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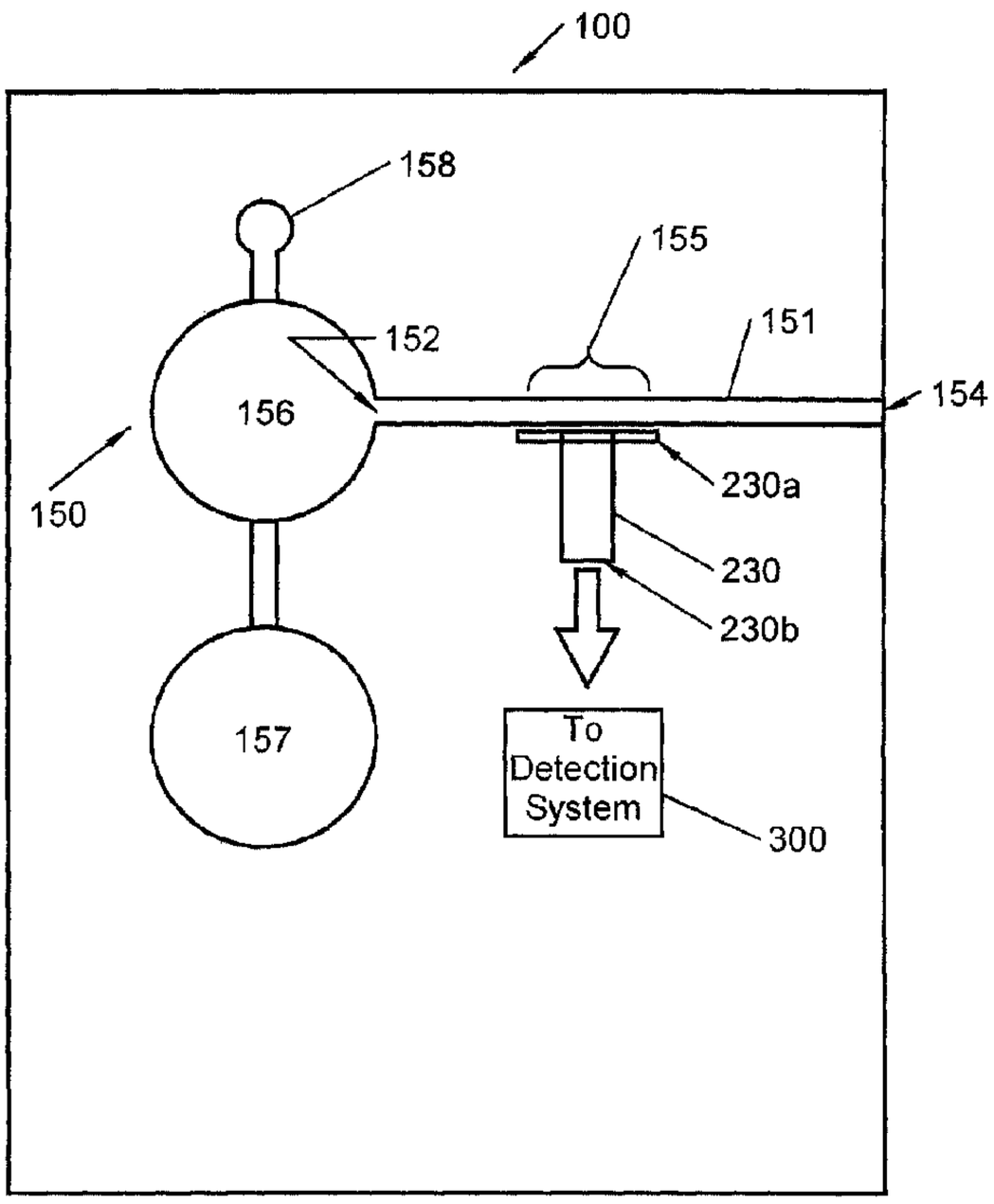
(54) Titre : SYSTEME ET PROCEDE PERMETTANT DE DETECTER ET D'IDENTIFIER DES EVENEMENTS MOLECULAIRES DANS UN ECHANTILLON D'ESSAI

(54) Title: SYSTEM AND METHOD FOR DETECTING AND IDENTIFYING MOLECULAR EVENTS IN A TEST SAMPLE




(57) Abrégé/Abstract:
A molecular detection system for detecting the presence or absence of a molecular event within a test sample includes a fluid reservoir, a signal source, a measurement probe, and a signal detector. The measurement probe includes a probe head and a connecting end. The probe head is configured to electromagnetically couple an incident test signal to the test sample within the detection region of the fluid reservoir. The interaction of the incident test signal with the test sample produces a modulated test signal, at least a portion of which the probe head is configured to recover. The system further includes a signal detector that is coupled to the connecting end of the measurement probe and configured to recover the modulated test signal.

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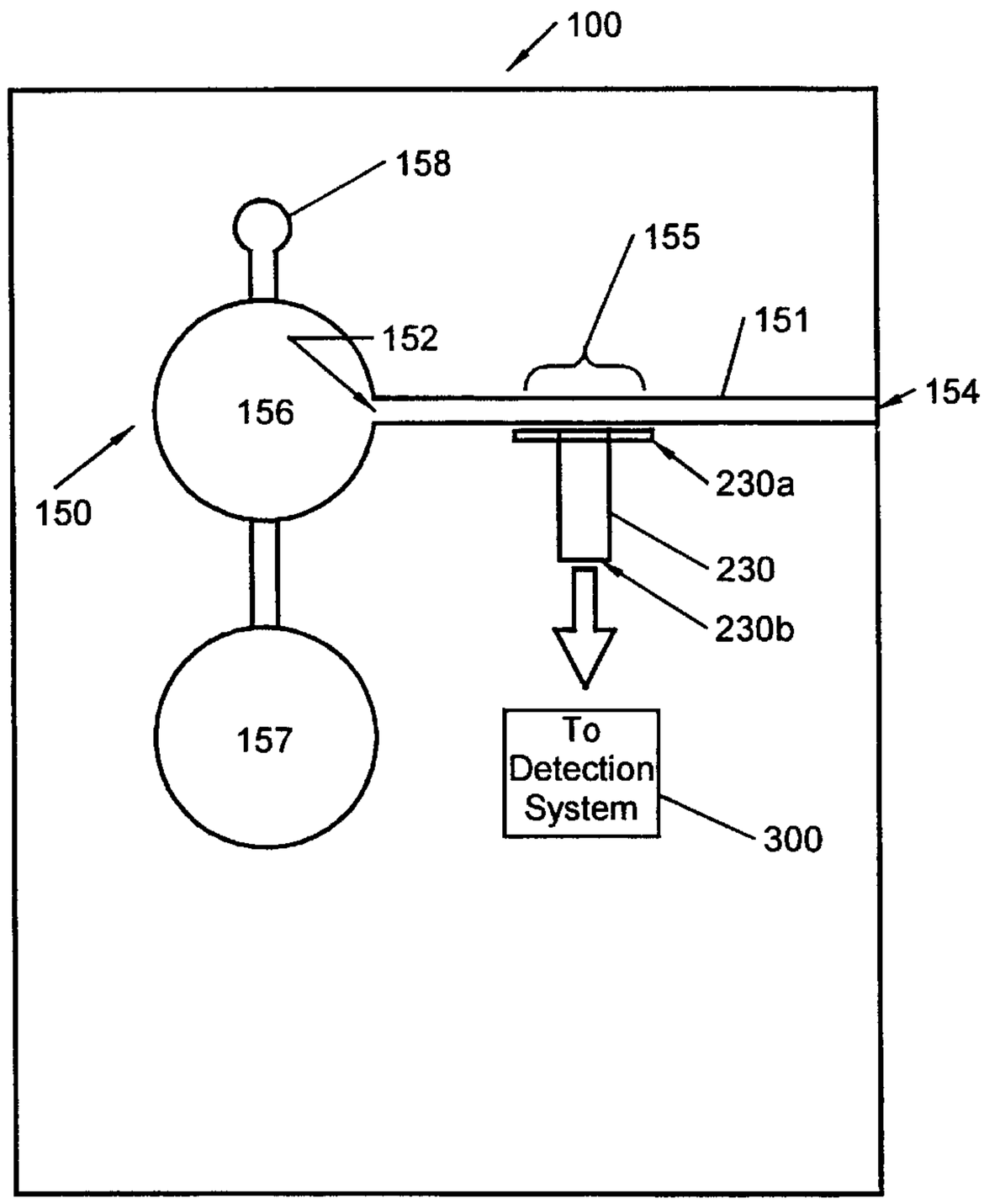
(54) Title: SYSTEM AND METHOD FOR DETECTING AND IDENTIFYING MOLECULAR EVENTS IN A TEST SAMPLE




(57) Abstract: A molecular detection system for detecting the presence or absence of a molecular event within a test sample includes a fluid reservoir, a signal source, a measurement probe, and a signal detector. The measurement probe includes a probe head and a connecting end. The probe head is configured to electromagnetically couple an incident test signal to the test sample within the detection region of the fluid reservoir. The interaction of the incident test signal with the test sample produces a modulated test signal, at least a portion of which the probe head is configured to recover. The system further includes a signal detector that is coupled to the connecting end of the measurement probe and configured to recover the modulated test signal.

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# PATENT APPLICATION

# SYSTEM AND METHOD FOR DETECTING AND IDENTIFYING MOLECULAR EVENTS IN A TEST SAMPLE

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## CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Applications 60/159,175, filed October 13, 1999, entitled "The Use of a Dielectric Interface for the Detection of Molecular Interactions," and 60/191,702, filed March 23, 2000, entitled "Method and Apparatus for Detecting Molecular Entities in a Sample."

## BACKGROUND OF THE INVENTION

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. Such molecular interactions, as well as the detection of functional and physical properties of molecules, are referred to here as molecular events. The need to detect molecular 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 *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 know 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 (*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.

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-Immunoassays (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 analytes, for instance, the extent, function and type of 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 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.

One recent trend in biological and biochemical research has been the miniaturization of assays, often resulting in an assay carried out on a "chip," a device of the size used in the electronics industry (from which the name is derived). Among the so-called chip devices are those that carry out their analysis in arrays of multiple, simultaneous analyses on a chip (which is often referred to as array technology) and those that carry out their analysis by transport of fluids on a chip past an analysis site (often referred to as microfluidics technology). Many companies have recently come into existence using these technologies, exemplified by Affymetrix , Inc., of Santa Clara, California; Incyte Pharmaceuticals, Inc., of Palo Alto, California; and Human Genome Sciences of Rockville, Maryland, in the array field and Caliper of Mountain View, California, and Aclara BioSciences, Inc., of Mountain View, California, in the microfluidics field. Many patents have issued in both of these fields, including USPN 6,033,546 entitled "Apparatus and method for performing microfluidic manipulations for chemical analysis and synthesis," USPN 5,126,022 entitled "Method and device for moving molecules by the application of a plurality of electrical fields," USPN 6,004,755 entitled "Quantitative microarray hybridization assay," USPN 5,874,219 entitled "Methods for concurrently processing multiple biological chip assays," and USPN 5,593,839 entitled "Computer-aided engineering system for design of sequence arrays and lithographic masks." A number of different array configurations and methods for their production are known to those of skill in the art and disclosed in U.S. Pat. Numbers 5,445,934; 5,532,128; 5,556,752; 5,242,974; 5,384,261; 5,405,783; 5,412,087; 5,424,186; 5,429,807; 5,436,327; 5,472,672; 5,527,681; 5,529,756; 5,545,531; 5,554,501; 5,561,071; 5,571,639; 5,593,839; 5,599,695; 5,624,711; 5,658,734; and 5,700,637; the disclosures of which are herein incorporated by reference. In the microfluidics field, exemplary U.S.

patents (with titles) include 6,012,902, Micropump; 6,011,252, Method and apparatus for detecting low light levels; 6,001,231, Methods and systems for monitoring and controlling fluid flow rates in microfluidic systems; 5,989,402, Controller/detector interfaces for microfluidic systems; 5,976,336, Microfluidic devices incorporating improved channel geometries; 5,972,187, Electropipettor and compensation means for electrophoretic bias; 5,965,410, Electrical current for controlling fluid parameters in microchannels; 5,965,001, Variable control of electroosmotic and/or electrophoretic forces within a fluid-containing structure via electrical forces; 5,964,995, Methods and systems for enhanced fluid transport; 5,959,291, Method and apparatus for measuring low power signals; 5,958,694, Apparatus and methods for sequencing nucleic acids in microfluidic systems; 5,958,203, Electropipettor and compensation means for electrophoretic bias; 5,957,579, Microfluidic systems incorporating varied channel dimensions; 5,955,028, Analytical system and method; 5,948,227 Methods and systems for performing electrophoretic molecular separations; 5,942,443 High throughput screening assay systems in microscale fluidic devices; 5,885,470, Controlled fluid transport in microfabricated polymeric substrates; 5,882,465, Method of manufacturing microfluidic devices; 5,880,071, Electropipettor and compensation means for electrophoretic bias; 5,876,675, Microfluidic devices and systems; 5,869,004, Methods and apparatus for in situ concentration and/or dilution of materials in microfluidic systems; 5,852,495, Fourier detection of species migrating in a microchannel; 5,842,787; Microfluidic systems incorporating varied channel dimensions; 5,800,690, Variable control of electroosmotic and/or electrophoretic forces within a fluid-containing structure via electrical forces; 5,779,868, Electropipettor and compensation means for electrophoretic bias; and 5,699,157, Fourier detection of species migrating in a microchannel, all of which are herein incorporated by reference. Many other publications, including other patents, that are indicative of the existing skill of the art in these fields, are listed in the patents named here, either in their specifications or in their lists of cited references.

Assay systems that use individual containers (*e.g.*, test tubes) or arrays of individual wells (*e.g.*, microtitre plates and other types of multiwell plates) to hold samples for individual analysis are also well developed. Microtitre plates having 96 well are commonly available, as are plates with other numbers of wells, such as 384-well plates. Numerous automated apparatuses have been developed to manipulate and analyze the contents of individual containers, whether as individual containers, single multiwell

plates, or multiple plates being handled at the same time. Other multiple-well systems (in addition to the common microtitre plate systems) exist. Examples of U.S. patents describing systems designed for handling and conducting assays in various types of contains for individual samples include 6,033,911, Automated assaying device; 6,024,920; Microplate scanning read head; 5,993,746, Plate holder; 5,988,236, Multiple syringe pump assembly for liquid handler; 5,985,214, Systems and methods for rapidly identifying useful chemicals in liquid samples; 5,976,470, Sample wash station assembly; 5,972,295, Automatic analyzing apparatus; 5,968,731, Apparatus for automated testing of biological specimens; and 5,952,240, Discrete matrix plate positioner. Many other publications, including other patents, that are indicative of the existing skill of the art in these fields, are listed in the patents named here, either in their specifications or in their lists of cited references.

Among the approaches used historically for studying biochemical systems have been various types of dielectric measurements. In the 1950's, experiments were conducted to measure the dielectric properties of biological tissues using techniques for the measurement of dielectric properties of materials known at the time. Since then various approaches to carrying out these measurements have included frequency domain measurements, and time domain techniques such as Time Domain Dielectric Spectroscopy. In these approaches, the experiments were commonly carried out using various types of coaxial transmission lines or other transmission lines and structures of typical use in dielectric characterization of materials. This included studies to look at the use and relevance of the dielectric properties of a broad range of biological systems. The interest has ranged from whole tissue samples taken from various organs of mammalian species, to cellular and sub-cellular systems including cell membrane and organelle effects. Most recently, there have been attempts to miniaturize the above-mentioned techniques (see *e.g.,* U.S. Patent Nos. 5,653,939; 5,627,322 and 5,846,708) for improved detection of changes in the dielectric properties of molecular systems in an array environment. Typically these use the biological sample—be it tissues, cellular systems, or molecular systems—as a shunt or series element in the electrical circuit topology.

Much art exists describing larger-scale field probes used for sensing radiation from an object and for measuring local material properties, as exemplified in Misra et al., "Noninvasive electrical characterization of materials at microwave frequencies using an open-ended coaxial line: test of an improved calibration technique," IEEE Transactions on Microwave Theory and Techniques, 38 8-14 (1990); Chevalier et

al., "High temperature complex permittivity measurements of composite materials using an open-ended waveguide," Journal of Electromagnetic Waves and Applications, 6 1259-75 (1992); Osofsky and Schwarz, "Design and performance of a non-contacting probe for measurements on high-frequency planar circuits," IEEE Transactions on Microwave Theory and Techniques, 40 1701-8 (1992); Xu, Ghannouchi et al., "Theoretical and experimental study of measurement of microwave permittivity using open ended elliptical coaxial probes," IEEE Transactions on Microwave Theory and Techniques, 40 143-50 (1992); Jiang, Wong et al., "Open-ended coaxial-line technique for the measurement of the microwave dielectric constant for low-loss solids and liquids," Review of Scientific Instruments, 64 1614-21 (1993); Jiang, Wong et al., and "Measurement of the microwave dielectric constant for low-loss samples with finite thickness using open-ended coaxial-line probes," Review of Scientific Instruments, 64 1622-6 (1993). There are also publications describing electron microscopes that use radio-frequency or microwave signals in addition to the electron current at the microscope tip, as exemplified by Keilmann, van der Weide et al., "Extreme sub-wavelength resolution with a scanning radio-frequency transmission microscope," Optics Communications, 129 15-18 (1996); Vlahacos, Black et al., "Near-field scanning microwave microscope with 100 .mu.m resolution," Applied Physics Letters, 69 3272-4 (1996); and Wei, Xiang et al., "Scanning tip microwave near-field microscope," Applied Physics Letters, 68 2506-8 (1996). Accordingly, the use of probes (both waveguides and coaxial probes) to test the properties of a dielectric material is well-known and commonly practiced in the art in other fields of technology, such as image collection in microscopy and dielectric measurements of insulators and other materials in the electronics industry.

One type of relatively large-scale instrument that has been used to analyze and control chemical processes, such as fermentations, appears to have arisen originally in the oil processing and drilling industry. For example, U.S. Patent 5,025,222 describes a system and method for monitoring conditions in a fluid medium. A stream of the fluid medium is flowed through a fluid container which is electrically configured as a transmission line segment and which is electrically connected to load to a UHF or microwave oscillator. The oscillator is not isolated from the load, and is operated free-running, at a starting frequency which is chosen to provide a particularly strong shift in permittivity of the fluid medium, as the chemical reaction progresses. Preferably the frequency and insertion loss of the oscillator are monitored, to gauge the progress of the reaction. Two later patents in this series are U.S. Patents 5,748,002 and 5,966,017, which

describes systems, methods, and probe devices for electronic monitoring and characterization using single-ended coupling of a load-pulled oscillator to a system under test. However, none of these patents appears to have looked specifically for molecular binding or for characterization of molecules, although detection of chemical species produced by fermentation (and by other processes) is an object of these patents.

Accordingly, there exists a need for further development of methods of detecting molecular events that do not require labels such as fluorophores or radioisotopes, that are quantitative and qualitative, that are specific to the molecule of interest, that are highly sensitive, and that are relatively simple to implement. The present invention fulfills many of the needs discussed above and others as well, as described herein.

## SUMMARY OF THE INVENTION

Earlier applications in the laboratories of the present inventors have dealt with an improvement in measurement techniques for detecting changes in the dielectric properties of molecular structures using a molecular binding region coupled along a signal path. These applications describe many of the general principles used in the present invention. See, for example, PCT WO99/39190, published 5 August 1999, and co-assigned with the present invention. The present invention differs in being generally directed detection of molecular events in fluid reservoirs, such as those present in microtitre plates, or enclosed fluid channels, such as those present in microfluidics devices. In these cases the samples are typically separated from the electromagnetic probe by an air gap and/or a dielectric material, such as would be present in the wall of a fluid container, in order to avoid contamination of the probe, as described below.

In general, the present invention provides a single port measurement system and methods for detecting and characterizing a molecular structure, either as itself or as a participant in a molecular binding event, particularly in an aqueous phase, using dielectric properties of the molecular structure and its interaction with an illuminating signal. The systems and methods of the present invention also provides for identification of the molecular structures and/or their binding with other molecules in a continuous fluid stream, which allows automation of the process. This permits application of the method to various screening processes, such as the identification of molecules of biological and pharmaceutical interest because of the binding of molecules to each other in an aqueous environment under physiological conditions. The method and apparatus allow such

detection to take place without the presence of a label on either of the binding partners, making the method particularly suitable to the detection of desirable pharmaceutical candidates, as there is no need to account for alteration of binding by the label.

In a first embodiment of the invention, a method for detecting a molecular structure in an aqueous sample is presented. The presented method includes the processes of (a) introducing a first sample into a fluid channel of a fluid transport system, the fluid transport system having a fluid movement controller and the fluid channel having a sample entry end, a detection region, and a sample exit end; (b) causing the sample to move through the channel from the sample entry end toward the sample exit end under the control of the fluid controller; (c) applying a test signal between 10 MHz and 1000 GHz to the detection region of the fluid channel; and (d) detecting a change in the test signal as a result of interaction of the test signal with the sample. Non-limiting examples of fluid movement control include mechanical pumps and movement of liquids under the control of electric fields, such as electrophoretic pumping, as well as surface effects (capillarity) and gravity and inertial effects (*e.g.*, through tilting or spinning operations). The frequency (or, in some case, a set of multiple frequencies or a continuum, *e.g.*, spectrum) is selected so that a sample that contains the molecular structure produces a detected electromagnetic field different from a detected electromagnetic field that is produced when the molecular structure is not present.

In another embodiment, the method just described further comprises (e) introducing a spacer material into the channel after the first sample; (f) introducing a further sample into the channel after the spacer material; (g) causing the further sample to move through the channel under the control of the fluid controller, whereby a plurality of different samples separated by spacer material is transported through the channel without intermixing different samples; and (f) repeating steps (c)-(d) for the further sample. In this manner, a series of sample can be presented to a single detector, therefore greatly simplifying the design of the detection system, which leads to greatly improved reproducibility, as well as easier design of a disposable analysis chip.

The methods described above can be carried out in a variety of apparatuses. In one embodiment of the invention, a molecular detection system for detecting the presence or absence of a molecular event within a test sample includes a fluid reservoir, a signal source, a measurement probe, and a signal detector. The fluid reservoir can be a discrete container (open or closed) or a channel that includes a test sample entry end, a detection region, and an exit end. The signal source is operable to

transmit an incident test signal. The measurement probe is coupled to the signal source and includes a probe head and a connecting end. The probe head is configured to electromagnetically couple the incident test signal to the test sample within detection region. The interaction of the incident test signal with the test sample produces a modulated test signal, at least a portion of which the probe head is configured to recover. The system further includes a signal detector that is coupled to the connecting end of the measurement probe, the signal source configured to recover the modulated test signal.

Accordingly, the present invention can be described generally as involving the interaction of electromagnetic signals, typically between about 1 MHz and 1000 GHz, with a molecular event in a fluid reservoir to determine properties of the molecular structure, such as structural and functional properties of the molecule itself and ability of the molecule to bind other molecules.

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

Fig. 1A illustrates an integrated detector assembly in accordance with one embodiment of the present invention.

Fig. 1B illustrates a fluidic transport system in accordance with one embodiment of the present invention.

Figure 2A illustrates a measurement probe in accordance with one embodiment of the present invention.

Fig. 2B illustrates a coaxial probe top in accordance with one embodiment of the present invention.

Fig. 2C illustrates a second embodiment of the measurement probe in cross-sectional view in accordance with the present invention.

Fig. 2D illustrates a top view of the measurement probe shown in Fig. 2C.

Fig. 2E illustrates a non-resonant coaxial measurement probe in accordance with one embodiment of the present invention.

Fig. 3A illustrates a molecular detection system in accordance with one embodiment of the present invention.

