample chambers 220 may be used in both the top and bottom dielectric layers 220. The top, center, and bottom layers may be formed from a variety of materials know in the art of high frequency circuit design. For instance, all three layers may consist of woven RT/duriod (typically .008" to .060" in thickness) available from the Rodgers Corporation of Rodgers, Connecticut. In a specific embodiment, the top and bottom dielectric layers 220 consist of alumina (εr ≈ 10) and each is ≈ 2000 um thick, the center dielectric layer 230 is omitted and the transmission line 250 is sputtered gold 3 um thick and approximately 300 um wide. Those skilled in the art of high frequency design will understand that other dimensions are possible. In alternative embodiments, the dielectric
layers may consist of alumina, glass, polyimide, quartz, materials used in semiconductor fabrication such as silicon dioxide and gallium arsenide, as well as other materials used in the art of high frequency circuit design. The reader will appreciate that the aforementioned dimensions can be scaled down and the detector fabricated using miniature photolithographic or semiconductor processing techniques, thereby enabling the construction of very high density detectors.

[0043] Fig. 3A illustrates a top view of a test fixture 300 operable to house the stripline detector 200 shown in Figs. 2A and 2B. The test fixture 300 includes a fixture housing 310 having a recessed area 320. Housing 310 may be constructed or machined from a variety of materials including brass, aluminum, and the like. Connectors 330, preferably microwave frequency coaxial connectors such as SMA-type, K-type, 2.4 mm and others, are used to supply and recover tests signals to and from the stripline detector 200. The illustrated test fixture 300 is but one possible example and those of skill in the art of high frequency design will appreciate that other high frequency fixtures designs are possible under the present invention.

[0044] Fig. 3B illustrates a side view of the stripline detector 200 located within the test fixture 300. The transmission line 250 is formed on the top surface of the center layer 230 and is connected between coaxial connectors 330 via center contacts 340, which, in one embodiment, are high frequency connector pins extending from the coaxial connectors to the transmission line 250 on the center layer 230. The sample chamber 240 forms a cut-out of the ground plane 210 and extends into the top dielectric layer 220. In alternative embodiments, the sample chambers may extend to and expose the transmission line 250.

[0045] Fig. 3C illustrates side and bottom views of a cap plate assembly for use with the test fixture shown in Fig. 3A. The bottom surface of the cap plate 350 includes an area 352 having two sample holes circumscribed by a grooved channel. The sample holes (the number of which may vary from the illustration) operate to direct sample into and out of the sample chamber 240. The bottom surface further includes attachment holes 344 through which screws or other attachment means extend to secure the assembly within the recessed area 320.

[0046] The cap plate 350 further includes a grooved channel 352 located on the bottom surface and sample capillaries 354 extending from the top surface. The grooved channel 352 is designed to depressingly retain the o-ring 370 around the periphery of the
sample chamber 240. In this configuration, the o-ring 370 forms a substantially hermetic chamber to contain the sample when it is introduced.

[0047] The capillaries 354 may be constructed from a variety of materials, such as those commonly used in microfluidic applications (some examples being glass, PTFE, hard plastic or polymers). In a specific embodiment, the cap plate 350 and the capillaries 354 may be formed from the same material, although different materials may be used in alternative embodiments.

[0048] Fig. 3D illustrates a side view of an assembled test fixture in accordance with one embodiment of the present invention. When assembled, sample tubes 354 supply the test sample to the sample chamber 240 which is located above the transmission line 250 of the stripline detector 200. In one embodiment, the sample chamber 240 extends to expose the transmission line 250, in which case the test sample will come into direct contact with the transmission line 250. In this embodiment, the transmission line 250 may be formed from a substantially inert metallization (e.g., gold) and include immobilized molecules, cells or other material operative to bind to a specific ligand contained or suspected of being contained within the supplied test sample. Alternatively, the transmission line may be coated with a thin passivation or other transitional binding layer(s) to seal the transmission line from the sample and/or promote ligand binding.