Fig. 3B illustrates a method for detecting an analyte in accordance with one embodiment of the present invention.

Fig. 3C illustrates an exemplary analyte signal response in accordance with one embodiment of the present invention.

Fig. 3D illustrates a method for detecting molecular binding events occurring within a test sample in accordance with the present invention.

Fig. 3E illustrates embodiments of a baseline buffer response, a first test sample response, a second test sample response, and two possible embodiments of a third sample signal response corresponding to bound and unbound conditions.

Fig. 3F illustrates a second method for detecting molecular binding events occurring within a test sample in accordance with the present invention.

Fig. 3G illustrates embodiments of first, second, and third sample response indicating the absence of binding events using the method of Fig. 3F in accordance with the present invention.

Fig. 4A illustrates a second embodiment of the molecular detection system in accordance with the present invention.

Fig. 4B illustrates a third embodiment of the molecular detection system in accordance with the present invention.

Fig. 5A illustrates one embodiment of a computer system operable to execute a software program designed to perform each of the described methods in accordance with the present invention.

Fig. 5B illustrates the internal architecture of the computer system shown in Fig. 5A in accordance with one embodiment of the present invention.

Fig. 6A illustrates a molecular detection system implemented in specific experiments in accordance with the present invention.

Figs. 6B-6G illustrate $S_{11}$ signal responses made in detecting specific analytes in accordance with the present invention.

Figs. 6H illustrates an $S_{11}$ signal responses made in detecting differing levels of concentration of NaCl in accordance with the present invention.

Figs. 6I-6J illustrate $S_{11}$ signal responses made in detecting specific molecular binding events using the method of Fig. 3D in accordance with the present invention.

Figs. 6K-6L illustrate $S_{11}$ signal responses made in detecting specific molecular binding events using the method of Fig. 3F in accordance with the present invention.

Fig. 6M illustrates a dose response curve made in accordance with the present invention.

## DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Table of Contents

I Definition of Terms

II. General Overview

III. Detector Assembly

IV. Exemplary Molecular Detection Systems

V. Exemplary Applications

VI. Software Implementation

VII. Experiments

### I. Definition of Terms

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."

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.

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).

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."

The methodology and apparatuses 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).

As used herein, the term "analyte" refers to a molecular entity whose presence, structure, binding ability, etc., is being detected or analyzed. Suitable analytes for practice of this invention include, but are not limited to antibodies, antigens, nucleic acids (*e.g.* natural or synthetic DNA, RNA, gDNA, cDNA, mRNA, tRNA), lectins, sugars, glycoproteins, receptors and their cognate ligand (*e.g.* growth factors and their associated receptors, cytokines and their associated receptors, signaling molecules and their receptors), small molecules such as existing pharmaceuticals and drug candidates (either from natural products or synthetic analogues developed and stored in combinatorial libraries), metabolites, drugs of abuse and their metabolic by-products, co-factors such as vitamins and other naturally occurring and synthetic compounds, oxygen and other gases found in physiologic fluids, cells, cellular constituents cell membranes

and associated structures, other natural products found in plant and animal sources, other partially or completely synthetic products, and the like.

Although most measurements described herein are made on individual molecules or pairs of molecules 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 channel receiving electromagnetic radiation while test compounds are allowed to flow past 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 can be considered to be the ligand and the antigen, the antiligand. Additionally, once an antiligand has bound to a ligand, the resulting antiligand/ligand complex can be considered an antiligand for the purposes of subsequent binding.

As used herein, the terms "molecule" refers to a biological or chemical entity that exists in the form of a chemical molecule or molecules, as opposed to salts or other non-molecular forms of matter. Many molecules are of the type referred to as organic molecules (compounds containing carbon atoms, among others, connected by covalent bonds), although some molecules do not contain carbon (including simple molecular gases such as molecular oxygen and more complex molecules such as some sulfur-based polymers). The general term "molecule" includes numerous descriptive classes or groups of molecules, such as proteins, nucleic acids, carbohydrates, steroids, organic pharmaceuticals, receptors, antibodies, and lipids. When appropriate, one or more of these more descriptive terms (many of which, such as "protein," themselves describe overlapping groups of compounds) will be used herein because of application of the method to a subgroup of molecules, without detracting from the intent to have such compounds be representative of both the general class "molecules" and the named subclass, such as proteins. When used in its most general meaning, a "molecule" also includes bound complexes of individual molecules, such as those described below. An ionic bond can be present in a primarily covalently bound molecule (such as in a salt of a carboxylic acid or a protein with a metal ion bound to its amino acid residues), and such molecules are still considered to be molecular structures. Of course, it is also possible that salts (*e.g.*, sodium chloride) will be present in the sample that contains a molecular

structure, and the presence of such salts does not detract from the practice of the invention. Such salts will participate in the overall dielectric response, but a molecular binding event or property can be detected in their presence.

As used herein, the terms "binding partners," "ligand/antiligand," or "ligand/antiligand complex" refers to pairs (or larger groups; see below) of molecules that specifically contact (*e.g.* bind to) each other to form a bound complex. Such a pair or other grouping typically consists of two or more molecules that are interacting with each other, usually by the formation of non-covalent bonds (such as dipole-dipole interactions, hydrogen bonding, or van der Waals interactions). The time of interaction (sometimes referred to as the on-off time) can vary considerably, even for molecules that have similar binding affinities, as is well known in the art. Examples include antibody-antigen, lectin-carbohydrate, nucleic acid-nucleic acid, and biotin-avidin pairs. Biological binding partners need not be limited to pairs of single molecules. Thus, for example, a single ligand can be bound by the coordinated action of two or more anti-ligands, or a first antigen/antibody pair can be bound by a second antibody that is specific for the first antibody. Binding can occur in liquid (also referred to as solution) or solid (also referred to as surface) phase and can include complex binding that involves the serial or simultaneous binding of three or more separate molecular entities. Possible examples include GPCR-ligand binding, followed by GPCR/G-protein binding; nuclear receptor/cofactor/ligand/DNA binding; or the binding complex chaperone proteins to a target, along with a small molecule ligand. Other examples will be readily apparent to those skilled in the art.

The word "ligand" is commonly used herein to refer to any molecule for which there exists another molecule (*i.e.* an "antiligand") that binds to the ligand, owing to a favorable (*i.e.*, negative) change in free energy upon contact between the ligand and antiligand. There is no limit on the size of the interacting substances, so that a ligand (or an antiligand) in this broad sense can consist of either an individual molecule or a larger, organized group of molecules, such as would be presented by a cell, cell membrane, organelle, or synthetic analogue thereof. As used herein, "ligand" and "antiligand" both have this broad sense and can be used interchangeably. However, it is recognized that there is a general tendency in the field of biology to use the word "ligand" to refer to the smaller of the two binding partners that interact with each other, and this convention is followed whenever possible.

As used herein, the term "ligand/antiligand complex" refers to the ligand bound to the antiligand. The binding can be specific or non-specific, and the interacting ligand/antiligand complex are typically bonded to each other through noncovalent forces such as hydrogen bonds, Van der Waals interactions, or other types of molecular interactions.

As used herein, the term "specifically binds," when referring to a protein, nucleic acid, or other binding partner as described herein, refers to a binding reaction which is selective for the ligand of interest in a heterogeneous population of potential ligands. Thus, under designated conditions (*e.g.*, immunoassay conditions in the case of an antibody), the specified antiligand binds to its particular "target" and does not bind in an indistinguishable amount to other potential ligands present in the sample. For example, a cell surface receptor for a hormonal signal (*e.g.*, the estrogen receptor) will selectively bind to a specific hormone (*e.g.*, estradiol), even in the presence of other molecules of similar structure (such as other steroidal hormones, even similar steroids such as estriol). Similarly, nucleic acid sequences that are completely complementary will hybridize to one another under preselected conditions such that other nucleic acids, even those different in sequence at the position of a single nucleotide, do not hybridize.

As used herein, the terms "isolated," "purified," and "biologically pure" refer to material which is substantially or essentially free from components that normally accompany it as found in its native state.

As used herein, the term "nucleic acid" refers to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and, unless otherwise limited, encompasses such polymers that contain one or more analogs of natural nucleotides that can hybridize in a similar manner to naturally occurring nucleotides.

As used herein, the terms "polypeptide," "peptide," and "protein" are generally used interchangeably to refer to a polymer of amino acid residues. These terms do not appear to have a consistent use in the art in reference to the size of molecules, although the order given generally refers to increasing size and complexity. All of these terms apply to amino acid polymers in which one or more amino acid residue or peptide bond is an artificial chemical analogue of a corresponding naturally occurring amino acid or bond, as well as to naturally occurring amino acid polymers.

As used herein, the term "antibody" refers to a protein consisting of one or more polypeptide chains substantially encoded by immunoglobulin genes or fragments of immunoglobulin genes. The recognized immunoglobulin genes include the kappa,

lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

An "antigen-binding site" or "binding portion" refers to the part of an immunoglobulin molecule that participates in antigen binding. The antigen binding site is formed by amino acid residues of the N-terminal variable ("V") regions of the heavy ("H") and light ("L") chains. Three highly divergent stretches within the V regions of the heavy and light chains are referred to as "hypervariable regions" which are interposed between more conserved flanking stretches known as "framework regions" or "FRs". Thus, the term "FR" refers to amino acid sequences that are naturally found between and adjacent to hypervariable regions in immunoglobulins. In an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen binding "surface". This surface mediates recognition and binding of the target antigen. The three hypervariable regions of each of the heavy and light chains are referred to as "complementarity determining regions" or "CDRs" and are characterized, for example by Kabat et al. Sequences of proteins of immunological interest, 4th ed. U.S. Dept. Health and Human Services, Public Health Services, Bethesda, MD (1987).

As used herein, the terms "immunological binding" and "immunological binding properties" refer to the non-covalent interactions of the type that occur between an immunoglobulin molecule and an antigen for which the immunoglobulin is specific.

As used herein, the term “enzyme” refers to a protein that acts as a catalyst and reduces the activation energy of a chemical reaction occurring between other compounds or of a chemical reaction in which one compound is broken apart into smaller compounds. The compounds that undergo the reaction under the influence of the enzyme are referred to as “substrates.” The enzyme is not a starting material or final product in the reaction, but is unchanged after the reaction is completed.

As used herein, the term “test sample” refers to the material being investigated (the analyte) and the medium/buffer in which the analyte is found. The medium or buffer can included solid, liquid or gaseous phase materials; the principal component of most physiological media/buffers is water. Solid phase media can be comprised of naturally occurring or synthetic molecules including carbohydrates, proteins, oligonucleotides, $SiO_2$, GaAs, Au, or alternatively, any organic polymeric

material, such as Nylon®, Rayon®, Dacryon®, polypropylene, Teflon®, neoprene, delrin or the like. Liquid phase media include those containing an aqueous, organic or other primary components, gels, gases, and emulsions. Exemplary media include celluloses, dextran derivatives, aqueous solution of d-PBS, Tris, deionized water, blood, cerebrospinal fluid, urine, saliva, water, and organic solvents.

As used herein, a "biological sample" is a sample of biological tissue or fluid that, in a healthy and/or pathological state, is to be assayed for the structure(s) or event(s) of interest. Such biological samples include, but are not limited to, sputum, amniotic fluid, blood, blood cells (*e.g.,* white cells), tissue or fine needle biopsy samples, urine, peritoneal fluid, pleural fluid, and cells from any of these sources. Biological samples also include cells grown in cultures, both mammalian and others. Biological samples further include sections of tissues such as frozen sections taken for histological purposes. Although a biological sample is often taken from a human patient, the meaning is not so limited. The same assays can be used to detect a molecular event of interest in samples from any mammal, such as dogs, cats, sheep, cattle, and pigs, as well as samples from other animal species (*e.g.*, birds, such as chickens or turkey) and plants (*e.g.*, ornamental plants and plants used as foods, such as corn or wheat). The biological sample can be pretreated as necessary by dilution in an appropriate transporting medium solution or concentrated, if desired, and is still referred to as a "biological sample." Any of a number of standard aqueous transporting medium solutions, employing one of a variety of transporting media, such as phosphate, Tris, or the like, preferably at physiological pH can be used. As with biological samples, pretreatment of a more general sample (by dilution, extraction, etc.) once it is obtained from a source material do not prevent the material from being referred to as a sample.

As used herein, the term "fluid reservoir" refers to any location, without regard to physical size or shape, where fluid is being maintained in a position that is coupled to a signal path, so that a signal resulting from interaction of a test signal with the sample in the detection region of the "fluid reservoir" can be detected. "Fluid reservoir" refers more to the fluid itself than to the container in which the fluid is located. In its simplest form, therefore, "fluid reservoir" can refer to a fluid droplet or layer formed on a flat surface and maintained at that location by inertia and/or surface tension. Such arrangements are sometimes used in various "chip" designs commonly used in genomics in which a sample fluid is washed across the surface of a chip that has specific molecular probes (usually DNA fragments of know sequence) attached at known locations on the

surface. The "fluid reservoir," however, can be and often is contained within physical walls that restrain movement of the fluid, such as vertical walls that constrain gravitational spreading (as in the side walls of test tube or microtitre plate), completely surrounding walls (as in a sealed container), or partially surrounding walls that direct and/or permit motion in a limited number of directions (such as the walls of a tube or other channel). The last of these named possibilities is often referred to herein as a "fluid channel" and occurs commonly in situations were a fluid is being moved from one location to another (such as in a microfluidics chip) to allow interaction with other samples and/or solutions containing reagents or to allow multiple samples to be transported past a single detection region.

As used herein, the term "signal path" refers to a transmission medium that supports the propagation of the desired test signal. In one embodiment the transmission path is a two-conductor structure such as a coaxial cable capable of supporting a traverse electromagnetic (TEM) signal. Other multiple-conductor, TEM structures such as microstrip line, stripline, suspended substrate, slot line, coplanar waveguide, are also included within the present definition. Other transmission media, such as wire, printed circuit board traces, conductive or dielectric waveguide structures, mutlipolar (*e.g.*, quadrapolar, octapolar) structures, are also included in the present definition.

As used herein, the term "detection region" refers to the region (all or a portion) of a fluid reservoir (*e.g.*, a fluid transport channel in a microfluidics chip or a well of a multiwell plate) that receives and interacts with the electromagnetic signal radiated from the signal path in a manner that is detected by the apparatus being used. Thus, while some signal may interact with sample at another location (*e.g.*, an adjacent well of a microtitre plate impinged by stray electromagnetic radiation from a probe head), such extraneous interactions, if not detected by the apparatus being used, would not cause the adjacent region to be part of the "detection region." On the other hand, if a signal were to interact with a portion of a bulk sample, all of the volume of the bulk sample that interacts with the signal so as to produce a modified signal that could be detected by the apparatus would be considered to be part of the "detection region." Detection regions of an apparatus used for the primary purposes of the present invention typically have relatively small. This is particularly true when the purpose is testing for potential candidate drugs from a library of test compounds for ability to interact with a target receptor, as the amount of each individual compound available for a specific assay is often low. Accordingly, detection region volumes of less than 1 ml ($1 \times 10^{-6}$ $m^3$) are

preferred. Even smaller detection regions are more preferred, such as 1 μl ($1 \times 10^{-9}$ $m^3$), 1 nl ($1 \times 10^{-12}$ $m^3$), or 1 pl ($1 \times 10^{-15}$ $m^3$), and ranges between all of these individually named volumes. Smaller volumes can be used but are not preferred, as smaller volumes are unlikely to contain a statistically significant number of molecules of interest under the conditions of temperature, pressure, and concentration normally used with physiological samples.

As used herein, the term "coupling" refers to the transfer of electromagnetic energy between two structures either through a direct or indirect physical connection or through any form of signal coupling. The general term "coupling" includes both signal coupling that occurs when a molecular event is in direct physical contact with a electrically conductive portion of the signal path (*e.g.*, a molecule of interest binding to a surface of a signal path) and signal coupling that occurs when a molecular event of interest is physically separated from any surface of the signal path (*e.g.*, such as the operations described herein using a probe that couples to the sample through a wall of a fluid reservoir). These two type of coupling are typically referred to as "direct coupling" and "indirect coupling" when they need to be distinguished.

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 ($10 \times 10^6$ Hz) and at or below 1000 GHz ($1 \times 10^{12}$ Hz), such as 10 MHz, 20 MHz, 45 MHz, 100 MHz, 500 MHz, 1 GHz ($1 \times 10^9$ Hz), 2 GHz, 5 GHz, 7.5 GHz, 10 GHz, 12 GHz, 15 GHz, 18 GHz, 20 GHz, 25 GHz, 30 GHz, 44 GHz, 60 GHz, 110 GHz, 200 GHz, 500 GHz, or 1000 GHz and range anywhere therebetween. A preferred region is from 10 MHz to 40 GHz, a more particularly from 45 MHz to 20 GHz.

## II. General Overview

The present invention makes use of the observation that a vast number of molecules can be distinguished and their structural properties and binding abilities measured based upon their unique dielectric properties in a region of the electromagnetic spectrum not previously used to detect molecular events. These dielectric properties are observed by initially coupling a test signal to a test sample that includes the analyte of interest. The dielectric properties of the analyte modulate the test signal and produce a distinguishable signal response. This response can be recovered, stored, and used to detect and identify the molecule in other test samples. Additionally, interactions of other molecules with the first molecule (*e.g.*, molecular binding events) can also be detected, as

the test signal is further modified by the interaction of a second molecule with the first. Detection and identification of the molecule properties and of binding events can occur in the liquid, gas, or solid phase, but are preferably carried out in an aqueous physiological environment in order to identify properties of the molecule associated with its function in a biological environment.

The detector assembly of the present invention provides a measurement probe operable to couple a test signal to a test sample in which a molecular event is taking place. The test sample is in a fluid reservoir, often a fluid channel or a well of a multiwell plate. A portion of the fluid reservoir, referred to as the detection region, is illuminated with the test signal. The dielectric properties of the molecules involved in the molecular event operate to modulate the test signal, providing a reflected signal having a signal response that is different from the signal response that would be detected if the same test signal were applied to a sample, otherwise identical, that did not contain the molecular event. The signal response is then recovered and provides information as to one or more properties of the molecule or molecules involved in the molecular event

III. Detector Assembly

Fig. 1A illustrates one embodiment of an integrated detector assembly 100 in accordance with the present invention. The detector assembly 100 includes a fluid transport system 150 integrated with a measurement probe 230 assembly. The sample transport system 150 includes a fluid channel 151, with a entry end 152 and an exit end 154. Motion of the test sample through the channel 151 is controlled by a fluid controller 156, which acts to move the test sample through the channel at times and under conditions selected by the user. Optionally, reservoir 158 can include a second analyte or test sample that can be mixed with the test sample stored in reservoir 157 as they are being introduced to the fluid channel 151. The ability to mix two test samples in close proximity to the detector makes it easy for the kinetics of binding events to be determined from this type of data. The fluid controller 156 can move the test sample in one direction, in forward and reverse directions, or pause the test sample for a predetermined duration, for instance over the detection region in order to improve sensitivity.

The probe assembly 230 includes a probe head 230a and a connecting end 230b. The probe head 230a is positioned proximate to the detection region 155 of the fluid channel 150 and is operable to electromagnetically couple an incident test signal to the test sample flowing through the detection region 155. The test sample modulates the

test signal, a portion of which is reflected to the probe head 230a. The reflected modulated signal is subsequently recovered by the detection assembly, further illustrated and described below. In one embodiment, the probe head can be an open-ended section of a coaxial cable operable to transmit test signals to and recover modulated reflected signals from the test sample flowing within the detection region 155. Those of skill in the art of microwave engineering will appreciate that other terminations (such as shorted or loaded terminations) and circuit architectures (such as stripline, microstrip, coplanar waveguide, slot line suspended substrate, or waveguide) can be used in alternative embodiments under the present invention.

The connecting end 230b is electrically connected (directly or via intervening circuitry) to a measurement port of a molecular detection system, further described below. In the exemplary embodiment in which the measurement probe is a coaxial type structure, the connecting end 230b can be a coaxial cable which extends from the molecular detection system, a compatible coaxial type connector such as a SMA-type connector or other connector type familiar to those skilled in the art of high frequency measurement. In alternative embodiments of the invention in which a different type of probe architecture is used (*i.e.*, microstrip, etc.), the connection port can comprise a compatible connection to provide signal communication to the molecular detection system.

Fluid Transport System

As illustrated in Fig. 1A, the fluid transport system 150 includes a fluid channel 151 through which the test sample flows. Depending upon the application, the fluid channel 151 can take on a variety of forms. For instance in one embodiment, the fluid channel 151 is a Teflon® (polytetrafluoroethylene; PTFE) or other hard plastic or polymer tube (for example TEZEL™ (ETFE) tube) operable to transport the test sample to and from the detection region 151. In another embodiment, the channel 151 consists of one or more etched channels (open or enclosed) in a microfluidic transport system, further described below. Two or more channels can be used to provide a larger detection region 155 to improve detection sensitivity. In another embodiment, the fluid channel 151 is formed through well known semiconductor processing techniques. Those of skill in the art will appreciate that other construction and architectures of the fluid channel 151 can be adapted to operate under the present invention.

The transporting medium can consist of a variety of solutions, gases, or other mediums depending upon the particular analyte therein. For example, when the

detection system of the present invention is used to detect the presence and/or binding of biological analytes, Dulbecco's phosphate-transporting medium saline (d-PBS) or a similar medium can be used as a transporting solution to provide an environment which resembles the biological molecule's natural environment. As appreciable to those skilled in the art, other transporting media such as DMSO, sodium phosphate ($Na_3PO_4$), MOPS, phosphate, citrate, glycine, Tris, autate, borate as well as others can be used in other embodiments under the present invention.

The fluid channel 151 includes a detection region 155 over which the probe 230 illuminates the sample. The area of the detection region 155 will be influenced by several factors including the architecture and material composition of the fluid channel 151, concentration of the analyte, desired detection time, the rate at which the test sample advances through the channel and other factors as appreciable to those skilled in the art. In those embodiments in which immobilized detection over the detection region 155 is employed, a binding surface is formed within the detection region 155, the area of which will be influenced by binding surface chemistry, the material and morphology of the binding surface, and other factors appreciable to those skilled in the art. Exemplary dimensions of the binding surface will be on the orders of $10^{-1}m^2$, $10^{-2}m^2$, $10^{-3}m^2$, $10^{-4}m^2$, $10^{-5}m^2$, $10^{-6}m^2$, $10^{-7}m^2$,$10^{-8}m^2$, $10^{-9}m^2$, $10^{-10}m^2$, $10^{-11}m^2$,$10^{-12}m^2$, $10^{-13}m^2$, $10^{-14}m^2$, $10^{-15}m^2$ or any range within these limits. The larger numbers in this range are preferably achieved in a small volume by using a convoluted or porous surface. Smaller numbers within those listed will be more typical of microfluidic devices and systems fabricated using semiconducting processing technology. The detection region 155 can alternatively be modified to accommodate other diagnostic applications, such as proteomics chips, known in the art. The size or shape of detection region need only be such that signal propagation thereto and analyte passage therethrough are possible, subject to other constraints described herein.

In the illustrated embodiment of the detector assembly 150, the fluid controller 156 is connected to a reservoir 157. Fluid controller 156 uses fluid from the reservoir 157 to move the test sample through channel 151, which requires less test sample than simple pumping of sample alone through the channel.

A second reservoir 158 can be used to store a second analyte or test sample for mixture with the reservoir 157 test sample. In such an embodiment, the fluid controller 156 can be further configured to rapidly mix the two test samples and supply the resulting mixture to the detection region 155. This technique (known as stopped-flow

kinetics in the art of fluidic movement systems) permits the operator to observe and record changes in the signal response as binding events occur between analytes of the two test samples. This data can also be used to determine the kinetics of binding events occurring between the analytes of the two samples. The fluidics of conventional stopped-flow kinetic systems, such as model no. Cary 50 available from Varian Australia Pty Ltd. of Victoria, Australia, can be adapted to operate with the present invention or integrated within the detector assembly 150. See www.hi-techsci.co.uk/scientific/index.html for additional information about stopped-flow fluidic systems.

Other components can be included to regulate the test sample flow through the channel 151. The fluid controller 156, fluid reservoirs 157 and 158 and other components associated with fluidic movement can comprise discrete components of the fluid transport system 150 or alternatively be partially or completely integrated.

Fig. 1B illustrates another embodiment of a fluid transport system . As shown, the fluid transport system 170 includes drive circuitry 172, a drive member 174, a syringe assembly 176, and a fluid channel 178. The fluid transport system 170 is preferably assembled and operated externally of the measurement probe assembly 230 (not shown), although some or all components of the fluid transport system 170 can be integrated into the measurement probe assembly 230 under other embodiments under the present invention.

During operation, the drive circuitry 174 receives commands from the operator to provide the test sample 175 to the measurement probe 230. The command can be instructions to provide a particular amount of sample and/or to provide the sample at a particular rate. In response, the drive circuitry 174 advances the drive member 176 (a screw in one embodiment), which, in turn, advances the plunger of the syringe assembly 176. The plunger supplies the desired amount (or rate) of sample to the fluid channel 178 (a PTFE tube in one embodiment) through which the sample is carried to the detection region 155 where it is illuminated by the test signal emanating from the measurement probe.

When the present invention is used to detect or identify molecules in liquid phase, several techniques can be used to separate different samples. In one technique, one or more sample plugs of small volume (*e.g.*, 5 μl) can precede and/or follow a main sample plug of greater volume (*e.g.*, 15 μl). The shorter duration sample plugs operate to insulate the main sample plug from changes in sample concentration. Air plugs can also

be introduced as the spacer material, before and/or after the sample plugs to further minimize mixing of fluids or changes in their concentration. Air plugs can also be used as indicators to inform the test system (or operator) of the test sample's position within the fluid channel.

In another technique, the transporting medium (which can be air) can be used as a spacer material to separate different test samples from each other, in a manner that is commonly practiced in devices using other detection systems, such as the microfluidic apparatuses and techniques described in U.S. Patent Nos. 6,033,546, 5,858,187, and 5,126,022. When operated in this manner, a spacer material is introduced into the channel after the first test sample, a further test sample (or samples) is introduced into the channel after the spacer material, and the test samples are moved through the channel under the control of the fluid controller, so that a series of different test samples separated by spacer material is transported through the channel. Such transport can occur without intermixing different test samples, so that the measurement over detection region 155 can take place individually for each test sample as it passes through (or is temporarily stopped in) the detection region 155.

Any pumping device of suitable dimensions can be used in the fluid movement controller system of the invention. Such pumps can include microelectromechanical systems (MEMS) such as reported by Shoji et al., "Fabrication of a Pump for Integrated Chemical Analyzing Systems," Electronics and Communications in Japan, Part 2, 70: 52-59 (1989); Esashi et al., "Normally closed microvalve and pump fabricated on a Silicon Wafer," Sensors and Actuators, 20: 163-169 (1989); or piezo-electric pumps such as described in Moroney et al., "Ultrasonically Induced Microtransport," Proc. MEMS, 91: 277-282 (1991). In many of the microfluidics devices, however, the pumps (fluid motion controllers) have no moving parts but used electrodes and electrostatic forces to move liquids. At least two types of such electrode-based pumping has been described, typically under the names "electrohydrodynamic pumping" (EHD) and "electroosmosis" (EO). EHD pumping has been described by Bart et al., "Microfabricated Electrohydrodynamic Pumps," Sensors and Actuators, A21-A23: 193-197 (1990) and Richter et al., "A Micromachined Electrohydrodynamic Pump," Sensors and Actuators, A29:159-168 (1991). EO pumps have been described by Dasgupta et al., "Electroosmosis: A Reliable Fluid Propulsion System for Flow Injection Analysis," Anal. Chem., 66: 1792-1798 (1994). Practical considerations for operating pumps that move fluids by means of electrodes in a liquid distribution system, such EO and EHD

pumping, are discussed in PCT Application No. WO95/14590. This same WO95/14590 application describes suitable electrodes, methods for forming such electrodes, and presents theoretical considerations that are believed to provide further guidance on how to operate such pumps. For additional guidance on fluid movement in microfluidic apparatuses, see any of the many patents that have issued in this field. When the fluid movement controller utilizes electrophoretic movement of the fluid, as described in the microfluidic apparatuses and techniques described in U.S. Patent Nos. 6,033,546, 5,858,187, and 5,126,022, very small samples (0.001 μl or smaller) can be handled with ease.

Microfluidic apparatuses (both the analysis chips themselves with channels to allow various types of fluid movement, such as test sample introduction, mixing with additional test compounds or reagents, and separations of mixed components, as well as the associated equipment, such as sampling devices, temperature controls, and detectors and analysis electronics) are well developed. As the present invention is related to a new detection system that can be applied to existing (and modified) fluidic movement systems, both microfluidics and the earlier, larger-scale apparatuses that transported milliliter-sized samples using mechanical pumps and bubble spacers are not themselves aspects of the invention, but are instead devices and methods to which the detection techniques of the invention can be readily applied. The reader is directed to existing literature on fluid management, such as the patents recited above (and the background patents and other references cited therein), for the basics of this well-established field. The present specification will be primarily directed to the combination of these known systems with the detector and analysis system described here, which combination provides the new results described herein and demonstrated in the following examples. A few general comments will be made, however, to show how the current method is applied to the earlier fluid transport systems.

For example, when substantially uncharged samples are used in an electrophoretic microfluidics device, a spacer material having a relatively high ionic strength relative to the samples is often used in order to allow the electric field of the microfluidics device easily move the test samples as plugs injected into a stream of spacer fluid. In some cases, it is useful to have a spacer fluid that is substantially immiscible with the test samples. However, when the fluids are moved relatively rapidly (so that there is no time for diffusion effects to mix adjacent test samples to an extent that adversely affects the desired measurement), mixing is not generally a problem as the

entire contents of a microfluidics channel, which usually has a cross-section with a diameter of from 0.1 to 500 μm (preferably 5 to 50 μm), tends to move along as a continuous stream without turbulent mixing. Immiscible spacer material is more common in larger operations which are of a sufficient scale so that turbulent mixing occurs. The most common spacer in such systems is a gaseous bubble, with the fluid movement controller providing a physical pumping of the fluid, often in the form of a peristalic pump. Such large-scale systems were once common in the medical analysis system (for example, the commercial clinical analysis systems known as the SMA1260), but have to a large extent been replaced by microfluidics. However, microfluidic systems also use bubbles as spacers, as described in U.S. Patent No. 5,992,820 entitled "Flow control in microfluidics devices by controlled bubble formation."

When these fluidic systems are intended for analysis of multiple test samples in series (although more than one series measurement can occur in a single physical device), the systems generally provides one or more channels that intersect the first channel in the fluidic transport system. The system provides separate control of fluid movement in the second fluid channel, which contains a test compound or a series of test compounds separated by spacer material. A test compound(s) from the second fluid channel is introduced into a test sample in the first fluid channel sufficiently upstream from the detection region 155 so that the test compound has time to interact with a molecular structure in a test sample in the first fluid channel before the test sample reaches the detection region 155. Whether or not the interaction has taken place is then determined by a further change in the test signal. The test signal can be used to detect the presence of the test compound in the second channel or the molecular analyte in the first channel, if the interaction is one that is known to occur between the two molecular species (such as when they are the two members of a known binding pair). Additionally, molecules not known to be members of a binding pair can be tested for binding (or other interactions, such as enzymatic reaction with a substrate), as is common in pharmaceutical screening.

In a preferred apparatus, the fluid transport system further comprises an automatic sampling device that introduces a series of test samples into the detection channel separated by a spacer material, which can be a liquid (*e.g.*, wash transporting medium) or gas (*e.g.*, bubble). Handling of multiple test samples is described in many of the patents and other publications relating to microfluidics listed above, and many automatic systems are known. For example, multiple test sample wells can be provided

on a chip, and an automatic pipetting system can apply different test samples to each of the test sample wells. In such detector assemblies, individual channels lead from the individual wells to a common location at the beginning of the detection channel (or some earlier location, if mixing with further reagents or test compounds is desired), and test samples are sequentially moved to the common location by the fluid control system. The fluid controller can then move test samples in sequence from the common location to other portions of the apparatus. Other types of automatic handling of small test samples have been described for use with other types of detection apparatuses and can be readily adapted to supply test samples to a device of the invention; see, for example, U.S. Patent No. 4,468,331, entitled "Method and system for liquid chromatography separation."

Measurement Probe

The measurement probe operates to launch a test signal toward the test sample occupying the detection region 155. The dielectric properties of the test sample modulate the test signal, and at least a portion of which is reflected back toward the measurement probe 230. The reflected modulated signal is recovered by the measurement probe and routed to a detection system, further described and illustrated below. The detection system then compares the incident test signal with the reflected modulated signal and generates a return loss or "$S_{11}$" response, as it is commonly referred to in the art of microwave engineering. Because the dielectric properties of most molecular events differ, the return loss response of each analyte will also be distinguishable and can serve to detect and identify the molecular event in an unknown test sample.

The measurement probe 230 can be realized in a variety of different forms suitable to support the propagation of the test signal at the desired frequency or frequencies. In a specific embodiment, the measurement probe 230 is a coaxial cable, although other configurations, such as microstrip, stripline, suspended substrate, coplanar waveguide, slot line, waveguide, as well as others can be used alternatively under the present invention. For some exemplary embodiments of the various forms, see R. E. Collins Foundations for Microwave Engineering, McGraw-Hill Publishing Co., 1966; and S. March, Microwave Transmission Lines and Their Physical Realizations, Les Besser and Associates, Inc., 1986.

The frequency or frequencies over which the measurement probe operates will depend upon the construction of the probe 230 but will generally be in the range of

10 MHz to 110 GHz. In a specific embodiment in which the measurement probe is realized in a coaxial configuration, the frequency of operation will typically range from 45 MHz to 20 GHz. Those of skill in the art will appreciate that probes constructed in accordance with the present invention can be used in other frequency ranges in alternative embodiments under the present invention.

Figure 2A illustrates a first embodiment of the measurement probe 230 realized in a resonant coaxial form in accordance with one embodiment the present invention. As illustrated, the probe 230 has two ports: a probe head 230a and a connecting end 230b. In a specific embodiment, the probe head 230a is an open-end coaxial cross section and the connecting end 230b is a coaxial-type connector, one embodiment of which is a SMA connector. Those of skill in the art will appreciate that other terminations (such as shorted or load terminations), as well as other circuit architectures (such as microstrip, stripline, coplanar waveguide, slotline, waveguide, etc.) can be used in alternative embodiments of the present invention.

The probe 230 further includes two coaxial sections 232 and 234, each having a center conductor 235, a dielectric insulator 236, and an outer conductor 237 (typically used to provide a ground potential reference). The first section 232 consists of the aforementioned probe head 230a and a first gap end 232a located opposite thereto, each realized as an open-end cross section of the coaxial cable. A shelf (preferably conductive) 231 is attached flush (preferably via solder, conductive epoxy or other conductive attachment means) with the outer conductor 237 of the probe head 230a.

The second section 234 is of similar construction as the first section 232, having a dielectric insulation 236 located between center and outer conductors 235 and 237. The second section 234 further includes a second gap end 234a and a connecting end 230b located opposite thereto. The second gap end is realized as an open-end cross section of the coaxial cable. The connecting end 230b is realized as a connector (SMA-type in a specific embodiment) operable to connect to the molecular detection system, further illustrated and described below. In the exemplary embodiment, the first and second sections each comprise RG401type semi-rigid coaxial cable, although larger or smaller diameter cables can be used as well. The length of the first section 232 is calculated to be approximately one-half wavelength in length at the desired frequency of resonance, as will be further described below. In the present illustration, the first section 232 is approximately 4 inches, which corresponds to approximately one-half of one wavelength at a 1 GHz test frequency.

In a specific embodiment of the invention, the probe 230 includes a tuning element 233 which is adjustably engaged between the first and second gap ends 232a and 234a to provide a variable gap distance therebetween. The gap provides a capacitive effect between the first and second sections 232 and 234, and it, in combination with the electrical length of the first section 332, is designed to provide a resonant signal response when the probe 230 illuminates the (analyte-free) transporting medium. The tuning element 233 can be rotated to expand or contract the gap (and according, decreasing or increasing the value of the capacitive effect) between the first and second sections 232 and 234, thereby changing the resonant frequency of the measurement probe 230 to the desired frequency.

In the exemplary embodiment, the gap distance is made variable, 0 inch to 0.050 inch, although other gap dimensions can be used in alternative embodiments under the present invention to adjust the resonant response to the desired frequency point. The resonant response sought is one in which the reflected portion of test signal is substantially nulled, *i.e.*, when the return loss or the magnitude of $S_{11}$ is minimal. As will be illustrated below, the presence of the analyte will significantly alter the resonant signal response, thereby allowing detection and identification of analyte binding and/or substructure.

The tuning element 233 is preferably a hollow tube constructed from a material (stainless steel in one embodiment) that exhibits relatively high conductively to maintain ground potential between the first and second sections at the test frequency of operation. Further, the tuning element can include internal threads 233a which mate with external threads 238 disposed on the outer conductors of the first and second sections near the first and second gap ends 232a and 234a. In alternative embodiments of the invention, the tuning element 233 can be omitted, in which case the first and second sections 232 and 234 can comprise one continuous coaxial transmission line structure.

Those of skill in the art will appreciate that the probe 230 can include other circuit elements to provide other signal responses in alternative embodiments under the present invention. Further, other circuitry in lumped element form, distributed form, or a combination of both can be included along the probe 230. For instance, impedance matching circuits and/or transporting medium amplifier circuits can be employed at the connecting end, within the tuning element, along the first and/or section sections 232 and 234, or at the probe head 230a. Alternatively or in addition, impedance matching

circuitry and one or more output amplifiers can be implemented to further enhance the output signal.

In one embodiment of the invention as illustrated in Fig. 1, the probe head 230a is closely positioned to but physically separated from the test sample by intervening materials. In this instance, the incident signal is transmitted to, and the reflected signal is recovered from the test sample via electromagnetic coupling. The intervening material(s) that physically separates the probe head 230a from the test sample can include solid phase materials, such as PTFE, alumina, glass, sapphire, diamond, Lexan®, polyimide, or other dielectric materials used in the area of high frequency circuit design; materials used in the fabrication of microfluidic devices or semiconductor processing; or other known materials which exhibit a relatively high degree of signal transparency at the desired frequency of operation. In a specific embodiment, the intervening material can be an electrically insulating material, some examples of which are described above. Alternatively or in addition, liquid and/or gaseous phase materials that exhibit a relatively high degree of test signal transparency can also comprise the intervening materials.

The thickness and dielectric properties of the intervening materials can vary depending upon the type of fluidic system implemented and measurement probe used. For instance, in systems in which the separation distance is great, a low loss, high dielectric material is preferred to provide maximum coupling between the test sample and the probe 230. In systems in which the separation distance is relatively short, materials of higher loss and lower dielectric constant can be tolerated. In a specific embodiment in which the channel 151 is PTFE tube having dimensions of 0.031 inch I.D., 0.063 inch O.D., wall thickness 0.016 inch, and a dielectric constant of approximately 2, the separation distance is approximately the tube's wall thickness, about 0.016 inch. In other detector assemblies, separation distances can be on the order of $10^{-1}$ m, $10^{-2}$ m, $10^{-3}$m, $10^{-4}$m, $10^{-5}$m, or $10^{-6}$m, and can be much smaller, *e.g.*, on the order of $10^{-9}$m in some cases (such as in a channel etched into the surface of a substrate and having a metallic signal path element with a thin polymer layer on the test sample side acting as the fourth side of the channel). Decreasing the separation distance or increasing the detection area 155, the sample volume, or analyte concentration will operate to increase detection sensitivity. The separation material, as illustrated above, can a solid phase material, or alternatively (or in addition) consist of a liquid or gaseous phase material or a combination thereof.

Fig. 2B illustrates a cover 240 that can be positioned opposite the probe 230 such that test sample is located between the cover and the probe. In one embodiment,

the cover 240 consists of an electrically conductive material (such as brass, copper, aluminum, etc.) which is set to ground potential over the frequency of operation so as to provide a ground plane on which the electromagnetic field radiating from the center conductor 235 terminate. The cover 240 further provides shielding from external sources that could interfere with the measurement.

In an alternative embodiment, the probe head 230a or the center conductor 235 of the measurement probe 230 extends into the channel 151 such that the center conductor 235 is in direct contact with the test sample flowing along the channel 151. In this embodiment, the center conductor 235 can be formed from a material which is capable of both supporting test signal propagation and which does not adversely affect the analyte. Such materials include, but are not limited to gold, indium tin oxide, copper, silver, zinc, tin, antimony, gallium, cadmium, chromium, manganese, cobalt, iridium, platinum, mercury, titanium, aluminum, lead, iron, tungsten, nickel, tantalum, rhenium, osmium, thallium or alloys thereof. These same materials can be used to form external probes, along with other materials that will be readily apparent to those of skill in the art.

Fig. 2C illustrates a cross-sectional view and Fig. 2D illustrates a top view of a second embodiment of the probe in accordance with the present invention. As illustrated in the cross-sectional view of Fig. 2C, the measurement probe 250 includes a first coaxial section 251, a bracket 252, an attachment platform 253, contact rings 255, a tuning gap 256, a second coaxial section 257, a conductive ground tube 258, and a fluidics shelf 259.

The first coaxial section 251 is coupled to signal source and signal detector (not shown) illustrated and described below. In one embodiment, the first coaxial section is RG401 semi-rigid cable. Those of skill in the art will appreciate that other types of semi-rigid cable as well as other transmission structures can be used in alternative embodiments under the present invention.

Securely held within the bracket 252, the first coaxial section 251 extends into the gap area 254 near the bottom of the fluidics shelf 259. Contact rings 255a and 255b can be optionally attached around the outer surface of the first coaxial section 251 to provide ground conductivity between the first coaxial section 251 and the inner surface of the ground tube 258. In one embodiment, the contact rings are highly conductive springs, although other structures can be used instead. In alternative embodiments, the outer surface of the first coaxial section 251 is brought into contact with the interior surface of

the ground tube 258 (copper in one embodiment) to a sufficient degree, thereby obviating the need for the contact rings 255.

The first and second coaxial sections 251 and 257 are separated by a tuning gap 256 that electrically operates to provide the resonant response described above. In the illustrated embodiment, the second coaxial section 257 is secured within the ground tube 258 within the fluidics shelf 259. The first coaxial section 251 is inserted into the gap region 254, the outer surface of the first coaxial section 251 making electrical contact with the interior surface of the ground tube 258, thereby providing a continuous ground potential therebetween. The tuning gap 256 formed between the first and second coaxial sections 251 and 257 is made either shorter or longer by moving the bracket 252 either up or down, respectively. The reader will appreciate that the position of the second coaxial section 257 within the conductive ground tube 258 can be adjustable, either alternatively or in addition to the first coaxial section 251. The attachment platform 253 attaches to and holds stationary the fluidics shelf 259, allowing the bracket to either insert or remove the first coaxial section 251 therefrom. In one embodiment, the bracket 252 is motor driven and included within a precision motorized translational stage assembly available from the Newport Corporation of Irvine, California.

Fig. 2E illustrates an embodiment of the measurement probe realized in non-resonant coaxial form in accordance with the present invention. In this embodiment, the probe 280 includes a section of open-ended coaxial line 281, an interaction fixture base 283, an interaction substrate 285, a fluid interface 287 having one or more fluid tubes 289 extending therefrom. In a specific embodiment, the probe 280 is coupled to a vector network analyzer or similar test equipment capable of measuring incident and reflected signal properties.

Fluid tubes 289 allows the introduction of sample into the fluid interface 287. An interaction substrate 285 may optionally be used to separate the supplied sample from the end of the coaxial section 281. The interaction substrate 285 may consist or a variety of materials, for example glass, quartz, polyimide, PTFE, materials such as silicon dioxide, gallium arsenide or other materials used in semiconductor processing. In alternative embodiments, interaction substrate 285 is removed and the sample comes into direct contact with the coaxial section 281. The base fixture 283 is used to securely attach and align the fluid interface 287 (and interaction substrate 285, if used) with the open end portion of the coaxial section 281. In a specific embodiment the base fixture is

aluminum, although other materials may be used in alternative embodiments of the present invention.

During operation, a volume of sample (which may be an analyte-free buffer used to make a baseline response) is introduced into the fluid interface 287 via fluid tubes 289. Subsequently, a test signal is applied to the coaxial section 281 from the test set 290. As described above, the dielectric properties of the supplied sample will modulate the incident signal. The open-end construction of the coaxial section 281 will reflect at least a portion of the modulated signal back towards the test set where the modulated signal is recovered. The modulation, which is typically exhibited by changes in the incident signal's amplitude and phase (or lack thereof), indicates the presence (or absence) of a molecular event.

Once the aforementioned fluidic transport system is provided, one of skill in the art will be generally familiar with the biological and chemical literature for purposes of selecting molecular samples with which to work. For a general introduction to biological systems, *see*, *Current Protocols in Molecular Biology*, F.M. Ausubel *et al.*, eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc, and John Wiley & Sons, Inc. (through 1997 Supplement) (Ausubel); Watson *et al.* (1987) Molecular Biology of the Gene, Fourth Edition, The Benjamin/Cummings Publishing CO., Menlo Park, CA; Alberts *et al.* (1989) Molecular Biology of the Cell, Second Edition Garland Publishing, NY; The Merck Manual of Diagnosis and Therapy, Merck & Co., Rathway, NJ. Product information from manufacturers of biological reagents and experimental equipment also provide information useful in assaying biological systems. Such manufacturers include, *e.g.*, the SIGMA chemical company (Saint Louis, MO), R&D systems (Minneapolis, MN), Pharmacia LKB Biotechnology (Piscataway, NJ), CLONTECH Laboratories, Inc. (Palo Alto, CA), Aldrich Chemical Company (Milwaukee, WI), GIBCO BRL Life Technologies, Inc, (Gaithersberg, MD), Fluka Chemica-Biochemika Analytika (Fluka Chemie AG, Buchs, Switzerland, Applied Biosytems (Foster City, CA), as well as many other commercial sources known to one skilled in the art.

Biological samples can be derived from patients using well known techniques such as venipuncture, lumbar puncture, fluid sample such as saliva or urine, or tissue biopsy and the like. When the biological material is derived from non-humans, such as commercially relevant livestock, blood and tissue samples are conveniently obtained from livestock processing plants. Similarly, plant material used in the invention

can be conveniently derived from agriculture or horticultural sources, and other sources of natural products. Alternatively a biological sample can be obtained from a cell or blood bank where tissue and/or blood are stored, or from an in vitro source, such as a culture of cells. Techniques for establishing a culture of cells for use as a source for biological materials are well known to those of skill in the art. Freshney, Culture of Animal Cells, a Manual of Basic Technique, Third Edition, Wiley-Liss, NY (1994) provides a general introduction to cell culture.