[0049] In another embodiment, the sample chamber terminates some distance above the transmission line 250. The separation distance between the transmission line 250 and sample chamber 240 can be controlled to provide the desired amount of coupling from the transmission line, the general relationship being that the thinner the separating dielectric layer and higher the dielectric constant of the separating material, the greater the coupling effect will be. Those of skill in the art of high frequency circuit design will appreciate that materials of various thicknesses and dielectric constants may be used, for instance, the aforementioned RT/duriod, as well as other materials known in the art.

[0050] Once the sample has been introduced into the sample chamber 240, a test signal is launched from one of the connectors 330 toward the other. Within (or below) the sample chamber 240, a portion of the test signal will electromagnetically couple from the transmission line 250 (either through direct or indirect coupling, as defined above) to the test sample contained within the sample chamber 240. The electromagnetically coupled signal is modulated by the dielectric properties (e.g., the permittivity, permeability, insertion loss, etc.) of the molecular events occurring within the test sample. A portion of the modulated signal is reflected from the sample to the transmission line in
the reverse direction where it can be detected using a return loss measurement. Alternatively or in addition to the return loss measurement, an insertion loss measurement may be made in which the remaining portion of the incident signal is recovered at the opposite connector and the recovered signal compared to the incident signal. Test signals are launched at varying frequencies to interrogate the test sample for molecular events over a broad spectrum of different frequencies.

[0051] Fig. 4A illustrates a schematic block diagram of a magic-t bioassay device 400 in accordance with the present invention (hereinafter “magic-t detector”). The magic-t detector 400 includes a magic-t coupler 410, an input/sum signal port 402, an output/delta port 404 and two load ports 406 and 408 coupled to sample loads 420 and 430, respectively.

[0052] Known to those of skill in the art of high frequency circuit design, the magic-t is a 180 degree power splitting device that allows the signal properties of two loads to be compared. In the present invention, the compared loads are reference and test sample solutions, and the specific signal properties measured are measurement parameters (s-parameters in one embodiment) which are affected by the differing dielectric properties of the molecular events occurring within the reference and test solutions. In exemplary embodiments, the reference sample may consist of a buffer solution such as PBS, a solution containing a known concentration of an unbound ligand, cellular structures, as well as other molecular events described or incorporated herein.

[0053] The sample loads (solutions in one embodiment) 420 and 430 may be supplied in a variety of ways, including microfluidic channels, embodiments of which have been described in applicant’s co-pending U.S. Patent Application serial no. 09/687,456 entitled “System and Method For Detecting Molecular Events in a Test Sample.” In this embodiment, portions of the microfluidic channels are positioned proximately to the load ports 406 and 408 such that the test sample within the channel is electromagnetically coupled (either directly or indirectly, as defined above) to the load ports. In another embodiment, the sample loads 420 and 430 are contained in respective vials, each of which are proximately located and indirectly coupled to the load ports 406 and 408. In a third embodiment, the sample loads 420 and 430 come into direct contact with the load ports 406 and 408 (or into direct contact with a sensor extending from the load ports 414 and 415). The reader will appreciate that the sample loads 420 and 430 may be presented to the load ports 406 and 408 in a number of other ways. All that is needed is that the sample loads 420 and 430 be electromagnetically coupled (directly or
indirectly) to the load ports 406 and 408. In the preferred embodiment, the degree of coupling between the sample loads 420,430 and their load ports 406, 408 is substantially the same.

[0054] Fig. 4B illustrates a (negative) mask layout of a planar magic-t coupler 410 shown in Fig. 4A in accordance with one embodiment of the present invention. The coupler 410 includes an input signal coplanar line 412, an output signal coplanar line 413, and two coplanar load lines 414 and 415. In the illustrated embodiment, the coupler 410 is constructed in a coplanar waveguide/slot-line configuration. The input, output, and load lines 412-415 are realized in the conventional ground-signal-ground coplanar waveguide architecture and connected to ports 402, 404, 406 and 408, respectively. Peripheral components (not shown) are connectable to ports 402, 404, 406,408 via high frequency connections such coaxial connections, wafer probe contacts, ribbon or wire bonding, or similar interfaces. The coplanar lines 412-415 feed the coupling ring 417 which is realized in a slot-line architecture. Coupler terminations 418 are tapered to provide improved broadband performance.