Detection Region Chemistry

In embodiments in which detection of analyte binding or substructure occurs without binding to the surfaces of the detection region 155, the chemistry of the detection region 155 is generally chosen to make the walls of the channel (a Teflon® tube in one of the illustrated embodiments) inert to the passage of test sample. In alternative embodiments in which detection occurs with binding to the detection region surfaces, the detection region surface can be functionalized to bind an anti-analyte so that one or more test samples can be transported by the fluid transport system for detection of potential binding interactions between the anti-analyte and an analyte. In such cases, the surfaces of the detection region 155 are prepared with a material possessing good molecular binding qualities. Ligands can bind directly, indirectly through other molecular structures, or through both configurations to bind to the walls of the channel. A list of possible types of binding interactions being identified includes but is not limited to protein/protein interactions, DNA/protein interactions, RNA/protein interactions, nucleic acid hybridization, including base pair mismatch analysis, RNA/RNA interactions, tRNA interactions, enzyme/substrate systems, antigen/antibody interactions, small molecule/protein interactions, drug/receptor interactions, membrane/receptor interactions, conformational changes in solid phase ligands, protein/saccharide interactions, and lipid/protein interactions. The chemistry of attachment can involve only a single species of molecules attached to the surface, a whole array of different species attached to the surface, or multiple binding events between species directly attached to the surface and ligands of interest in the solution.

IV. Exemplary Molecular Detection Systems

Fig. 3A illustrates one embodiment of a molecular detection system 300 in accordance with the present invention. The system 300 includes a signal source 302, a signal detector 304, and the detection assembly 100. The detection assembly 100 is coupled to the signal source 302 and to the detector 304 via a signal path, *e.g.*, a cable, transmission line or other medium 310 which can support the propagation of a signal at the desired test frequency. The source 302 is operable to transmit an incident test signal 312 toward the detector assembly 100. The incident test signal is modulated by the dielectric properties of the test sample and at least a portion of the modulated test signal is reflected back toward the resonant probe 230. The reflected signal couples to the probe and compared to the incident signal to produce a signal response. Those of skill in the art of microwave engineering will recognize this measurement as a one- port $S_{11}$ reflection measurement.

In a specific embodiment, the signal source 302 and signal detector 304 are included within a vector network analyzer test set, examples being model numbers 8510 and 8714 available from Agilent Technologies of Palo Alto, California. Other high frequency measurement systems, such as scalar network analyzers, or other systems that provide signal information based upon transmitted and reflected signals can be used in alternative embodiments under the present invention. While the exemplary detection system 300 is depicted as a one-port reflection measurement system, additional signal sources (and/or detectors) can be used to recover either reflected modulated signals or modulated signals propagating through the test sample (referred to as $S_{21}$ or "thru" measurements in the art) in alternative embodiments under the present invention.

In one embodiment of the present invention, the resonant probe 230 is used with the molecular detection system 300 to detect the presence or absence of a molecular event in a test sample. The method includes the process of initially determining a baseline response for the resonant probe 230 (either with air, buffer, or another analyte-free medium) and subsequently measuring a change in the baseline response when a molecular event is introduced in the detection region 155. This method is further described below and illustrated in Fig. 3B.

Initially at 320, the probe 230 is designed to have a resonant $S_{11}$ response at or near a predefined frequency. In a specific embodiment, this process is accomplished by defining the length of the first coaxial section 332 to be one-half of a wavelength at the

desired resonant frequency using the following calculation as known to those skilled in the art:

$$\lambda/2 = c/[2 \times f_{des} \times \varepsilon_r^{1/2}],$$

where:

$\lambda/2$ = length of the first section 332 (in meters)

c = the speed of light: $3 \times 10^8$ m/s

$f_{des}$ = the desired resonant frequency (in Hz)

$\varepsilon_r$ = the relative dielectric constant of the insulating material 336

In the illustrated embodiment in which the desired resonant frequency is 1 GHz and the relative dielectric constant of the coaxial insulating material (Teflon®) 336 is approximately 2.1, the length of the first coaxial section 232 is selected to be 4 inches. Those skilled in the art of high frequency circuit design will appreciate that the illustrated design technique and resulting resonant signal response is but one of many possible. Other well-known circuit designs (such as a short-circuited quarter-wavelength lines, etc.) can also be used to obtain a resonant signal response in an alternative embodiment.

Next at 321, the probe head 230a is positioned proximate to the detection region 155 so as to enable electromagnetic coupling thereto. Typically, this operation will involve placing the probe head 230a as close as possible to the detection region 155. As described above, intervening materials which separate the probe head 230a and the detection region 155 can be solid phase materials, such as PTFE, alumina, glass, sapphire, diamond, Lexan®, polyimide, or other dielectric materials used in the area of high frequency circuit design, materials used in the fabrication of microfluidic devices, materials used in semiconductor processing, or other known materials which exhibit a relatively high degree of signal transparency at the desired test signal frequency. In a specific embodiment, the intervening material can be an electrically insulating material, some examples of which are glass, PTFE or variations thereof, quartz, silicon dioxide, gallium arsenide, as well as those materials described above. Alternatively or in addition, liquid and/or gaseous phase materials which exhibit a relatively high degree of test signal transparency can be used as well.

Subsequently at 322, the fluid transport system 150 supplies the analyte-free transporting medium to the detection region 155. Next at 323, a test signal is coupled to the detection region 155, and the resulting baseline response is obtained. In the exemplary embodiment described herein, the baseline response is an $S_{11}$ response obtained by comparing the amplitude and phase data of the incident and reflected signals 312 and 314 when the analyte-free buffer occupies the detection region 155. Other signal responses using well-known data comparison techniques can be obtained in alternative embodiments under the present invention. During testing, fluid motion can be continuous (such as if measurement time is short, for instance when a signal at a single frequency or small group of frequencies is being measured or when detection sensitivity is high), or fluid flow can be halted. Alternatively, multiple scans can be performed and averaged to increase sensitivity.

Next at 324, the tuning element is adjusted (rotated clockwise or counter-clockwise, or advanced using a motorized assembly as described in Fig. 2D) until the magnitude of the baseline response reaches its lowest point, an example of which is illustrated as trace 334 in Fig. 3C. The frequency point at which the baseline $S_{11}$ response reaches it minimum point is herein referred to as $f_{res.}$ This point represents the frequency at which the least amount of signal power is reflected back to the detection system 330. Typically, the resonant frequency $f_{res}$ will be offset from the predefined frequency $f_{des}$ due to the dielectric effects of the apparatus and sample located at the open-end portion of the probe head 230a. The frequency point $f_{des}$ is chosen at a frequency or within a frequency range where the molecular event is expected to exhibit a dramatic change on the dielectric properties of the sample. While a frequency of 1 GHz was chosen in the exemplary embodiment to illustrate the detection process, the dielectric properties of many molecular events enable their detection at frequencies of 10 MHz, 20 MHz, 45 MHz, 100 MHz, 250 MHz, 500 MHz, 1 GHz, 2.5 GHz, 5 GHz, 7.5 GHz, 10 GHz, 12 GHz, 15 GHz, 20 GHz, 25 GHz, 30 GHz, 40 GHz, 50 GHz, 60 GHz, 80 GHz, 100 GHz, 110 GHz and frequencies ranging therebetween using the present invention. Those of skill in the art will appreciate that other frequencies and frequency ranges can be used in alternative embodiments under the present invention.

The return loss or $S_{11}$ response near the resonant frequency point $f_{res}$ will change quite rapidly. In order to obtain a more gradual response near the resonant frequency point, the probe can be detuned (by rotating the tuning element) away from the resonant point $f_{res}$. This step (and the tuning element 333 itself) can be omitted in

alternative embodiments (for instance, fabricated IC chips) that exhibit a tuned $S_{11}$ response.

Next at 325, the test sample is introduced into the detection region 155 using any of the aforementioned means. At 326, a test signal is coupled to the detection region 155 and the resulting test sample response is obtained.

In the exemplary embodiment described herein, the baseline response is an $S_{11}$ response made in the presence of the fluid channel and analyte-free buffer. Those of skill in the art will appreciate that other measurements (such as a two-port $S_{21}$ measurement) made be used as a baseline response as well. Further, different conditions may be used to measure the baseline response, for instance the buffer may contain a known analyte, or the response may be made in the absence of any buffer and/or the fluid channel. The reader will appreciate the numerous possibilities of baseline response that can be made. As described above, the test sample can be held stationary or move through the detection region 155 during the measurement process.

Fig. 3C illustrates a test sample response 335 when a molecular event occurs within the detection region 155. As illustrated, the test sample response 335 displays a shallower null. Electrically, the dielectric properties of the analyte within the test sample alters the resonant signal response, and decreases the amplitude of the test sample response 335 compared to the baseline response 334. In other embodiments, the test sample response 335 will exhibit a frequency shift, *i.e.* the frequency at which the minimum $S_{11}$ response occurs is shifted above or below $f_{res}$, and in some embodiments, both the amplitude and frequency of the baseline response will change. In the instance in which binding events occur within the detection region, the response can be monitored real-time (*i.e.*, as the reaction is occurring). Those of skill in the art will appreciate that the illustrated test sample response 335 is but one example of a possible response, and other responses can be observed as well under the present invention. For instance in some embodiments, the test sample can produce a deeper null than the buffer resonant point. This may be the case for instance when the initial baseline response 334 is tuned away from the minimum resonance point.

Subsequently at 327, a determination is made as to whether the baseline and test sample responses 334 and 335 differ by more than a predefined amount. This process can be accomplished by comparing the amplitude, frequency, and/or phase data of the baseline and test sample responses 334 and 335, and indicating a change has occurred when the difference exceeds a predefined or preprogrammed quantity.

Exemplary quantities at a $f_{res}$ of 1 GHz range from 0.1 dB, 0.5 dB, 1 dB, 3dB, 5 dB, 10 dB (or anywhere therebetween) in amplitude; 1 degree, 10 degrees, 25 degrees, 45 degrees, 90, degrees, 180 degrees (or range therebetween) in phase; 1 KHz, 3 KHz, 5KHz, 10 KHz, 100 KHz, 1 MHz, 10 MHz, 100 MHz (or range therebetween) in frequency; or a combination of two or more of these quantities. The aforementioned frequencies may be scaled accordingly with a higher or lower $f_{res}$ frequency. For example, the preprogrammed/predefined frequency may range from 10 Hz to 1 MHz (or range anywhere therebetween) for a $f_{res}$ at 10 MHz.

If at 327, the difference between the baseline and test sample response does not exceed the predefined quantity, the detection system 300 indicates that, within the specified frequency range $f_{start}$ to $f_{stop}$ , a molecular event has not been detected (process 328). This indication can be communicated to the user in a number of different ways, for instance communicating the aforementioned message to the user, graphically illustrating the measured difference in amplitude, frequency, and/or phase between responses 334 and 335, supplying measurement data or other output means. It is noted that the illustrated frequency range $f_{start}$ to $f_{stop}$ can encompass two or more smaller frequency ranges within which detection of the binding event or substructure can be expected.

If at 327, the difference between the baseline and test sample response exceeds the predefined quantity, the detection system 300 indicates that that a molecular event has been detected within the detection region 155 (process 329). This indication can be communicated to the user in ways similar to that described above. In a preferred embodiment, the test sample response 335 is subsequently stored for later retrieval and comparison with other test sample responses (process 330).

Information as to the identity of the detected analyte can be obtained in a variety of ways. If the detection region 155 has been functionalized to specifically bind a particular analyte, the identity of the bound analyte can be determined therefrom.

In another embodiment in which non-specific binding occurs within the detection region 155 or where no molecular binding occurs, identification of the detected binding event is a multiple-step process. Initially, a known analyte is added to a buffer and supplied over the detection region of the fluid channel. Next, using the aforementioned measurement probe and methodology, the signal response 335 of the known test sample is obtained and stored. The analyte's identifier (the analyte's name, alphanumeric code or sequence, or other identifier assigned thereto) is subsequently

associated with the test sample response 335 and stored in a database from which it can be retrieved and compared against another test sample response. In the instance in which another test sample response closely correlates to the stored response 335, the identity of the molecular event within the other test sample can be ascertained.

The process continues at 331 where a determination is made as to whether another measurement is to be made. This process can be employed for instance in high throughput automated molecular detection systems. If one or more measurements remain, the process returns to step 324 where another test sample is introduced into the detection region 155 using one of the aforementioned processes described herein. If no other measurements are to be made, the measurement process concludes at 332.

In an extension of the illustrated embodiment, the detection system 300 can include N measurement probes $230_i$, for instance, ninety-six probes, each probe coupled to a well in a ninety-six well micro titer plate. Each probe would be coupled to a respective N number of detection regions $155_i$, for instance the bottom portion of each of the wells in the micro titer plate. In this embodiment the detection system will preferably include a 1x N switch matrix to route the test signal to one of the N detection regions $155_i$, and a Nx1 switch matrix to route the modulated signal from one of possible N detection regions to the signal detector. In an alternative embodiment, each probe $230_i$ and detection region $155_i$ can be designed to exhibit a distinct resonant frequency, in which case all of the detection regions $155_i$ can be interrogated over a continuous frequency spectrum. Other possible configurations of the detection system 300 will be apparent to those skilled in the art.

Fig. 3D illustrates a method for detecting molecular binding events occurring within a test sample in accordance with the present invention. The process begins in a manner similar to Fig. 3B above: designing the measurement probe having a resonant response at/near a predefined frequency, positioning the probe head proximate to the channel's detection region, supplying the analyte-free buffer to the detection region, and tuning the probe to/near the buffer resonant point (steps 340-343).

Next at 344, the first test sample containing the first analyte is supplied to the detection region. In the preferred embodiment, the analyte is supplied in a 1x concentration, for instance 6 mg/ml. The response (353, illustrated in Fig. 3E) is obtained and stored at 345. Next at 346, a second test sample containing the second analyte is supplied to the detection region, preferably in the same concentration as the first analyte (6 mg/ml in the illustrated example). Preferably, the detection region is flushed with a

cleaning agent to remove any residual portions of the first analyte. This process may be performed using spacer material as described herein. The response for the second test sample (355, illustrated in Fig. 3E) is subsequently obtained and stored (step 347).

Next at 348, the first and second analytes mixed in a third sample. Preferably, the first and second analytes are mixed at 0.5x concentration (each at 3 mg/ml in the illustrated example) to maintain the same total concentration of analytes relative to the measured first and second samples. The third sample is then supplied to the detection region, the response obtained and stored (step 349). Alternatively, the two analytes may be introduced and mixed at/near the detection region (for instance using the stopped-flow systems described above) in order to monitor the change in signal response in real time.

Detection of binding events occurring between the first and second analytes is determined by comparing the buffer baseline response, the first test sample response 353, the second test sample response 355, and the third test sample response (either 357 or 359).

Fig. 3E illustrates embodiments of the baseline buffer response 351, the first test sample response 353, the second test sample response 355, and two possible embodiments of the third sample signal response, 357 corresponding to an unbound condition and 359 corresponding to an bound condition between the first and second analytes. As can be seen from the traces 353, 355, and 357, when the two analytes are unbound within the third test sample, the third test sample response 357 indicates substantially an average value between the first and second test sample responses 353 and 355. When the first and second analytes bind within the third test sample, the response 359 is distinguishable in magnitude and frequency (as well as phase) from the average of the two samples.

The test system can be preprogrammed to calculate an average response given the first and second test sample responses, calculate the difference between the calculated response and the measured test sample response, and determine from that difference whether binding has occurred (the more closely correlated the calculated and measured responses are, the more likely binding has not occurred). In a specific embodiment, a predefined amplitude and frequency window is computed around the calculated average value of the two responses. Measured responses occurring outside of the predefined amplitude/frequency window indicate binding, and measured responses occurring within the window are indicative of binding. The amplitude/frequency window at 1 GHz may range in amplitude from 0.1 dB, 0.3 dB, 0.5 dB, 1 dB, 3 dB, 5 dB, 10 dB,

and in frequency from 0.1 KHz, 0.5 KHz, 1 KHz, 3 KHz, 5 KHz, 10 KHz, 30 KHz, 50 KHz, 100 KHz, 1 MHz, 3 MHz, 10 MHz, 100 MHz (or range anywhere therebetween). The aforementioned frequencies may be scaled accordingly with a higher or lower $f_{res}$ frequency. For example, the preprogrammed/predefined frequency may range from 10 Hz to 1 MHz (or range anywhere therebetween) for a $f_{res}$ at 10 MHz.

In an alternative embodiment of the process of Fig. 3D, the measurement step of 345 includes re-adjusting the tuning element 233 such that the probe is tuned to a resonant response in the presence of the first test sample. This response can be subsequently stored and arithmetically subtracted from second test sample measurement 347 and/or the combined test sample measurement 349.

Fig. 3F illustrates a second method for detecting molecular binding events in a sample comprising two analytes. This method closely parallels that of Fig. 3D, except that the first and second analytes are mixed in the third sample at 1x concentrations (*i.e.*, 6 mg/ml each) at step 367.

In this embodiment, the absence of binding is detectable by analyzing the shift in the resonant frequency. In particular, when binding does not occur between the first and second analytes in the third sample, the signal response of the third sample will exhibit a resonant frequency that is substantially $f_{res}$ plus the sum of the changes in the resonant frequency points of the first and second samples responses. An example of the first, second and third sample responses are shown in Fig. 3G. When binding occurs the resonant frequency response of the third sample will vary substantially from the resonant frequency summation shown in Fig. 3G. An amplitude/frequency window such as that described above may be calculated from the measured responses of the first and second samples. A signal response occurring within the predefined window will indicate that binding between the two analytes has not occurred.

Detection and identification of analytes have been described above using a resonant probe by monitoring changes in the probe's resonant signal response. Those of skill in the art in high frequency circuits and systems will appreciate that a non-resonant probe (one embodiment of which is shown in Fig. 2E) can be used to detect and identify analytes. The process closely parallels the steps illustrated in Figs. 3B and 3D, the exception being that the baseline and test sample responses will be taken over a broad frequency range, typically on the order of hundreds of MHz or GHz. The broader band response provides advantages in that the dielectric properties of the sample will be

interrogated over a wide range of frequencies, causing its signal response to vary significantly over the broad frequency range. A significant variation in a measured response can be used to indicate that a molecular event is occurring within the test sample. Additionally, once the signal response is correlated to the molecular event, the response can be used to identify the particular molecular event in a subsequently tested unknown sample.

Fig. 4A illustrates another embodiment of the molecular detection system comprising a time domain measurement system 400. The system 400 includes a pulse signal source 410 and a detector 412 coupled to a detector assembly via a signal path such as a coaxial cable, transmission line or other transmission medium 420. An additional pulse source and detector can be used to provide a complete two-port measurement capability. In a specific embodiment, the pulse signal source 410 and the detector 412 are integrated within a time domain reflectometer system, such as model number 11801 manufactured by the Tektronix Corporation of Beaverton, Oregon. Other high frequency measurement systems, such as network analyzers having a time domain measurement mode can alternatively be used.

During a time domain measurement, an incident signal 422 consisting of a pulse is produced and launched along the transmission line 420 towards the detector assembly 100. In one embodiment the pulse consists of a square wave, although other pulse shapes can be used in alternative embodiments under the present invention. The dielectric properties of the molecule(s) in solution will cause a portion of the incident pulse to be reflected toward the signal detector 412. The reflected signal 424 will exhibit a unique shape and/or time delay which is characteristic of the molecules dielectric properties. Thus, the pulse shape and delay of the reflected signal 424 can be used to characterize and identify the molecule(s) in solution. The time domain test system 400 can be used separately or in conjunction with a scalar or vector network analyzer to identify one or more unknown molecules in solution.

As known in the art, the dielectric relaxation frequency of molecule is the rate at which the dielectric properties of the molecular level changes when an electric field is applied to the molecule. As with the dielectric properties of the molecule, the dielectric relaxation frequency is primarily defined by the structure and binding geometries unique to each molecule. Thus once measured, the dielectric relaxation frequency of a molecule can be used to identify it.

The dielectric relaxation frequency can be determined by measuring the rate at which the analyte absorbs power over frequency. Fig. 4B illustrates one embodiment of a system 450' for making this type of measurement. The system 450 is similar to the time domain measurement system 450 illustrated in Fig. 2A and includes a pulse signal source 460 and a detector 462 coupled to the detector assembly 100. An additional pulse source and detector can be used to provide a complete two-port measurement capability. In a specific embodiment, the measurement system 450 consists of a time domain reflectometer such as describe above wherein the input signal comprises a pulse train having an adjustable pulse interval. Those of skill in the art will appreciate that other instruments capable of generating similar pulse trains and detecting their corresponding resultant signals can be used in alternative embodiments under the present invention.

The incident signal 480 consists of separate pulse groups 482 and 484, each group having two or more incident pulses and a different pulse interval. The pulse groups 482 and 484 are launched along the transmission line toward the portion of the transmission line that is to the detection region 155. If the pulse group 482 has a pulse interval substantially equivalent to the dielectric relaxation period of the analyte (the reciprocal of the relaxation frequency), the analyte will absorb successively less energy in succeeding pulses. The decrease in signal absorption can be measured in the reflected response 490 at detector 462. As an alternative measurement quantity, the remaining signal power can be measured at the detector 462 as well.

The rate of change of signal absorption and the pulse interval at which the change occurs can then be plotted and used to characterize and identify the unknown analyte and/or a binding event involving the analyte. This system characterization can be used independently or in conjunction with the above-described time and/or frequency domain test systems.

In all of the above systems, one of skill in the art will readily appreciate that such systems can be scaled down to the chip level using such technologies as Microwave Monolithic Integrated Circuit (MMIC) and the like. Such miniaturized systems can be readily extended to highly parallel systems capable of detecting and measuring hundreds, thousands, or tens of thousands of compounds simultaneously. These systems can be configured to yield "logic gates" which are switched by the binding event itself, such as by changing a characteristic impedance and thus the transmission and/or reflection coefficients, or by changing the band pass properties of such a circuit, and using this as the on/off gate.

V. Exemplary Applications

Using the above described systems and methods, the present invention can be used in a variety of applications. In one aspect, the present invention can be used to identify substructures or binding events involving analytes, for example proteins, in the primary binding stage. In the calibration phase the responses of a large number of known proteins can be determined and stored. After introducing an unknown protein to the detection region, the dielectric properties of the system can be measured and the dielectric properties of the signal used to identify the protein's properties. Because each protein's fingerprint response is stored, the unknown response can be compared with the stored responses and pattern recognition can be used to identify the unknown protein.

In another embodiment, the invention can be used in a parallel assay format. The device will have multiple addressable channels, each of which can be interrogated separately. After delivering a test sample or samples to the device, responses at each site will be measured and characterized. As an example, a device of this type can be used to measure and/or identify the presence of specific nucleic acid sequences in a test sample by attaching a unique nucleic sequence as the antiligand to the detection region or a part thereof. Upon exposure to the test sample, complementary sequences will bind to appropriate sites. The response at each site will indicate whether a sequence has bound. Such measurement will also indicate whether the bound sequence is a perfect match with the antiligand sequence or if there are one or multiple mismatches. This embodiment can also be used to identify proteins and classes of proteins.

In another embodiment, this invention can be used to generate a standard curve or titration curve that would be used subsequently to determine the unknown concentration of a particular analyte or ligand binding curve. For example, an antibody could be attached to the detection region. The device could be exposed to several different concentrations of the analyte and the response for each concentration measured. Such a curve is also known to those skilled in the art as a dose-response curve. An unknown test sample can be exposed to the device and the response measured. Its response can be compared with the standard curve to determine the concentration of the analyte in the unknown test sample. Similarly, binding curves of different ligands can be

compared to determine which of several different ligands has the highest (or lowest) affinity constant for binding to a particular protein or other molecule.

In another embodiment, this invention can be used to internally self-calibrate for losses due to aging and other stability issues. For example with antibody-antigen systems, this invention allows one to measure the amount of active antibody in a test sample by measuring a primary response before exposure to the test sample of unknown activity. The responses are compared to determine the amount of active antibody that remains.

The detector assembly can used to provide information about numerous properties of the test sample, such as the detection and identification of molecular binding events, analyte concentrations, changes in dielectric properties of the bulk test sample, classification of detected binding events, and the like. In addition, the detector assembly includes a self-calibration capability, which is useful in point-of-use quality control and assurance. Each of these methods and capabilities are further described below. Based upon the described methods and structures, modifications and additional uses will be apparent to those skilled in the art.

Detecting Molecular Structures

The present invention enables the detection of the presence of a molecular structure or of molecular binding events in the detection region 155 of the detection system. Detectable binding events include primary, secondary, and higher-order binding events. For instance, mixing of two test solutions can lead to the detection of binding between ligand/antiligand pairs. For example, a solution is provided which contains the subject molecule or molecular structure. A test signal is propagated along the signal path. Alternatively, the test signal can be launched during or shortly after a mixing operation in order to observe in real time the signal response occurring as a result of binding events. The test signal is recovered, the response of which indicates detection of the analyte, substructure, or binding event.

The dielectric properties of the test sample can contribute to induce any number of signal responses, each of which can be indicative of molecular binding. For instance, the dispersive properties of the test sample can vary dramatically over frequency. In this instance, the test signal response will exhibit large changes in the amplitude and/or phase response over frequency when molecular events occur in the

detection region, thereby providing a means for detecting molecular binding events or other time dependent events after the mixing of test samples.

In another embodiment, the dielectric relaxation properties of the test sample in the detection region will vary as a function of pulse period of the input signal. In this instance, the test signal response will indicate a change in the amount of power absorbed, or change in some other parameter of the test signal like phase or amplitude, at or near a particular pulse period. By observing a change in the absorbed power or other parameters, binding events can be detected. Other quantities such characteristic impedances, propagation speed, amplitude, phase, dispersion, loss, permittivity, susceptibility, frequency, and dielectric constant are also possible indicators of molecular presence or binding events. Important information regarding molecular properties can also be determined by measuring signals, such as these, during changes in the environment of the molecular structure being detected (such as changes in pH or ionic strength).

The above-described method can be used to detect the primary binding of an antiligand and ligand. Similarly, the process can also be used to detect secondary binding of a ligand to an antiligand. The method not limited to detection of primary or secondary binding events occurring along the signal path. Indeed, tertiary, and higher-order binding events occurring either along the signal path or suspended in solution can also be detected using this method.

For example, initially a primary binding event is detected and the signal response measured, as described herein. Subsequently, the primary binding event signal response is stored and used as a baseline response. Next, a second molecular solution is added to the assay device. Detection steps are repeated to obtain a second signal response. Next, the second signal response and the baseline response are compared. Little or no change indicates that the two signal responses are very close, indicating that the structural and dielectric properties of the test sample have not been altered by the addition of the molecules within the new solution. In this case, secondary binding has not occurred to a significant degree. If the comparison results in a change outside of a predetermined range, the structure and/or dielectric properties of the test sample have been altered, thereby indicating secondary binding events. Quantities which can be used to indicate secondary binding events will parallel the aforementioned quantities, *e.g.*, amplitude, phase, frequency, dispersion, loss, permittivity, susceptibility, impedance,

propagation speed, dielectric constant as well as other factors. Tertiary or high-order binding events can be detected using this approach.

An alternative method of detecting secondary or higher order binding events does not required prior knowledge of the specific primary binding event. In this embodiment, the assay device is designed in the assay development stage to operate with known parameters, so that whenever a pre-defined change in one of these parameters is detected, for example at the point-of-use, the binding event or events are then known to have occurred. In this embodiment, the pre-measurement of a primary binding event is not necessary, as the initial characterization has already been done either at the time of fabrication or at the time of design.

Secondary binding events can also be achieved by detecting changes in the structure of the primary molecules structure. When a molecule becomes bound, it undergoes conformational and other changes in its molecular structure relative to its unbound state. These changes affect the primary binding molecule's dielectric properties as well as inducing changes in the surrounding solution, the variation of which can be detected as described above. Quantities that can be monitored to indicate a change in the dielectric properties of the primary bound molecule include the aforementioned quantities, *e.g.*, amplitude, phase, frequency, dispersion, loss, permittivity, susceptibility, impedance, propagation speed, and dielectric constant, as well as other factors.

Detecting Changes in the Dielectric Properties of the Test sample

The detection systems described herein can also be used to measure the dielectric changes of the test sample as a result changes in temperature, pH, ionic strength and the like. The process closely parallels the disclosed method for identifying binding events, the exception being that the method allows for the detection and quantitation of changes in dielectric properties of the test sample without reference to a binding event.

The process when a solution having an initial dielectric property is added to the detector assembly. The signal response is measured and recorded, as previously described. After a predetermined time or operation, a second measurement is made and a second signal response is recorded. A comparison is then made between the first and second signals to determine whether the two signals correlate within a predefined range. If so, the properties of the solution are deemed to not have undergone any dielectric changes.

If the signal responses do not correlate within a predefined range, at least dielectric property of the solution will have undergone a change. Optionally, the change in dielectric properties can be quantitated. For example, the second signal is stored and correlated to a known signal response. The closest correlated response will identify the dielectric property of the solution and the first signal response can be correlated to the initial value of the dielectric property, the difference of which can be used to determine the amount by which the identified dielectric property has been altered.

Identifying Molecular Structures

Using the described detector assemblies, it is possible to characterize a known analyte and subsequently identify it in a solution having an unknown analyte make-up. For example, a large number of molecular structures and/or substructures are measured and their responses stored using one or more of the measurement systems, described below. Each stored response will correspond to a single structure/substructure occurring within the solution or multiple structures/substructures occurring within the same solution. Subsequently, a measurement is made of an unknown solution. Next, the signal response of the solution is compared to the stored signal responses to determine the degree of correlation therewith. The unknown molecular structure is identified by selecting the stored response which exhibits the closest correlation to the unknown response. The comparison can be performed using one or more data points to determine the correlation between one or more stored responses, and can involve the use of pattern recognition software or similar means to determine the correlation. The process can be used to identify an individual structure/substructure, as well as primary, secondary or higher-order bound molecular structures.

Identifying Classes of Molecular Structures

It is also possible to characterize known molecular sub-structures such as domains or other structural homologies that are common to similar classes of proteins or sequence homologies in nucleic acids. In one embodiment, the process proceeds as shown in section D immediately above, except that a number of molecular sub-structures are measured and their responses stored. Each stored signal response will correspond to one or more sub-structures. The process continues until a sufficient number or structures have been detected and characterized to identify the unknown compound. Once a

sufficient number of correlations occur, it is then possible to classify the unknown molecular structure.

There are other processes by which unknown analytes can be classified. One process identifies the unknown analyte by detecting binding to structural motifs on the unknown compound. Initially, a detector assembly can be provided which has multiple addressable parallel channels, each of which has a antiligand for a specific ligand sub-structure bound in the detection region. Next, the presence of particular sub-structures is detected by the binding of each to its respective antiligand and subsequent characterization. In one embodiment, this step is performed as described above, but other variations can be carried out as well. Subsequently, each of the binding events is then characterized by identification of qualities such as affinity, kinetics, and spectral response. A correlation is then made between the known and unknown responses. If each of the unknown responses correlates to known responses, the ligand is identified as the ligand corresponding to the known response. If the sub-structures exhibit both correlated and uncorrelated responses, the correlated responses can be used to construct a more general classification of the unknown ligand. This process can be used to identify any molecular structure, for example proteins, which occur within the same class or have re-occurring structural homologies.

It is also possible that an intensive spectral analysis of a given unknown compound could lead to insights on structure and function, as comparisons can be made to known structures, and extrapolation will lead to some level of classification.

Specific vs. Non-Specific Binding

Specific binding can be distinguished from non-specific binding by the spectral fingerprint of the binding events. Indeed, any two binding events, such as the binding of one molecular structure on separate occasions with two similar but different molecular partners, can generally be distinguished by the spectral fingerprints of the two binding events. For example, a given binding event of interest, such as antibody binding to antigen, can be first characterized in a purified solution containing just the ligand of interest and the antiligand specific to the ligand. A broad spectral study is then carried out to see when in the spectrum the strongest responses are found. The assay is then repeated in the solutions typically found in the dedicated applications, for example whole blood, to determine what effects non-specific binding has on the response. Then various points are found which are determinate of specific binding, and a separate set of points

are found which are determinate of non-specific binding, and a subset of these frequency points are chosen for the actual assay application. By comparing the response due to specific binding with those due to the non-specific binding, the extent of specific binding can be determined.

Characterization of a Given Analyte

Often it is desirable to determine certain qualities of a given molecule. Examples in include determining the class to which a protein belongs, or which type of polymorphism a given gene or other nucleic acid sequence is. This can be done in a number of ways. Proteins are often classified by number and types of structural homologies, or particular substructures which are found in the same or similar classes of proteins. For example, G-Proteins commonly found in cell membranes and which mediate signal transduction pathways between the extra-cellular environment and the intra-cellular environment, always have a structure which traverses the cell membrane seven times. Such a structure is virtually definitive of a G-Protein. Other classes of proteins have similar structural homologies, and as such, any method which can distinguish one class of proteins from another on the bases of these homologies is of enormous use in many of the biomedical research fields. Given that the dielectric properties of a given molecule is determined by the geometry of the charge distribution of the molecule, and further given that most proteins have a unique structure or geometry, then each protein can be uniquely determined by measuring the dielectric properties of the protein. Thus a simple dielectric signature, such as the ones generated by the present invention, can serve to uniquely identify a given protein, and further, can allow classification of the protein into some previously known class of proteins. A further refinement can be added to the classification methodology by using a group of anti-ligands on the detector assembly which are specific for particular sub-structures of a given protein. For example, a group of antibodies that are specific for particular sub-structures, such as domains, can be utilized for the determination of the existence or absence of the sub-structures. Thus, any given protein can be characterized by determining both the presence and absence of certain sub-structures as well as the dielectric properties of the protein itself. Further refinements to this classification strategy can include looking at temperature, pH, ionic strength, as well as other environmental effects on the above-mentioned properties.

Nucleic acids can also be characterized by following a similar paradigm. For example, a given gene can be known to have a certain base pair sequence. Often

times in nature there will be small variations in this sequence. For example, in the gene which codes for a chloride ion transport channel in many cell membranes there are common single base-pair mutations, or changes. Such changes lead to a disease called cystic fibrosis in humans. Thus characterizing a given nucleic acid sequence with respect to small variations is of enormous importance. Such variations are often called polymorphisms, and such polymorphisms are currently detected by forming complementary strands for each of the known polymorphisms. Since any given gene can take the form of any one of hundreds or even thousands of polymorphisms, it is often an arduous task to generate complementary strands for each polymorphism. Using the invention described herein, non-complementary binding or hybridization can be detected and distinguished by measuring many of the same physical properties as were described in the previous paragraph: The dielectric properties of the hybridization event can be characterized and correlated to known data, thereby determining the type of hybridization which has occurred—either complete or incomplete. Thus with an antiligand comprised of a given nucleic acid sequence, hundreds of different polymorphisms (as ligands) can be detected by the characterization of the binding event. One of skill in the art will appreciate that further refinements are possible, such as modifying the stringency conditions to alter the hybridization process, or varying the temperature and determining the melting point, which serves as another indicator of the nature of the hybridization process.

In a similar manner, drug-receptor interactions can be characterized to determine is a given binding event results in the receptor being turned on or off, or some other form of allosteric effect. For example, a given receptor can be used as an antiligand, and a known agonist can be used as the first ligand. The interaction is then characterized according to the dielectric response, and this response is saved. Subsequently, compounds that are being screened for drug candidates are then observed with respect to their binding properties with the receptor. A molecule that binds and yields a similar dielectric response is then known to have a similar effect on the receptor as the known agonist, and therefore will have a much higher probability of being an agonist. This paradigm can be used to characterize virtually any type of target-receptor binding event of interest, and represents a significant improvement over current detection strategies which determine only if a binding event has occurred or not. Those of skill in the art will readily appreciate that there are many other classes of binding events in which the present invention can be applied.

Examples of sub-structures which can be used in the above method include: Protein secondary and tertiary structures, such as alpha-helices, beta-sheets, triple helices, domains, barrel structures, beta-turns, and various symmetry groups found in quaternary structures such as $C_2$ symmetry, $C_3$ symmetry, $C_4$ symmetry, $D_2$ symmetry, cubic symmetry, and icosahedral symmetry. [ G. Rose (1979), Heirarchic Organization of Domains in Globular Proteins, *J. Mol. Biol.*134: 447-470] Sub-structures of nucleic acids which can be analyzed include: sequence homologies and sequence polymorphisms, A, B and Z forms of DNA, single and double strand forms, supercoiling forms, anticodon loops, D loops, and TψC loops in tRNA, as well as different classes of tRNA molecules. [ W. Saenger (1984) *Principles of Nucleic Acid Structure.* Springer-Verlag, New York; and P. Schimmel, D. Soll, and J. Abelson (eds.) (1979) *Transfer RNA*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.]

Quantitating Concentrations

The detector assemblies described herein can also be used to quantitate the concentrations of analytes. In one such one embodiment of this process, in which the device is not pre-calibrated, initially anti-ligands are chosen having the appropriate binding properties, such as binding affinity or kinetics, for the measured analyte. These properties are selected such that the anti-ligand's equilibrium constant is near the center of its linear operating region. For applications where the range of concentration is too wide for the use of a single antiligand, several anti-ligands can be used with differing affinities and/or linear operating ranges, thereby yielding a value for the concentration over a much wider range.

Next, the anti-ligands are added or attached to the detector assembly or chip and the device is connected to the measurement system. A decision is then made as to whether the response requires characterization for maximum specificity. If so, a spectral analysis is performed in which the frequency or frequencies where analyte binding has maximal effect on the signal are determined, the regions where the non-specific binding has maximal effect are determined, and the response due to analyte binding is determined. If characterization is not required, or if so, after its completion, the device is calibrated. This step is performed in one embodiment by supplying a known concentration of ligands to the detector assembly and measuring the resulting response. Alternatively, if more data points are needed for the calibration, then a test sample can be

chosen with a different concentration, and the response against this concentration can be measured. Subsequently, an extrapolation algorithm is generated by recording the calibration points from the foregoing response. Next, a test sample of unknown analyte concentration is measured. This step is accomplished in one embodiment by supplying the unknown test sample to the detector assembly, correlating the response to the titration algorithm, and determining therefrom the analyte concentration.

In the event that a given detector assembly is either pre-calibrated, or calibrated by design, the only step required is to mix the binding pairs and measure the response. Such a detector assembly can be realized in many different ways. For example, some circuit parameter, such as impedance or characteristic frequency of a resonant circuit, can be designed to change in a pre-determined way when the binding event occurs, and the amount by which the parameter changes can further be designed to have a dose-response. Thus, a measurement of the circuit parameter will, when analyzed via a suitable algorithm, immediately yield a quantitative value for the concentration of a given analyte or ligand.

Detector Assembly Self-Calibration

The detector assembly possess a self-diagnostic capability and thus a point-of-use quality control and assurance. For a given dedication application, a particular antiligand (primary binding species) will act as an antiligand for some ligand (the secondarily binding species) of interest in the solution. The primary binding species can be attached at the point of fabrication, and the secondary binding species can be attached at the point-of-use. Thus, variations in fabrication—especially the attachment of the primary species—will cause variations in the ability of the device to bind its specific ligand. However, the amount of ligand bound will be in direct proportion to the amount of antiligand bound, thus a ratiometic measurement of the two is possible.

In one embodiment of the process, a molecular binding surface is formed along the signal path by binding the appropriate antibody at various concentrations and characterizing the resulting response for each of these concentrations, yielding some value "x" for each concentration. Next, a similar titration curve is generated for the ligand by measuring the antibody/ligand binding response for several different concentrations of ligand, and a ligand titration curve is pre-determined. Next, a scale factor A is generated by taking the ratio of responses of antibody binding to ligand binding. At the point-of-use, the uncalibrated assay is then first probed to determine the amount of bound antibody

"x" and the scale factor "y" resulting therefrom. The ligand is then applied to the assay and the response is measured, and the response and predetermined titration curve are scaled by the scale factor "y" to determine unknown concentration.

The process can also be modified to allow quantitating the amount of binding in the solution. In the modification, the binding surface of the detector assembly includes antiligands having a predefined affinity and ligand specificity. The solution is subsequently applied to the device, and a response is measured. The signal response will be proportional to the amount of the ligand that has bound. Thus, a titration of any given ligand can be carried out by choosing an antiligand with an appropriate linear operating range—the range in which the equilibrium constant is within a couple of log units of the desired range of concentrations to be detected. The same ratiometic analysis as described above can be applied to yield a robust and precise quantitative assay with internal controls and calibration necessary to insure reliability.

## VI. Software Implementation

Each of the measurement and detection methods described herein can be practiced in a multitude of different ways (*i.e.*, software, hardware, or a combination of both) and in a variety of systems. In one embodiment, the described method can be implemented as a software program.

Fig. 5A illustrates a simplified block diagram of a computer system 510 operable to execute a software program designed to perform each of the described methods. The computer system 500 includes a monitor 514, screen 512, cabinet 518, and keyboard 534. A mouse (not shown), light pen, or other I/O interface, such as virtual reality interfaces can also be included for providing I/O commands. Cabinet 518 houses a CD-ROM drive 516, a hard drive (not shown) or other storage data mediums which can be utilized to store and retrieve digital data and software programs incorporating the present method, and the like. Although CD-ROM 516 is shown as the removable media, other removable tangible media including floppy disks, tape, and flash memory can be utilized. Cabinet 518 also houses familiar computer components (not shown) such as a processor, memory, and the like.

Fig. 5B illustrates the internal architecture of the computer system 510. The computer system 510 includes monitor 514 which optionally is interactive with the I/O controller 524. Computer system 510 further includes subsystems such as system

memory 526, central processor 528, speaker 530, removable disk 532, keyboard 534, fixed disk 536, and network interface 538. Other computer systems suitable for use with the described method can include additional or fewer subsystems. For example, another computer system could include more than one processor 528 (*i.e.*, a multi-processor system) for processing the digital data. Arrows such as 540 represent the system bus architecture of computer system 510. However, these arrows 540 are illustrative of any interconnection scheme serving to link the subsystems. For example, a local bus could be utilized to connect the central processor 528 to the system memory 526. Computer system 510 shown in Fig. 6 is but an example of a computer system suitable for use with the present invention. Other configurations of subsystems suitable for use with the present invention will be readily apparent to of skill in the art.

## VII. Experiments

Using the systems and methods of the present invention, several experiments were performed to detect and identify analytes in a transporting medium. Figs. 6A illustrates the molecular detection system employed and Figs. 6B-6F illustrate the measured results.

Fig. 6A illustrates the molecular detection system used in the following experiments and includes a vector network analyzer model number HP 8714 available from Agilent Technologies, Inc. (formerly the Hewlett Packard Corporation), a computer, the measurement probe, including a grooved cover piece which over which a detection region is formed, and a length of PTFE tube (Cole-Parmer Instrument Company of Vernon Hills, IL) used as a fluid channel to transport the transporting medium and test sample to the detection region of the measurement probe. The resonant probe 230 illustrated in Fig. 2A was used in the experiments. The resonant probe was designed with a $f_{des}$ at 1 GHz and exhibited an $f_{res}$ of about 1.163 GHz assembled and in the presence of the fluid channel (PTFE tube) containing the buffer solution.

The computer executes Labview® software to control the operation of the network analyzer and to display and store data resulting therefrom. A coaxial-type measurement probe as described and illustrated in Fig. 2A above was used. The PTFE tube (0.031" I.D., 0.063" O.D., wall 0.016") was placed over the detection region of the measurement probe and was secured using a grooved top cover which was screwed into the shelf of the measurement probe. The tubing extends from the measurement probe in

two directions. One end of the tubing was connected to a syringe pump (not shown) while the other end was immersed in the fluidic test sample to be analyzed. The syringe pump provided negative pressure that was applied to pull the test sample through the tube and over the detection region. In the following experiments, the spectra of the various test samples were measured while the test sample was moving over the detection region 155. With the syringe pump aspirating fluid at a rate of ~ 0.05 mL/min, different fluidic test samples were introduced in the tubing by removing the end of the tubing from the first test sample, waiting ~1 second to introduce a gap of air ~1 cm in length, and finally by placing the end of the tube into the second test sample. The gap of air is used to prevent mixing of two test samples in the tube. Similar results were obtained when the measurements were made while the fluid was held stationary over the detection region (*i.e.*, the syringe pump was turned off) or when the tubing described above was substituted with a smaller I.D. tubing, such as PTFE, 0.020" I.D., 0.063" O.D., wall 0.021".

The analytes used for the experiment shown in Figs. 6B-6H were carbonic anhydrase II (CA, bovine), fibrinogen (type I-S, bovine), lysozyme (egg white), pepsin (porcine stomach mucosa), ferritin (type I, horse spleen), bovine serum albumin (BSA), water (18 mega ohm), sodium dodecylsulfate (SDS) and transporting medium phosphate transporting medium saline solution (PBS, pH 7.4) were purchased from Sigma (St. Louis, MO). Sodium phosphate monobasic and sodium phosphate dibasic were purchased from EM Science (Gibbstown, NJ). PBS transporting medium was used as received, 25 mM sodium phosphate transporting medium, pH 7.8 was prepare using water (18 mega ohm just prior to use. 1.0% solutions (w/w) of CA, fibrinogen, lysozyme, BSA and pepsin were prepared in 25 mM sodium phosphate transporting medium, pH 7.8 just prior to use. 1.0% solutions (w/w) ferritin and 5% (w/w) SDS were prepared in PBS just prior to use.

In a first experiment, PBS transporting medium was introduced into the tube and transported to the detection region where a $S_{11}$ (return loss) measurement was taken. Next, a Ferritin test sample (1% w/w in PBS) was transported to the detection region and a second $S_{11}$ measurement was taken. The Ferritin measurement was taken four times to indicate measurement repeatability. The $S_{11}$ response of the PBS solution and four Ferritin responses is illustrated in Fig 6B (magnitude in dB) and Fig. 6C (phase in degrees).

As illustrated in Fig. 6B, the magnitude of the $\bar{S}_{11}$ response associated with the PBS solution has a deep null (-60 dB) at 1.163 GHz. Also observable from Fig. 6B are the differences between the PBS and Ferritin $S_{11}$ responses. In particular, the Ferritin $S_{11}$ response illustrates a frequency shifted, less pronounced null compared to the PBS response. One or both of these differences can be used as parameters to detect the presence of analytes as described in the processes above. In addition, the Ferritin $S_{11}$ response (magnitude and/or phase) can be stored and used as an identifying signature to identify an unknown analyte in another test sample as described in the above processes.

Fig. 6C is a further illustration of the difference in magnitude between the PBS and Ferritin $S_{11}$ responses over frequency. At its maximum point, the difference approaches 20 dB, a sufficiently large magnitude to be indicative of analyte presence in the test sample. Further observable is the small measurement variation between Ferritin measurements, which is an indication of high measurement repeatability and stability.

Fig. 6D illustrates the phase of the $S_{11}$ response of the PBS and Ferritin samples and Fig. 6E illustrates the difference therebetween. The phase difference between the PBS and Ferritin measurements is evident, as is the repeatability of the Ferritin sample measurements. Accordingly, phase information can also be used as a parameter to detect analytes in solution in accordance with the process described above.

Next, the above described molecular detection system was used to demonstrate the detection of different analytes. Figs. 6F and 6G illustrates the magnitude and phase $S_{11}$ measurements taken of six analytes: carbonic anhydrase, fibrinogen, lysozyme, BSA, and pepsin and that of the transporting medium (25 mM sodium phosphate transporting medium, pH 7.8). As can be seen, each analyte provides a different and identifiable signal response, thereby enabling detection and identification of each in a test sample as described herein.