[0055] In one embodiment, the planar coupler 410 is designed to operate over a frequency of 4 GHz to 7 GHz and is fabricated on 1.5 mm thick section of alumina having a relative dielectric constant of generally 9.5. The width of the center transmission line of coplanar lines 412-415 is 0.25 mm and the gap separating the transmission line and ground plane sections is 0.24 mm. Those of skill in the art of high frequency circuit design will appreciate that other dimensions may be used in alternative designs under the present invention to cover different frequency ranges. Further, the reader will appreciate that the aforementioned dimensions can be scaled down and the coupler fabricated using miniature photolithographic or semiconductor processing techniques, thereby enabling the construction of very high density detectors.

[0056] In a specific embodiment, the measurement process using the aforementioned magic-t detector involves comparing (2) two-port measurements. In the first measurement, two reference samples are electromagnetically coupled to the load ports and a two-port measurement is made in which a test signal (launched at one frequency or over a broad spectrum of frequencies) is input to the sum port and the resulting output signal recovered at the delta port. In the second measurement, the test sample replaces one of the reference samples and the previous measurement is repeated. The two measurements are arithmetically compared (in amplitude and/or phase) and a comparison response plotted or otherwise output. A change in the amplitude (e.g., +/-
0.1, 0.3, 0.5, 1, 3, 5 or 10 dB) and/or phase (+/- 0.5, 1, 3, 5, 10, 22.5, 45, or 90 degrees) will be indicative of the presence of a molecular event in the test sample relative to the reference sample. The differences may be plotted (in amplitude and/or phase over frequency) to determine if the unknown sample displays a positive or negative correlation with the reference sample. In another embodiment, the comparison response may be monitored real-time while the test sample undergoes a change in molecular binding, analyte concentration or composition, or other molecular changes.

[0057] The reader will appreciate that the invention is not limited to the foregoing examples. For instance, in another embodiment in which the reference sample is a solution containing a binding event, a change in the amplitude or phase of the response will be indicative of the test sample containing unbound molecular structures. Other measurements are similarly possible. Further, the measurement process is not confined to the comparison of two-port measurements. One-port measurements, or alternatively three-port measurements or others may be made and compared. Other alternatives of the test sample and measurement process will be apparent to reader.

[0058] Fig. 4C illustrates a bottom view of a flow cell 440 operable to provide the sample solutions 420 and 430 to the coplanar magic-t coupler 410 illustrated in Fig. 4A. Each flow cell 440 includes a sample chamber 442 for containing the supplied solution and inlet and outlet ports 444 for transporting the solution. The sample chamber 442 is circumscribed by a gland 446 which seats a compressible O-ring. As the flow cell is attached to the top surface of the coupler 410, the O-ring becomes compressed, thereby forming a tight seal to contain the supplied solution.

[0059] In the preferred embodiment, each flow cell is positioned over the center transmission line of load lines 414 and 415, thereby permitting a signal traveling along the coplanar lines 414 and 415 to electromagnetically couple to the sample. In one embodiment, the center transmission line extends through the length of the sample chamber 442. In a second embodiment, the center transmission line terminates at the boundary of the sample chamber 442. In still another embodiment, the center transmission line terminates within the boundaries of the sample chamber 442. In the preferred embodiment, the sample comes into direct contact with the center transmission line (i.e., directly coupled as defined above). In an alternative embodiment, the sample and load lines 414 and 415 may be "indirectly coupled" (as defined above) through a barrier, such as a passivation layer, linker, or other molecular layer.
[0060] In a specific embodiment, the flow cell 440 is constructed from polycarbonate material and the sample chamber 442 holds between 2-500 μl of fluid. The input and output ports include needles operable to engage 0.020" ID PTFE tubing for transporting the sample to/from the sample chamber 442. Flow cell attachment to the coupler’s top surface and O-ring compression is achieved using two no. 4-40 screws.