Fig. 6H illustrates the $S_{11}$ response (magnitude) of five solutions of sodium chloride using the system described above. The solutions were prepared by serial dilution from a 1.0 M stock solution; all solutions were prepared using de-ionized water. The fluid samples were introduced to the detection region of the coaxial resonating fixture via the tubing setup described previously. Initially, de-ionized water was introduced to the top of the resonant probe operating at $f_{des}$ of 1.2 GHz. The probe was subsequently tuned to the illustrated $f_{res}$ to achieve a reflection measuring -65 dB in magnitude. Each of the

sodium chloride solutions was then introduced and the signal response was recorded. As Figure 6H illustrates, the test system is capable of distinguishing between a 0.10 mM NaCl solution and de-ionized water.

Figs. 6I and 6J illustrate $S_{11}$ signal responses made in detecting specific molecular binding events using the method of Fig. 3D in accordance with the present invention (using equal weight concentrations at ½ x). Fig. 6I illustrates the signal responses for denatured HSA, SAL, and the non-binding mixture, and Fig. 6J illustrates signal responses for native HSA, SAL, and the combined mixture of native HSA binding with SAL. Each of the measured responses exhibited an amplitude resonance (minimum amplitude point) and a frequency resonance (frequency at which the minimum amplitude is measured) described below.

Human serum albumin (HSA) and salbutamol (SAL) were obtained from Aldrich Chemical Company (Milwaukee, WI). Phosphate buffered saline (PBS) was obtained from Life Technologies, Inc. (Grand Island, NY). Stock solution of SAL was prepared in 1x PBS (pH 7.2) at 50 μM concentrations. Stock solution of HSA was prepared in 1x PBS (pH 7.2) at 50 μM concentration. 50 μM concentration HSA was denatured in a 1x PBS buffer for 15 minutes @ 60 degrees C. Newly prepared 50 μM HSA (native and denatured) was pre-incubated with an equal volume of 50 μM SAL for 10 mins. prior to experiments. Signal responses were made for a solution of 50 μM HSA (native and denatured) in 1x PBS (pH 7.2), a solution containing 50 μM SAL in 1x PBS (pH 7.2) and an equal volume mixture of solutions resulting in a final concentration of 25 μM HSA and 25 μM SAL in 1x PBS (pH 7.2). Signal responses were obtained at room temperature using model no 8714 vector network analyzer from Agilent Technologies, Inc. (Palo Alto, CA.) A resonant coaxial probe having a $f_{des}$ of 1.2 GHz was used as the measurement probe.

Referring to Fig. 6I, the buffer response exhibits a $f_{res}$ at of approximately 1232.86 MHz reaching an amplitude resonance at approx. –62 dB. The SAL solution exhibits a slight increase in resonant amplitude (to -60 dB) and a negligible change in the resonant frequency. The denatured HSA solution shows a decrease in amplitude to –73 dB and a shift of about +3.5 KHz from the buffer $f_{res}$ in frequency. The mixture shows a frequency resonance of approx. +1.9 KHz from buffer $f_{res}$ at an amplitude of approx. –67 dB. As can be seen, the non-binding signal response exhibits an amplitude and frequency

resonance at approximately the mean value between the denatured HSA and SAL responses.

Referring in comparison to Fig. 6J, the buffer and SAL responses are as shown in Fig. 6I. Native HSA shifted approx. +2.3 KHz from buffer $f_{res}$ and had a measured amplitude resonance of –55 dB. The combined (binding) mixture shows a frequency shift of approx. +0.7 KHz from the buffer $f_{res}$ and an amplitude resonance of -53 dB, these quantities being clearly distinguishable from the mean value of the SAL and native HSA responses.

Figs. 6K and 6L illustrate $S_{11}$ signal responses made in detecting specific molecular binding events using the method of Fig. 3F in accordance with the present invention (using equal volume 1x concentrations in the combined mixture). Fig. 6K illustrates the signal responses for denatured HSA, SAL, and the non-binding mixture, and Fig. 6L illustrates signal responses for native HSA, SAL, and the combined mixture of native HSA binding with SAL. Each of the measured responses exhibited an amplitude resonance (minimum amplitude point) and a frequency resonance (frequency at which the minimum amplitude is measured) described below.

Stock solutions of salbutamol and HSA were prepared in 1x PBS (pH 7.2) at 200 μM concentrations. HSA at a final concentration of 200 μM was denatured in a 1x PBS buffer with a pH adjusted to 2.73 overnight. Prior to the binding experiment, the PBS buffer was rapidly changed to pH 7.2 by membrane filtration. Newly prepared HSA at 100 μM final concentration was pre-incubated with salbutamol at a final concentration of 100 μM for 10 mins. Signal responses were made for a solution containing 100 μM HSA (either native or denatured) in 1x PBS (pH 7.2), a solution containing 100 μM SAL in 1x PBS (pH 7.2) and a solution containing 100 μM HSA and 100 μM SAL in 1x PBS (pH 7.2). Signal responses were obtained at room temperature using model no 8714 vector network analyzer from Agilent Technologies, Inc. (Palo Alto, CA). A resonant coaxial probe having a $f_{des}$ of 1.2 GHz was used as the measurement probe.

Referring to Fig. 6K, the buffer response exhibits a $f_{res}$ at approximately 1232.82 MHz reaching an amplitude resonance at approx. –62 dB. The SAL solution exhibits a slight increase in resonant amplitude (to -60 dB) and a negligible change in the resonant frequency. The denatured HSA solution shows a decrease in amplitude to –87 dB and a shift of about +7.2 KHz from the buffer $f_{res}$ in frequency. The non-binding

mixture shows a frequency resonance of approx. 7.2 KHz from buffer $f_{res}$ at an amplitude of approx. –63 dB. As can be seen, the non-binding signal response exhibits a frequency resonance ($f_{res}$ + 7.2 KHz) which is approximately equal to $f_{res}$ plus the sum of the frequency shifts due to the non-binding components of SAL (approx. 0 Hz) and HSA (approx. 7.2 KHz).

Referring in comparison to Fig. 6L, the buffer and SAL responses are substantially as shown in Fig. 6K. Native HSA shifted the frequency resonance approx. + 6.3 KHz from buffer $f_{res}$ and had a measured amplitude resonance of –61 dB. The binding mixture exhibits a frequency shift of 10.8 KHz from the buffer $f_{res}$, which is clearly outside of the range for the collective contributions of HSA (6.3 KHz) and SAL (0 Hz).

Fig. 6M illustrates a dose response curve made in accordance with the present invention. HSA was obtained from Aldrich Chemical Company (Milwaukee, WI), PBS was obtained from Life Technologies, Inc (Grand Island, NY), and 16-mecaptohexadeccanoic acid (C16) was purchased from Gateway Chemical Technology, Inc. (St. Louis, MO). Stock of C16 was prepared in DMSO at 1mM concentration. Stock solution of HSA was prepared in 1x PBS (pH 7.2) at 200 μM concentration. HSA was prepared at a final concentration of 10 μM and incubated with C16 at final concentrations range from 0.1 to 1000 μM for 10 min. All of the final solutions contained 5% DMSO. A baseline response was obtained for a solution of 1x PBS containing 5% DMSO (pH 7.2) . Measured responses were obtained for solutions containing various concentrations of C16 in 1x PBS containing 5% DMSO (pH 7.2). Signal responses were obtained at room temperature using model no 8714 vector network analyzer from Agilent Technologies, Inc. (Palo Alto, CA). A resonant coaxial probe having a $f_{des}$ of 1.2 GHz was used as the measurement probe.

Changes in the resonant frequency produced by HSA, C16, and the mixture of HSA with various amounts of C16 were measured, normalized to (divided by) $f_{res}$ of the baseline response of PBS/5%DMSO solution, and plotted versus the concentration of C16. The response (Fig. 6M) is clearly indicative of a saturation curve having a saturation point of approximately 50 μM for C16.

While the above is a complete description of possible embodiments of the invention, various alternatives, modifications, and equivalents can be used. For example,

other transmission mediums, such as conductive or dielectric waveguides, can alternatively be used, as well as other fluid transport systems. Further, 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. Specifically, this application is related to the following commonly owned, co-pending applications, all of which are herein incorporated by reference in their entirety for all purposes:

Serial No. 09/243,193 entitled "Method and Apparatus for Detecting Molecular Binding Events, filed February 1, 1999 (Atty Dkt No. 19501-000200US);

Serial No. 09/243,196 entitled "Method and Apparatus for Detecting Molecular Binding Events," filed February 1, 1999 (Atty Dkt No. 19501-000300US);

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

Serial No. 09/365,978 entitled "Test Systems and Sensors for Detecting Molecular Binding Events," filed August 2, 1999 (Atty Dkt No. 19501-000500);

Serial No. 09/365,581 entitled "Methods of Nucleic Acid Analysis," filed August 2, 1999 (Atty Dkt No. 19501-000600);

Serial No. 09/265,580 entitled "Methods for Analyzing Protein Binding Events," filed August 2, 1999 (Atty Dkt No. 19501-000700);

Serial No. 09/480,846 entitled "Method and Apparatus for Detecting Molecular Binding Events," filed January 10, 2000 (Atty Dkt No. 19501-000310);

Serial No. 09/480,315 entitled "Method and Apparatus for Detecting Molecular Binding Events," filed January 10, 2000 (Atty Dkt No. 19501-000320).

WHAT IS CLAIMED IS:

1. A molecular detection system for detecting a molecular event within a test sample, the system comprising:

(1) a fluid reservoir, the fluid reservoir comprising a detection region having of volume of less than 1.0 mL;

(2) a signal source operable to transmit an electromagnetic incident test signal at a frequency above 10 MHz and less than 1000 GHz;

(3) a measurement probe comprising:

(a) a probe head having:

(i) a wave guide coupled to the signal source, or

(ii) a transmission line, a ground plane, and a dielectric layer interposed between the transmission line and the ground plane, wherein the transmission line is coupled to the signal source;

wherein the probe head is configured to electromagnetically couple the incident test signal to the test sample within the detection region, the interaction of the incident test signal with the test sample producing a modulated test signal, the probe head further configured to recover a portion of the modulated test signal; and

(b) a connecting end; and

(4) a signal detector coupled to the connecting end of the measurement probe and configured to recover the modulated test signal.

2. The molecular detection system of claim 1, wherein the signal detector is configured at a sufficiently high sensitivity to detect that a first modulated test signal is different from a second modulated test signal when the first modulated test signal is obtained while an aqueous sample containing 0.3 μg or less of fibrinogen is present in the detection region and the second modulated test signal is obtained while a second aqueous sample is present in the detection region, the second aqueous sample being identical to the first aqueous sample except that it does not contain any fibrinogen.

3. The molecular detection system of claim 1, wherein the probe head is physically separated from, but electromagnetically coupled to, the test sample within the detection region.

4. The molecular detection system of claim 3, wherein the probe head comprises an open-ended cross section of a coaxial transmission line.

5. The molecular detection system of claim 3, wherein the measurement probe comprises:

a first coaxial section comprising the probe head and a first gap end;

a second coaxial section comprising a second gap end and the connecting end; and

a tuning element adjustably engaged between the first and second gap ends and configured to provide a variable gap distance therebetween.

6. The molecular detection system of claim 5, wherein the tuning element comprises a hollow, electrically conductive tube surrounding the gap ends.

7. The molecular detection system of claim 1, wherein the signal source and the signal detector are included within a vector network analyzer system, a scalar network analyzer system, or a time domain reflectometer.

8. The molecular detections system of claim 1, wherein the fluid reservoir comprises a well of a microtitre plate.

9. The molecular detection system of claim 1, wherein the fluid reservoir comprises a channel configured to transport fluid from one location to another.

10. The molecular detection system of claim 9, wherein the fluid channel comprises an interior channel of a PTFE tube.

11. The molecular detection system of claim 9, wherein the fluid reservoir comprises at least one channel in a microfluidic transport system.

12. The molecular detection system of claim 9, further comprising a fluid transport system configured to move fluid through the channel, wherein the fluid transport system further comprises:

a fluid reservoir configured to store a transport fluid; and

a fluid controller connected to the fluid reservoir and to the fluid channel, the fluid controller configured to control the rate of flow of the transport fluid from the fluid reservoir through the fluid channel.

13. A method for detecting a molecular event in an aqueous test sample, the method comprising:

(1) introducing a first sample into a fluid reservoir having a detection region with a volume of less than 1.0 mL;

(2) applying a test signal of greater than 10 MHz and less than 1000 GHz to the detection region utilizing:

(a) a measurement probe comprising:

(A) a probe head having:

(i) a wave guide coupled to the signal source, or

(ii) a transmission line, a ground plane, and a dielectric layer interposed between the transmission line and the ground plane, wherein the transmission line is coupled to the signal source;

wherein the probe head is configured to electromagnetically couple the incident test signal to the test sample within the detection region, the interaction of the incident test signal with the test sample producing a modulated test signal, the probe head further configured to recover a portion of the modulated test signal; and

(B) a connecting end; and

(b) a signal detector coupled to the connecting end of the measurement probe and configured to recover the modulated test; and

(3) determining whether a change in the test signal occurs as a result of interaction of the test signal with the sample.

14. The method of claim 13, wherein the molecular event is structural or functional similarity of a first molecular substance to a reference molecular substance, wherein the similarity is determined by comparing a test signal detected when the sample contains the first molecular substance to a test signal detected when the sample contains the reference molecular substance.

15. The method of claim 13, wherein the molecular event is binding of a first molecular substance to a second molecular substance.

16. A method for detecting a molecular event in an aqueous test sample, the method comprising:

(a) introducing a first sample into a fluid channel of a fluid transport system, the fluid transport system having a fluid movement controller and the fluid channel having a sample entry end, a detection region, and a sample exit end, the detection region having a volume of less than 1 mL;

(b) causing the sample to move through the channel from the sample entry end toward the sample exit end under the control of the fluid controller;

(c) applying a test signal of greater than 10 MHz and less than 1000 GHz to the detection region of the fluid channel; and

(d) detecting a change in the test signal as a result of interaction of the test signal with the sample.

17. The method of claim16, further comprising:

(e) introducing a spacer material into the channel after the first test sample,

(f) introducing a further test sample into the channel after the spacer material,

(g) causing the further test sample to move through the channel under the control of the fluid controller, whereby a plurality of different test samples separated by spacer material is transported through the channel without intermixing different test samples, and

(f) optionally repeating steps (c)-(d) for the further test sample.

18. The method of claim 17, wherein the spacer material comprises a solution of ionic strength sufficiently high to be transported by electroosmotic pumping and the fluid movement controller utilizes electroosmotic pumping of the fluid.

19. The method of claim 17, wherein the spacer material comprises a fluid that is substantially immiscible with the test samples.

20. The method of claim 17, wherein the spacer material comprises a gaseous bubble and the fluid movement controller utilizes physical pumping of the fluid.

21. The method of claim 16, further comprising:

providing a further fluid channel that intersects the first fluid channel in the fluidic transport system, the system providing separate control of fluid movement in the second fluid channel, the second fluid channel containing a test compound or a series of test compounds,

introducing a test compound from the second fluid channel into a test sample in the first fluid channel sufficiently upstream from the test signal so that the test compound has time to bind with a molecular structure in a test sample in the first fluid channel before the test sample reaches the test signal,

detecting binding by a change in the test signal.

22. A method for detecting a molecular event in a test sample in a detection region of a fluid reservoir, the method comprising:

locating a measurement probe that exhibits a resonant signal response at a predefined frequency in a range from 10 MHz to 1000 GHz proximate to the detection region to electromagnetically couple a signal thereto;

supplying a reference medium to the detection region;

coupling a test signal to the detection region and recording a baseline signal response;

supplying a test sample containing or suspected of containing the molecular event to the detection region;

coupling a test signal to the detection region and obtaining a test sample response;

determining the difference, if any, between the test sample response and the baseline response; and

relating the difference to the molecular event.

23. The method of claim 22, wherein the measurement probe exhibits an $S_{11}$ resonant response.

24. The method of claim 22, wherein coupling a test signal to the detection region and obtaining a baseline signal response comprises:

generating an incident signal;

coupling the incident signal to the detection region;

recovering a reflected signal from the detection region; and

comparing amplitude or phase of the incident signal to amplitude or phase of the reflected signal.

25. The method of claim 24, further comprising:

storing a first test sample response; and

comparing a later test sample response with the stored first test sample response.

26. A measurement probe configured to detect a molecular event in a test sample, comprising:

a first coaxial section comprising a longitudinally extending center conductor, a dielectric insulator disposed around the longitudinal axis of the center conductor, and an outer ground plane disposed around the longitudinal axis of the dielectric insulator, the first coaxial section having a probe head and a first gap end, the probe head comprising an open-end coaxial cross section;

a second coaxial section comprising a longitudinally extending center conductor, a dielectric insulator disposed around the longitudinal axis of the center conductor, and an outer ground plane disposed around the longitudinal axis of the dielectric insulator, the second coaxial section having a second gap end and a connecting end, the gap end comprising a open-end coaxial cross section and the connecting end comprising a coaxial connector; and

a tuning element adjustably engaged between the first and second gap ends and configured to provide a variable gap distance therebetween.

27. The measurement probe of claim 26, wherein the first section further comprises a shelf conductively attached to the outer conductor and substantially flush with the open end of the probe head.