[0061] In a second embodiment, the magic-t detector may be realized as a waveguide structure. In such an embodiment, each of the signal lines 412-415 will consist of waveguide sections, the dimensions of which are dictated by the desired frequency of operation as known to those of skill in the art of high frequency circuit design. The magic-t coupler may be any of a variety of commercially available waveguide magic-t couplers, such as model no. 8242 available from Penn Engineering (North Hollywood, CA., www.pennengineering.com).

[0062] Fig. 4D illustrates a side view of a waveguide magic-t detector 450 in accordance with the present invention. The illustrated view shows the load ports 406 and 408, each having a section of tubing 452 (PTFE in one embodiment) positioned across the waveguide aperture 454 of the feeding signal line 414 and 415, respectively. The tube 452 is operable to transport the sample solution to the waveguide aperture 454 where the test signal will be electromagnetically coupled to it. The tube 454 may be serpentinized across the aperture 454 to provide a higher sample volume across the aperture 454 in order to increase the measurement response. The input and output ports 402 and 404 are standard input and output waveguide transitions in one embodiment, but in others may include probes or similar transitions for connecting to coaxial connectors or similar interfaces.

[0063] Fig. 4E illustrates a top view of an encapsulated flow cell 460 for use with the waveguide magic-t detector shown in Fig. 4C. This flow cell may be used as an alternative to the tube configuration shown in Fig. 4D.

[0064] The flow cell 460 is sized to fit into the waveguide aperture 454 located at the load ports 406 and 408 and constructed from acrylic ([poly]methylmethacrylate) in one embodiment. The flow cell 460 includes a sample chamber 462 (holding 25 μl in one embodiment) and inlet/outlet needles 464 which are UV epoxied to the ends of the chamber 462. Preferably, the diameter of needles 464 is chosen to insert securely within a section of tubing (0.020" ID PTFE tube in one embodiment) which supplies the sample.
Those of skill in the art of fluidic design will appreciate that the
foregoing designs shown in Figs. 4C and 4E are only exemplary and that other flow cell
designs are possible in the present invention. The flow cell may be constructed from
materials other than acrylic. Preferably, materials (such as those listed herein) having a
high dielectric constant (> 3.0) and/or low loss tangent (< .001) are used. Further, the
flow cell may be formed to have minimal thickness over the detection region to permit
the greatest degree of electromagnetically coupling to the sample. The flow cell thickness
may range from 0.1 μm, e.g., when the flow cell is formed using photolithographic
process is used, to 500 μm, e.g., when a machined flow cell is used.

Fig. 5A illustrates a front perspective view of a microstrip bioassay
device 500 ("microstrip detector" hereinafter) in accordance with the present invention
(the rear portion is the mirror image of the front view). The microstrip detector 500
includes top and bottom dielectric plates 510 and 520 and a flow tube 530 interposed
therebetween.

Top and bottom dielectric plates 510 and 520 are preferably constructed
from a material exhibiting a low loss tangent at the desired frequency of operation.
Suitable materials include alumina, glass, quartz, sapphire, beryllium, diamond, PTFE or
variations thereof, materials used in semiconductor processing such as silicon dioxide and
gallium arsenide, woven dielectric materials such as Rodgers Duriod®, polystyrene,
polypropylene, polyethylene, or other similar materials. In the illustrated embodiment,
the dielectric plates 510 and 520 are each .030” thick of GML 1000 (available from Gil
Technologies of Collierville, TN) having a relative dielectric constant of approximately
3.2. While the dielectric plates 510 and 520 are of the same thickness and relative
dielectric constant, variation in one or both of these may be used in alternative
embodiments.

The top dielectric plate 510 includes a transmission line 512 deposited
on the top surface and a channel 514 formed on the bottom surface. The width of
transmission line 512 is chosen to provide a predetermined characteristic impedance
along the detection length 540 (further described below). The impedance calculation may
take into account the varying dielectric constants and dimensions introduced by channels
514 and 524 and flow tube 530. Alternatively, these features may be ignored and one
continuous dielectric plate assumed. The transmission line 512 may consist of any
material which exhibits high conductivity of the desired test frequency(ies). Such
materials include gold, copper, silver, indium tin oxide, or other similar metals. In the
illustrated embodiment, the transmission line consists of 1 ounce copper.

[0069] The second dielectric plate 520 includes a channel 524 formed on the top
surface and metallization deposited on the bottom surface. The channel 524 is aligned
with channel 514 to form a cavity within which the flow tube 530 extends. The
metallization 522 deposited on the bottom surface functions as the ground plane of the
microstrip detector and will typically consist of a highly conductive material such as
those described above. In an alternative embodiment, ground plane metallization may be
deposited on the top surface of the top dielectric plate 510 to form a coplanar waveguide
structure. Those of skill in the art of high frequency circuit design will appreciate that
other configurations are also possible. In the illustrated embodiment, .002” of copper is
used as the bottom surface metallization to provide the detector’s ground plane.

[0070] As shown, channels 514 and 524 are aligned to form a cavity which
retains the flow tube 530 in a substantially vertically aligned position between the
transmission line 512 and the ground plane 522. The flow tube is held between the
transmission line 512 and the ground plane 522 along the detection length 540. This
configuration results in the passage of a significant number of field lines emanating from
the transmission line through the flow tube (and accordingly, the test sample) before
terminating on the ground plane 522. As discussed previously in this and the related
applications, the dielectric properties of the molecular events within the sample will
modulate the signal propagating along the transmission line 512 (i.e., by altering the field
lines setup between the transmission line 512 and ground plane 522), thereby providing a
means to detect and identify the molecular events occurring in the test sample.

[0071] Fig. 5B illustrates a cross-sectional view of the microstrip detector along
the detection length 540. As illustrated, the flow tube 530 is generally centered between
the transmission line 512 and ground plane 522 to intersect field lines emanating from the
center of transmission line 512 and terminating on the ground plane 522. In an alternative
embodiment, two flow tubes are located near the longitudinal edges of the transmission
line 512 between the transmission line 512 and ground plane 522. In this configuration,
fringing field lines emanating from the longitudinal edges of the transmission line 512
intersect the flow tubes (and consequently, the sample solution flowing therethrough).
Other flow tube configurations and placement in which field lines emanating from the
transmission line intersect the flow tube will be apparent to those skilled in the art of
microwave engineering.
[0072] In one embodiment, the microstrip detector includes connectors (not shown) connected to the transmission line 512 and ground plane 522 on the near and far sides. Suitable connectors are selected based upon the desired test frequency, N-type connectors being suitable for low frequency tests (< 100 MHz), and SMA, K-type, 3.5 mm or 2.4 mm connectors being more suitable for higher frequency tests. Connection by other means, such as coplanar waveguide probes, may also be used in alternative embodiments.

[0073] In a specific embodiment, the microstrip detector is used as a one-port device with the far side connector terminated in an load closely matched to the characteristic impedance of the transmission line 512 in order to minimize reflections created by the termination. In this embodiment, portions of the modulated signal will be reflected back toward the signal source (transmitted to the near side connector, not shown) and is detectable through a directional coupler. Those of skill in the art will understand that other arrangements are possible, for instance, using a highly reflective termination, or using the microstrip detector as a two port to determine the insertion loss response.

[0074] The flow tube 530 supplies the test sample through the detection region 540 between the transmission line 512 and ground plane 522. In the preferred embodiment, the flow tube 530 is constructed from a material having a low loss tangent and a smooth, resilient surface morphology which inhibits analyte formation along the inner surface. A PTFE tube having an ID of .015” and OD of .030” is used in the illustrated embodiment, although other materials and/or sizes may be used as well. For example, materials such as ETFE or other materials described in this and the related cases may be used in alternative embodiments. Further, the flow tube 530 may consist of a microfluidic capillary such as those discussed in applicant’s co-pending U.S. Patent Application serial no. 09/687,456 entitled “System and Method For Detecting Molecular Events in a Test Sample.”