28. A computer-readable storage medium containing information obtained by the method of claim 13.

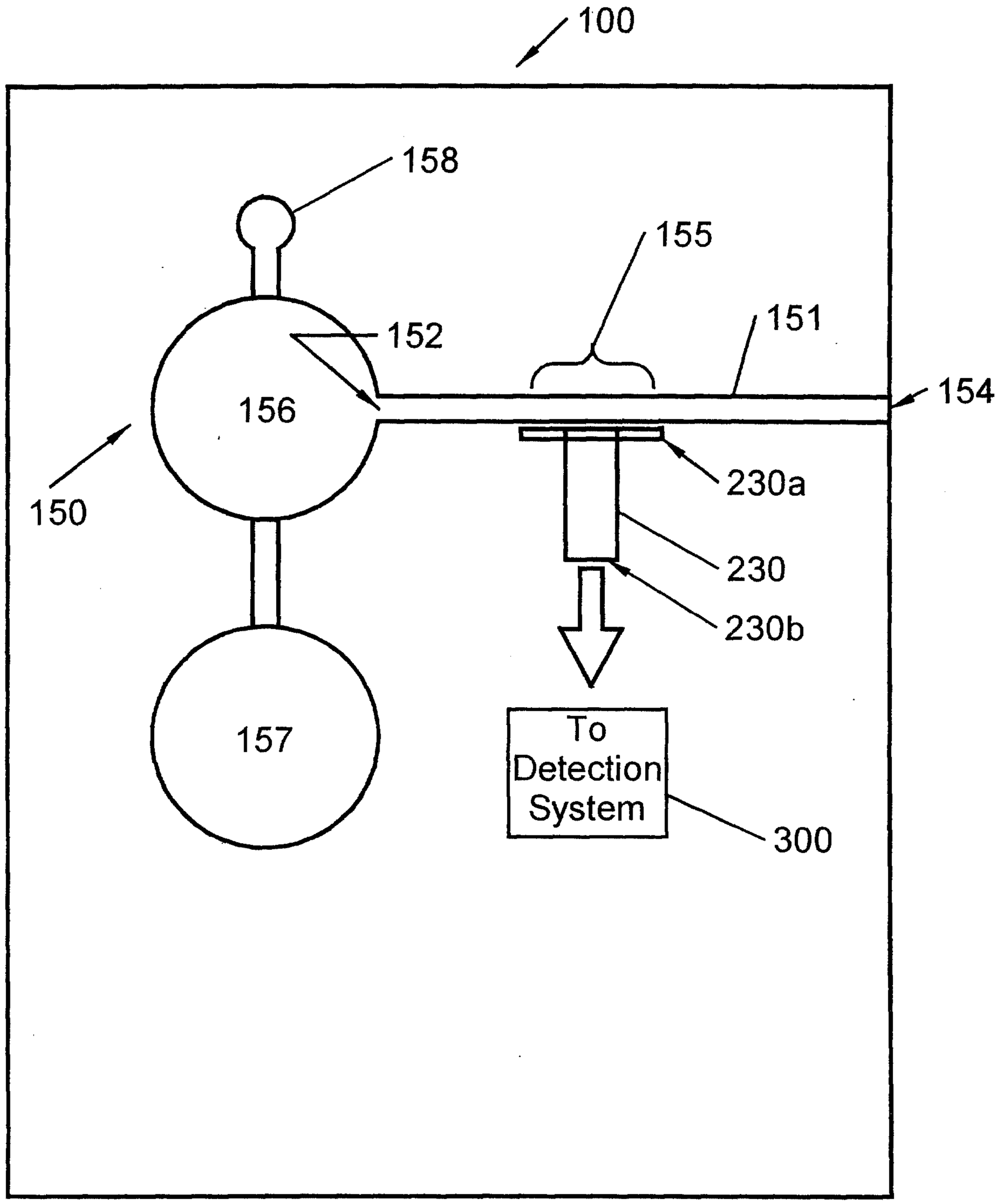

100
158
155
151
152
154
156
150
230a
230
230b
157
To
Detection
System
300

FIG. 1A

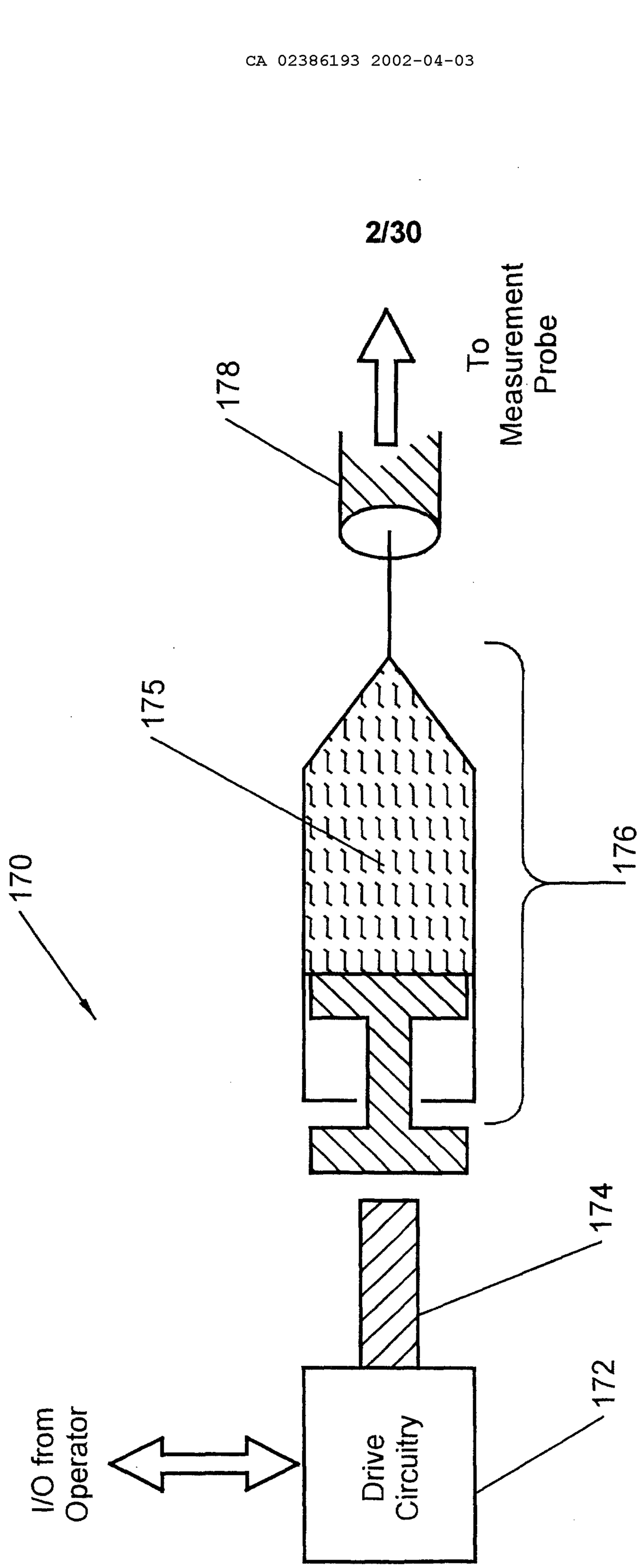

170
175
176
178
174
172
To Measurement Probe
I/O from Operator
Drive Circuitry

FIG. 1B

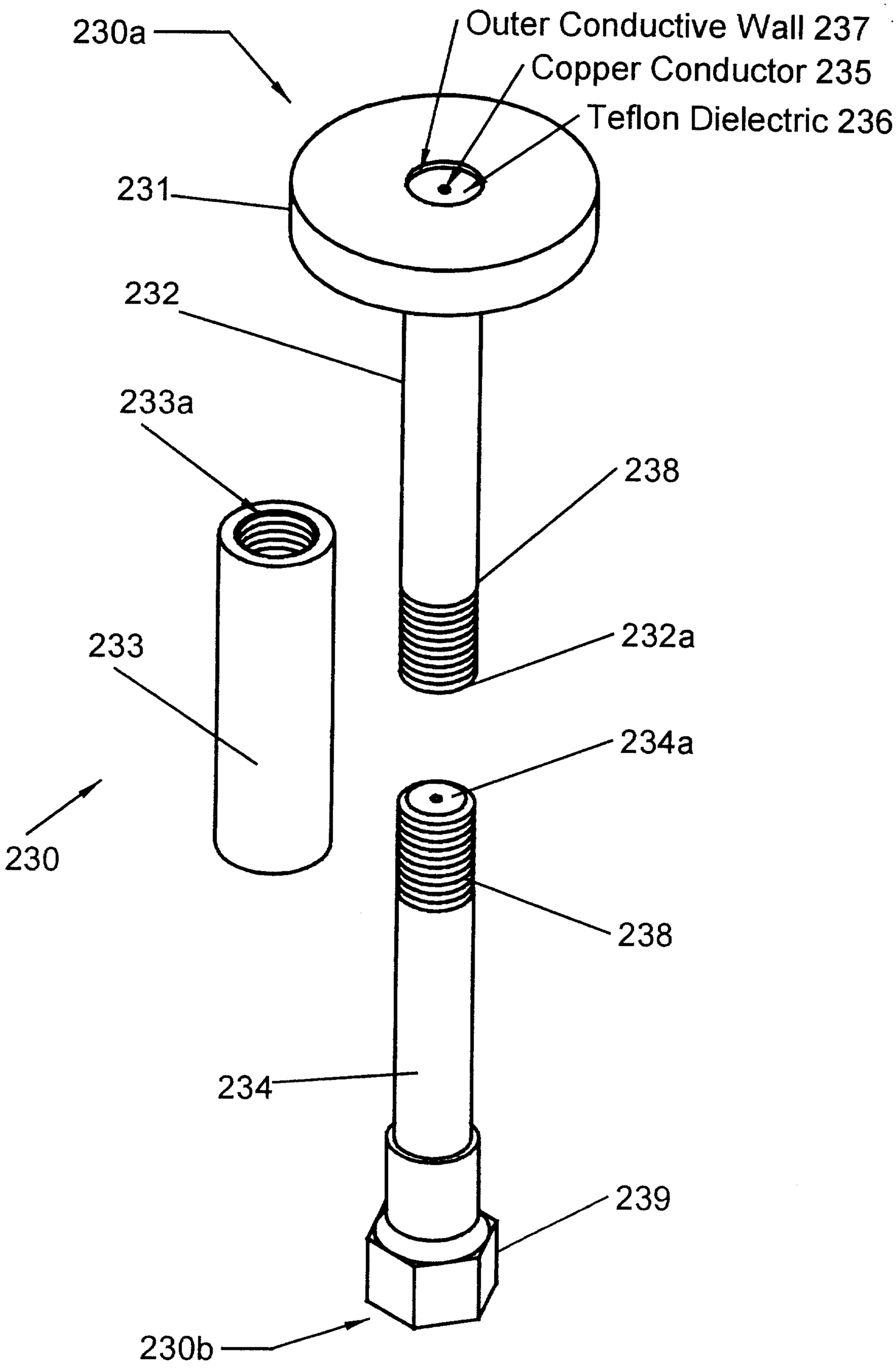

230a
Outer Conductive Wall 237
Copper Conductor 235
Teflon Dielectric 236
231
232
233a
238
233
232a
234a
230
238
234
239
230b

FIG. 2A

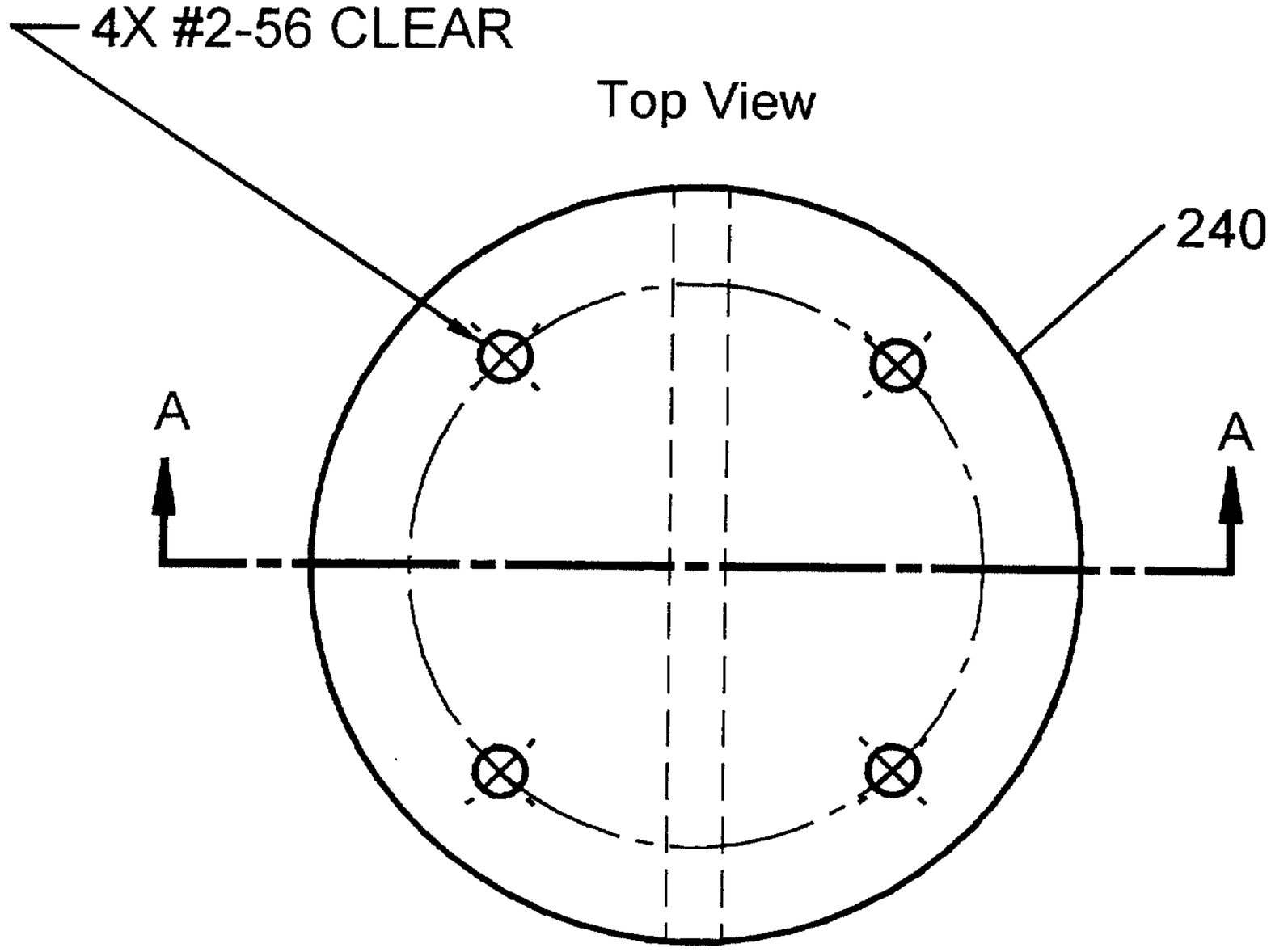



***FIG. 2B***

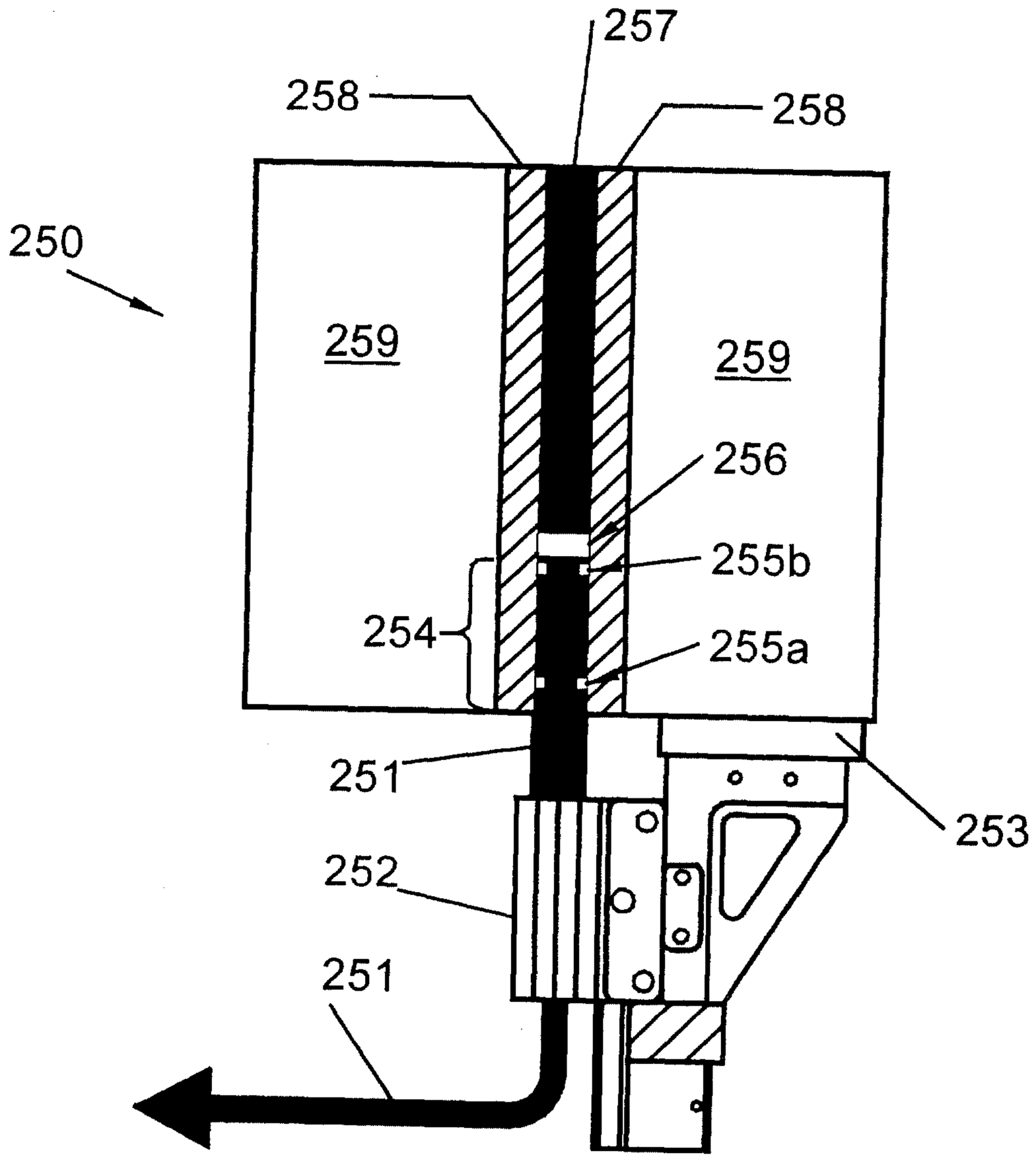

257
258
258
250
259
259
256
255b
254
255a
251
253
252
251
To Signal Source
+
Signal Decoder

*FIG. 2C*

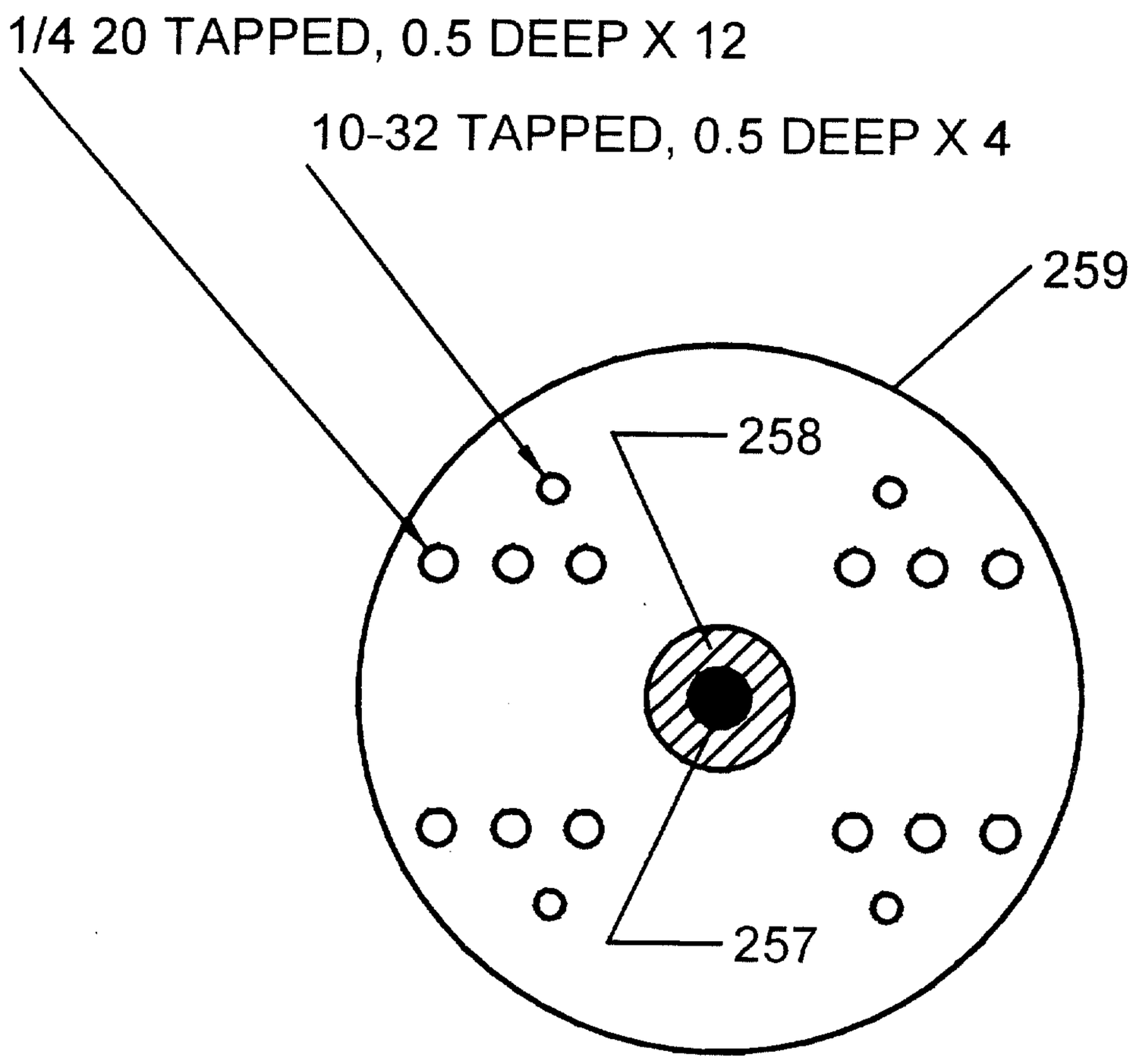



*FIG. 2D*

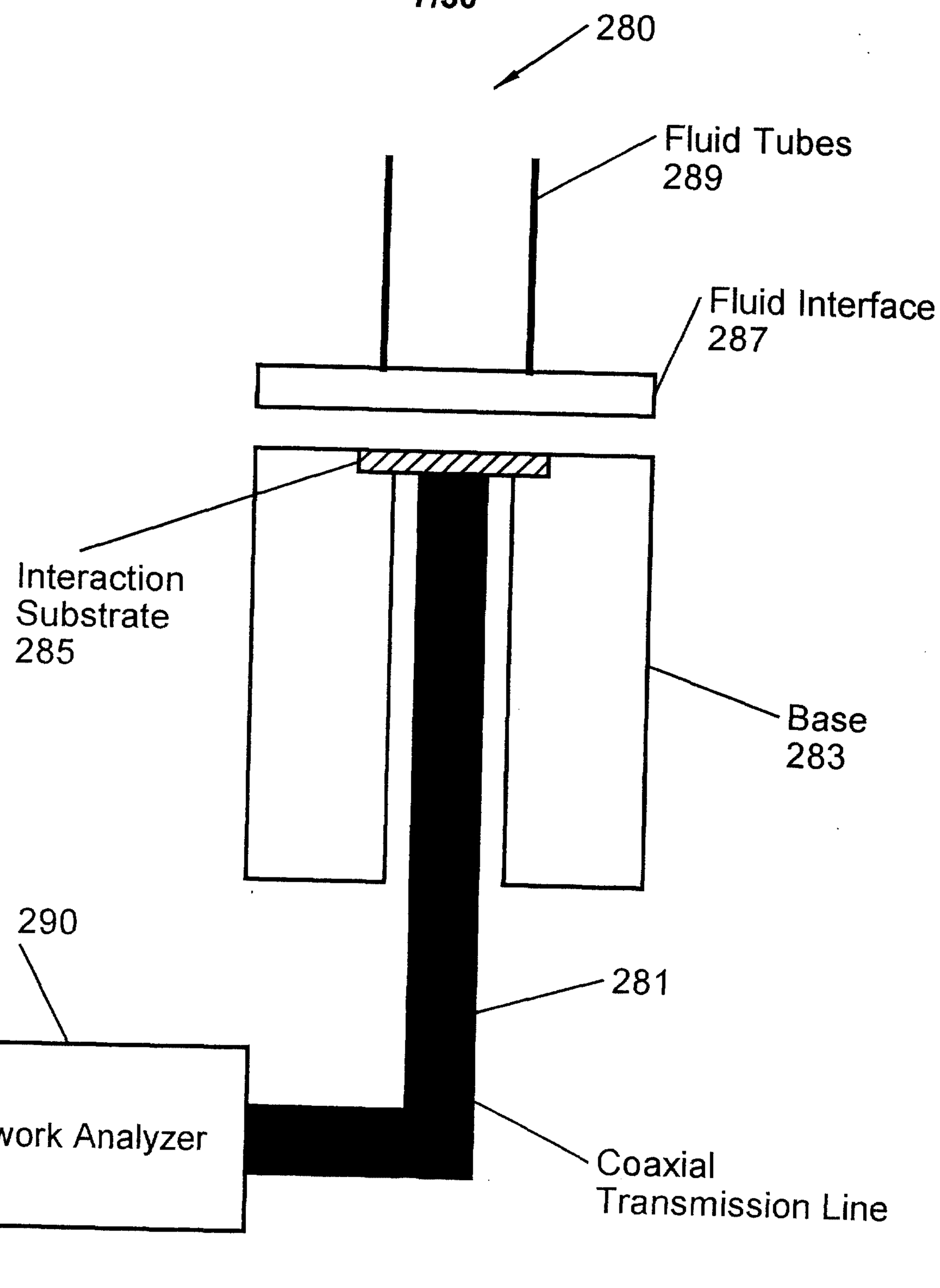

280
Fluid Tubes
289
Fluid Interface
287
Interaction
Substrate
285
Base
283
290
281
Network Analyzer
Coaxial
Transmission Line