[0075] The reader will appreciate that numerous variations may be implemented in alternative embodiments under the invention. For instance, additional dielectric plates may be used between the dielectric plate, or alternatively one dielectric plate having a transmission line and ground plane metallization is deposited on opposite sides and in which a cavity is bored to permit passage of the test sample therethrough. In another embodiment, the flow tube 530 may be omitted and the test sample supplied through the detection region via negative or positive (or both) pressure. Further, either of the
channels 514 or 524 may be omitted when the remaining channel is large enough to accommodate the flow tube or the test sample if the flow tube 530 is omitted. The reader will appreciate that other modifications are possible as well under the present invention.

While the above is a complete description of possible embodiments of the invention, various alternatives, modifications, and equivalents can be used. For example, other TEM transmission media, such as coaxial and suspended substrate, and non-TEM transmission media, such as conductive or dielectric waveguides, can alternatively be used.

Applicant’s commonly-owned U.S. patent application entitled “System and Method for Detecting and Identifying Molecular Events in a Test Sample using a Resonant Test Structure,” (Atty. Dkt. No. 16.0 US) is concurrently filed herewith. Further, the following commonly owned, co-pending patent applications, as well as all publications and patent documents recited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication and patent document was so individually denoted:

Serial No. 09/243,194, entitled: “Method and Apparatus for Detecting Molecular Binding Events,” filed February 1, 1999 (Atty Docket No 19501-000200),

Serial No. 09/365,578, entitled “Method and Apparatus for Detecting Molecular Binding Events,” filed August 2, 1999 (Atty Docket No. 19501-000210);

Serial No. 09/365,978, entitled: “Test Systems and Sensors For Detecting Molecular Binding Events,” filed August 2, 1999 (Atty Docket No. 19501-000500); and

WHAT IS CLAIMED IS:

1. A coplanar waveguide bioassay device operable to detect the presence or absence of a molecular event within a test sample, the coplanar waveguide bioassay device comprising:
   a dielectric substrate;
   a first transmission line section formed on the dielectric substrate and extending longitudinally between an input port and a first terminus;
   a second transmission line section formed on the dielectric substrate and extending longitudinally between a second terminus and an output port, wherein the first and second termini are spaced apart a predefined distance to form a center gap area; and two ground strips, each formed on the dielectric substrate and located laterally on each side of the first and second transmission lines sections, the two ground strips spaced apart from the first and second transmission line sections a predefined distance,
   wherein the center gap area is operable to contact the sample, and wherein the molecular event, if contained within the test sample, is electromagnetically coupled to the first and second transmission line sections.

2. The coplanar waveguide bioassay device of claim 1, wherein the molecular structure or binding event is in contact with, and directly coupled to, at least one of the first or second transmission line sections.

3. The coplanar waveguide bioassay device of claim 1, wherein the molecular structure or binding event is in contact with, and directly coupled to, at least one of the ground strips.

4. The coplanar waveguide bioassay device of claim 2, wherein an antiligand operable to bind the molecular structure is immobilized directly on the at least one of the first or second transmission line sections.

5. The coplanar waveguide bioassay device of claim 1, wherein the molecular structure or binding event is physically separated from, and indirectly coupled to, at least one of the first and second transmission line sections.
6. The coplanar waveguide bioassay device of claim 5, wherein a hermetic barrier is formed between the molecular structure or binding event and the at least one of the first and second transmission line sections.

7. The coplanar waveguide bioassay device of claim 1, wherein the first and second transmission lines are operable to support the propagation of a test signal between 10 MHz and 1,000 GHz.

8. The coplanar waveguide bioassay device of claim 1, wherein the first and second transmission lines are operable to support the propagation of a test signal between 45 MHz and 20 GHz.

9. A stripline bioassay device operable to detect the presence or absence of a molecular event within a test sample, the stripline bioassay device comprising:
   a first dielectric layer having a first major surface comprising a ground plane and a second major surface;
   a second dielectric layer having a first major surface comprising a ground plane and a second major surface;
   a transmission line interposed between the second surfaces of the first and second dielectric layers;
   wherein the first dielectric layer includes a sample chamber formed by a void extending through the ground plane of the first dielectric layer, the sample chamber operable to contain at least a portion of the supplied sample in proximity to the transmission line to enable electromagnetic coupling between the molecular event, if contained with the test sample, and the transmission line.

10. The stripline bioassay device of claim 9, wherein the sample chamber extends through the first dielectric layer to encompass the transmission line such that the contained sample is in contact with the transmission line.

11. The stripline bioassay device of claim 9, wherein the sample chamber does not encompass the transmission line, wherein the contained sample is physically separated from, and indirectly coupled to the transmission line.
12. The stripline bioassay device of claim 9, further comprising:
   a depressible o-ring located around the outer periphery of the sample
   chamber; and
   a cap plate depressably mounted over the o-ring, the cap plate comprising
   at least one fluid channel for supplying the sample to the sample chamber.

13. The stripline bioassay device of claim 9, wherein the transmission
   line is operable to support a test signal between 10 MHz and 1000 GHz.

14. The stripline bioassay device of claim 9, wherein the transmission
   line is operable to support a test signal between 45 MHz and 20 GHz

15. A magic-t bioassay device operable to detect the presence or
    absence of a molecular binding event within a test sample, the magic-t bioassay device
    comprising:
    a magic-t coupler having a sum/input port, a delta/output port, a first load
    port, and a second load port;
    a first load electromagnetically coupled to the first load port of the magic-t
    coupler, the first load located proximate to the first load port to enable electromagnetic
    coupled between a known molecular event contained within a reference sample and the
    first load port; and
    a second load electromagnetically coupled to the second load port of the
    magic-t coupler, the second load located proximate to the second load port to enable
    electromagnetic coupled between an unknown molecular event contained within the test
    sample and the second load port.

16. The magic-t bioassay device of claim 15, wherein magic-t coupler
    comprises a planar device.

17. The magic-t bioassay device of claim 15, wherein magic-t coupler
    comprises a waveguide device.

18. The magic-t bioassay device of claim 15, wherein the magic-t
    coupler is operable between 10 MHz and 1000 GHz.
19. The magic-t bioassay device of claim 15, wherein the magic-t coupler is operable between 45 MHZ and 20 GHz.

20. The magic-t bioassay device of claim 15, wherein the magic-t coupler is operable between 4 GHz and 7 GHz.

21. The magic-t bioassay device of claim 15, wherein the magic-t coupler comprises a coplanar waveguide/slotline magic-t coupler device.

22. A microstrip bioassay device operable to detect the presence or absence of a molecular event in a test sample, the microstrip bioassay device comprising:

   a transmission line;
   a ground plane;
   dielectric material positioned between the transmission line and the ground plane; and
   a flow tube disposed within the dielectric material and generally centered between the transmission line and the ground plane, the flow tube operable to permit the passage of the test sample therethrough.

23. The microstrip bioassay device of claim 22, wherein the dielectric material comprises a first dielectric plate, and wherein the transmission line is deposited on the top surface of the first dielectric plate.

24. The microstrip bioassay device of claim 23, wherein the dielectric material further comprises a second dielectric plate and wherein the ground plane metallization is deposited on the bottom surface of the second dielectric plate.

25. The microstrip bioassay device of claim 24, wherein the first dielectric plate comprises a channel formed on the bottom surface.

26. The microstrip bioassay device of claim 24, wherein the second dielectric plate comprises a channel formed on the top surface.
4X O-RING GLAND

FIG. 1C