*FIG. 2E*

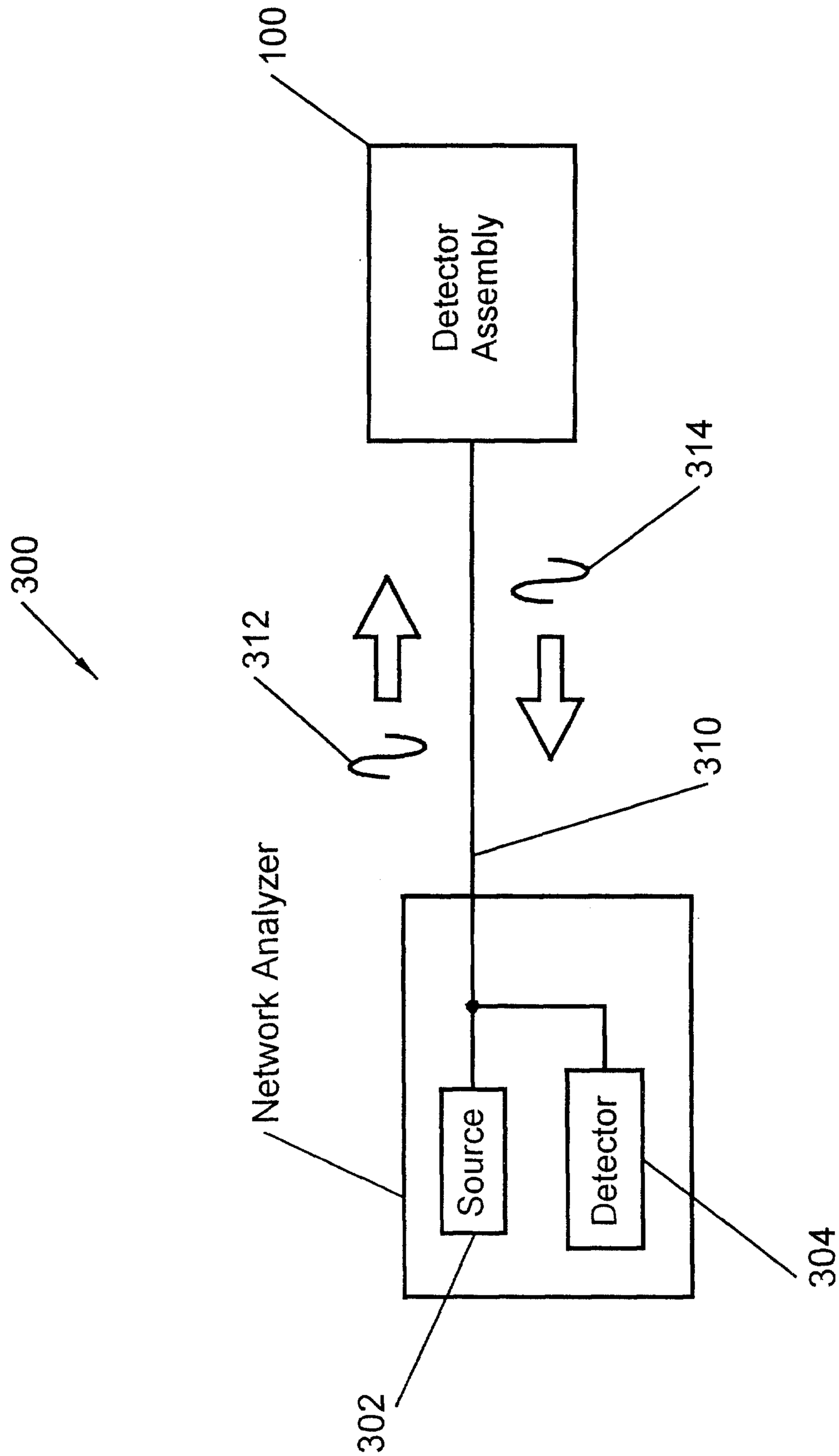

300
100
Detector Assembly
312
314
310
Network Analyzer
Source
Detector
302
304

FIG. 3A

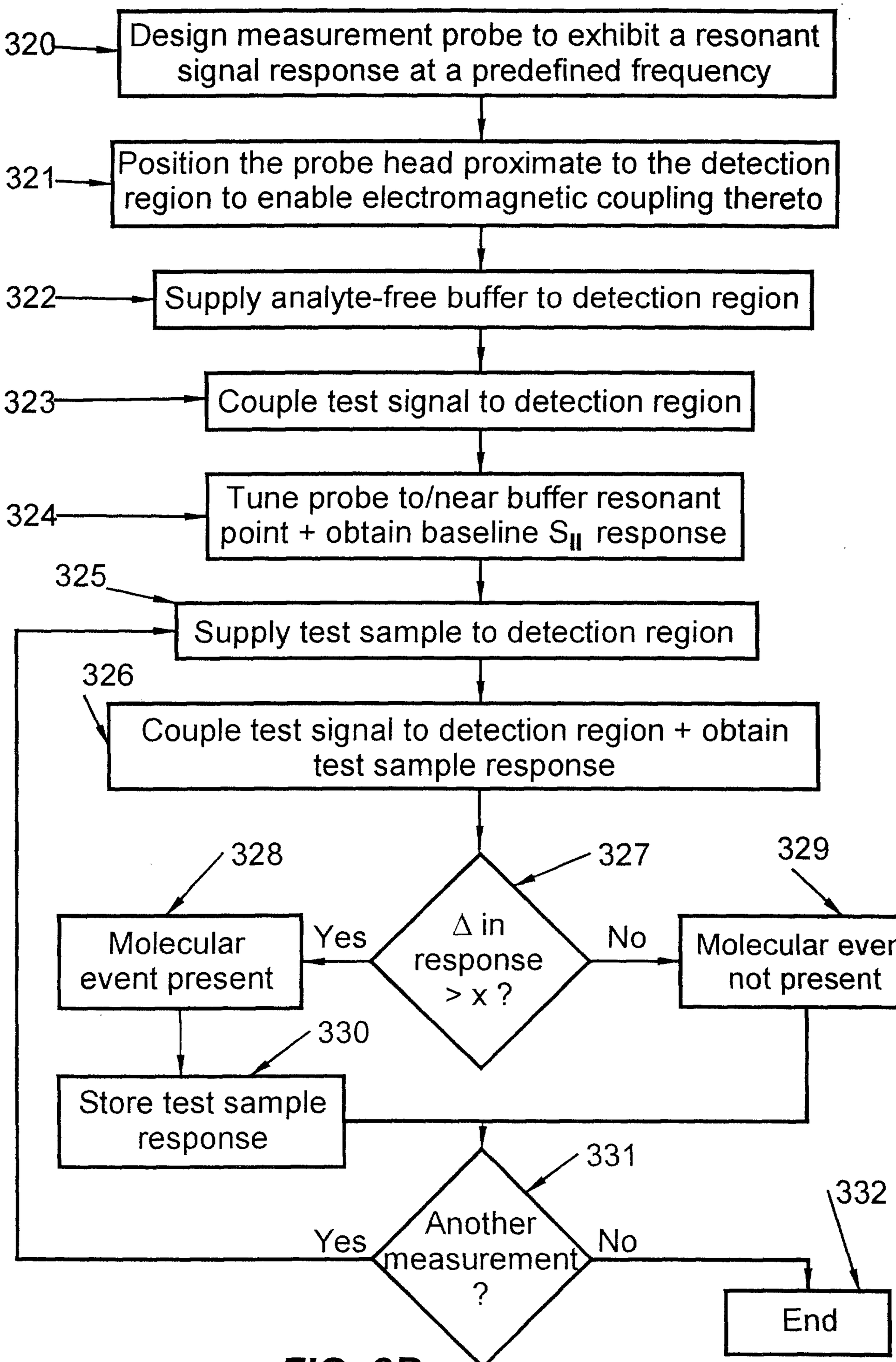

320
Design measurement probe to exhibit a resonant signal response at a predefined frequency
321
Position the probe head proximate to the detection region to enable electromagnetic coupling thereto
322
Supply analyte-free buffer to detection region
323
Couple test signal to detection region
324
Tune probe to/near buffer resonant point + obtain baseline $S_{11}$ response
325
Supply test sample to detection region
326
Couple test signal to detection region + obtain test sample response
327
Δ in response > x ?
Yes
No
328
Molecular event present
329
Molecular event not present
330
Store test sample response
331
Another measurement ?
Yes
No
332
End

*FIG. 3B*

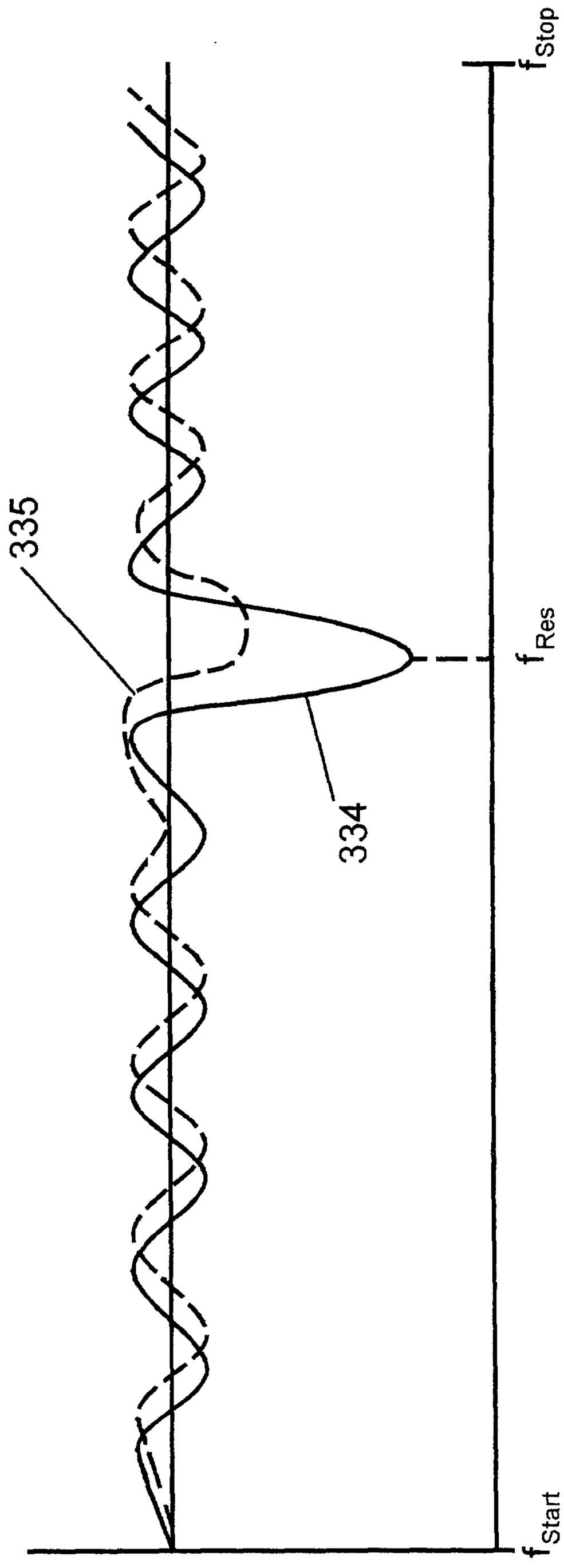



FIG. 3C

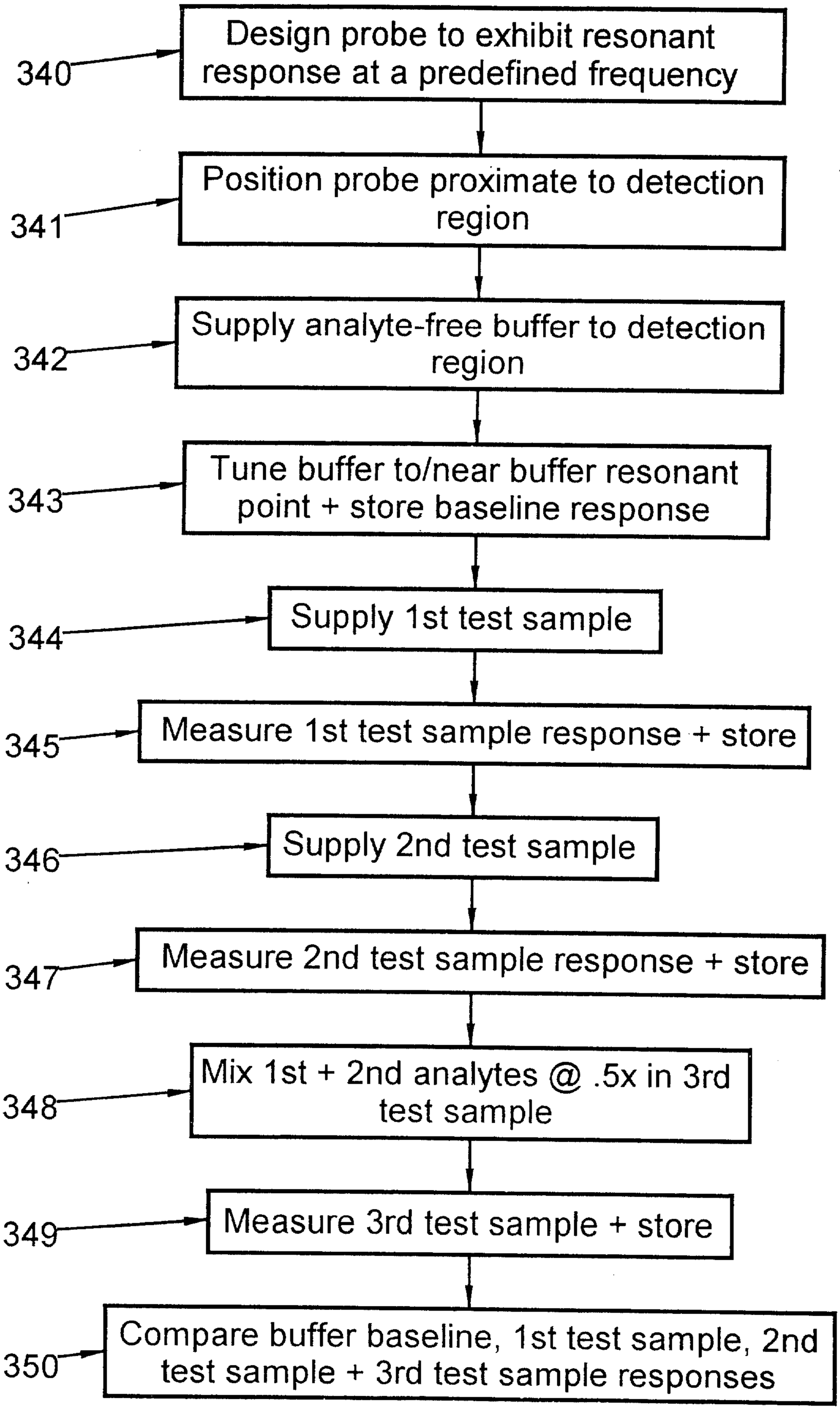

340
Design probe to exhibit resonant response at a predefined frequency
341
Position probe proximate to detection region
342
Supply analyte-free buffer to detection region
343
Tune buffer to/near buffer resonant point + store baseline response
344
Supply 1st test sample
345
Measure 1st test sample response + store
346
Supply 2nd test sample
347
Measure 2nd test sample response + store
348
Mix 1st + 2nd analytes @ .5x in 3rd test sample
349
Measure 3rd test sample + store
350
Compare buffer baseline, 1st test sample, 2nd test sample + 3rd test sample responses

*FIG. 3D*

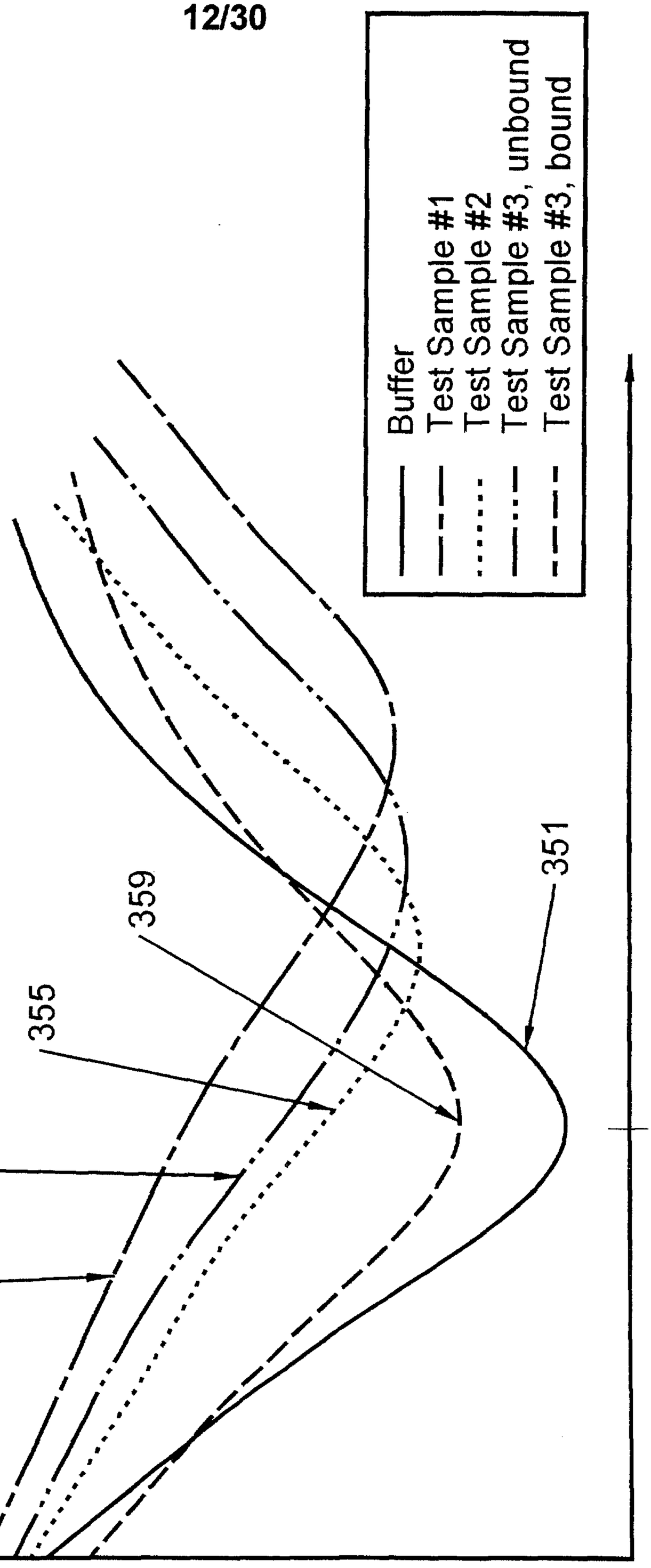

Buffer
Test Sample #1
Test Sample #2
Test Sample #3, unbound
Test Sample #3, bound
351
353
355
357
359
1
Frequency (GHz)
Magnitude S11

FIG. 3E

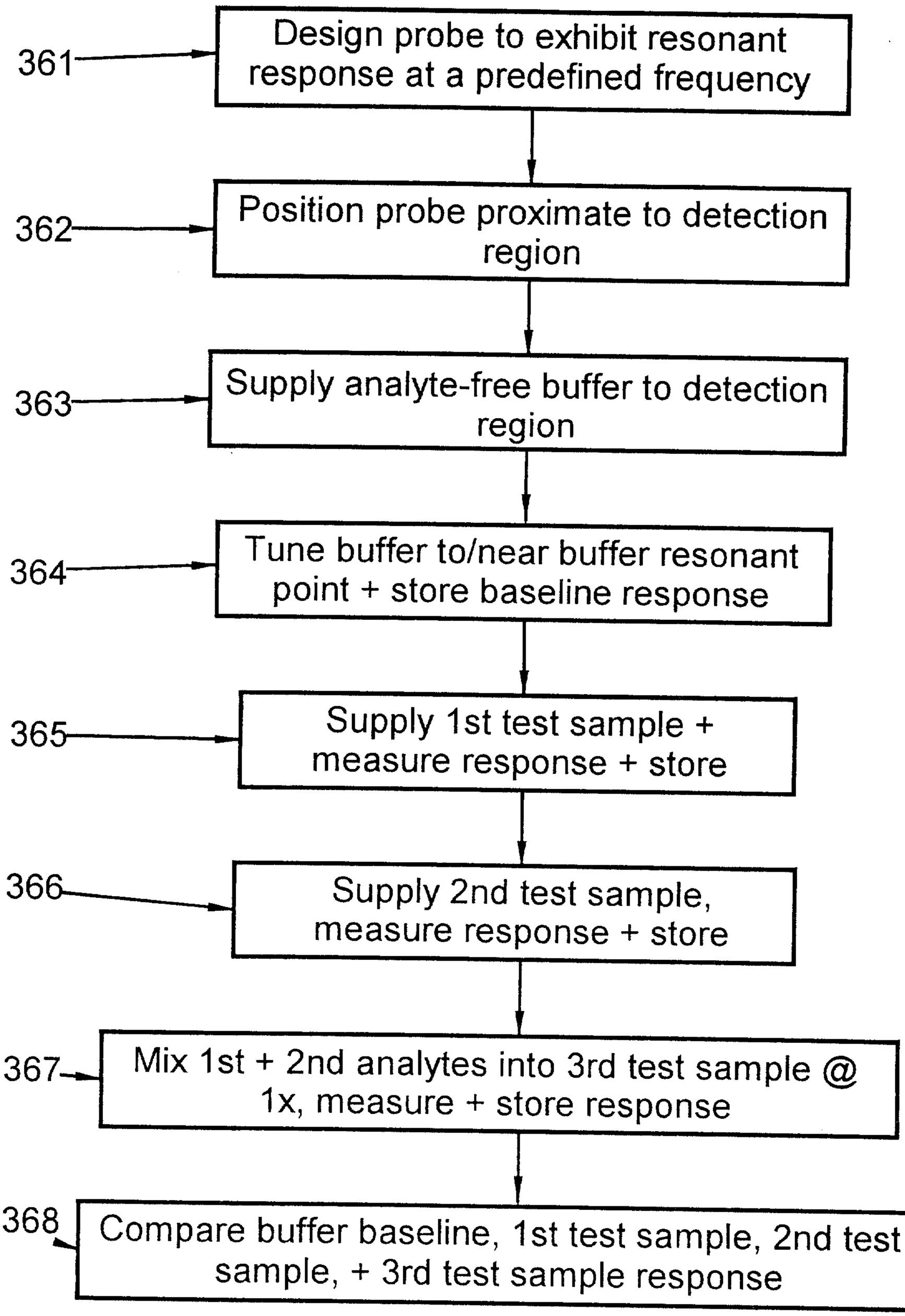

361
Design probe to exhibit resonant response at a predefined frequency
362
Position probe proximate to detection region
363
Supply analyte-free buffer to detection region
364
Tune buffer to/near buffer resonant point + store baseline response
365
Supply 1st test sample + measure response + store
366
Supply 2nd test sample, measure response + store
367
Mix 1st + 2nd analytes into 3rd test sample @ 1x, measure + store response
368
Compare buffer baseline, 1st test sample, 2nd test sample, + 3rd test sample response

*FIG. 3F*

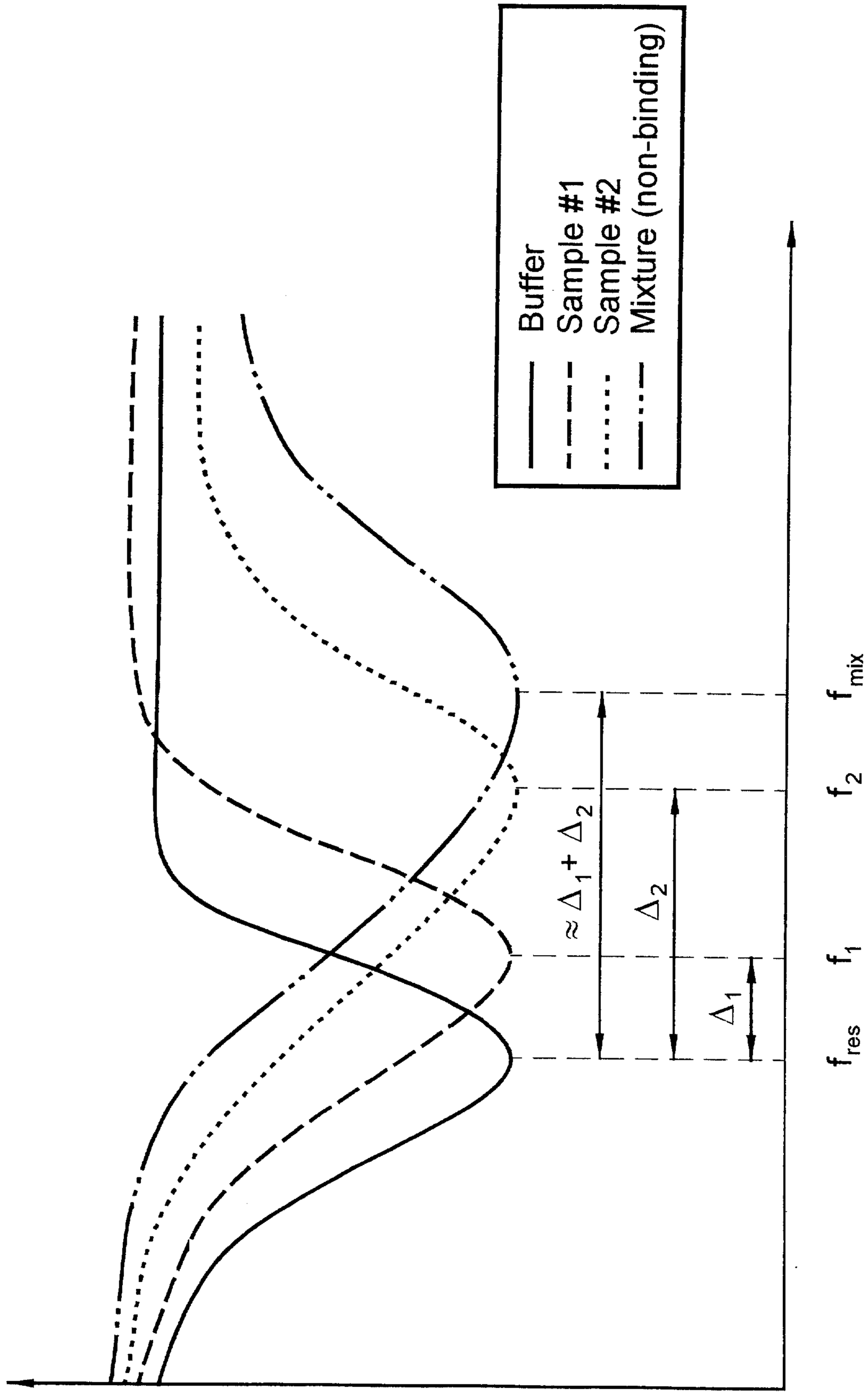

Buffer
Sample #1
Sample #2
Mixture (non-binding)
$f_{res}$
$f_1$
$f_2$
$f_{mix}$
$\approx \Delta_1 + \Delta_2$
$\Delta_2$
$\Delta_1$

FIG. 3G

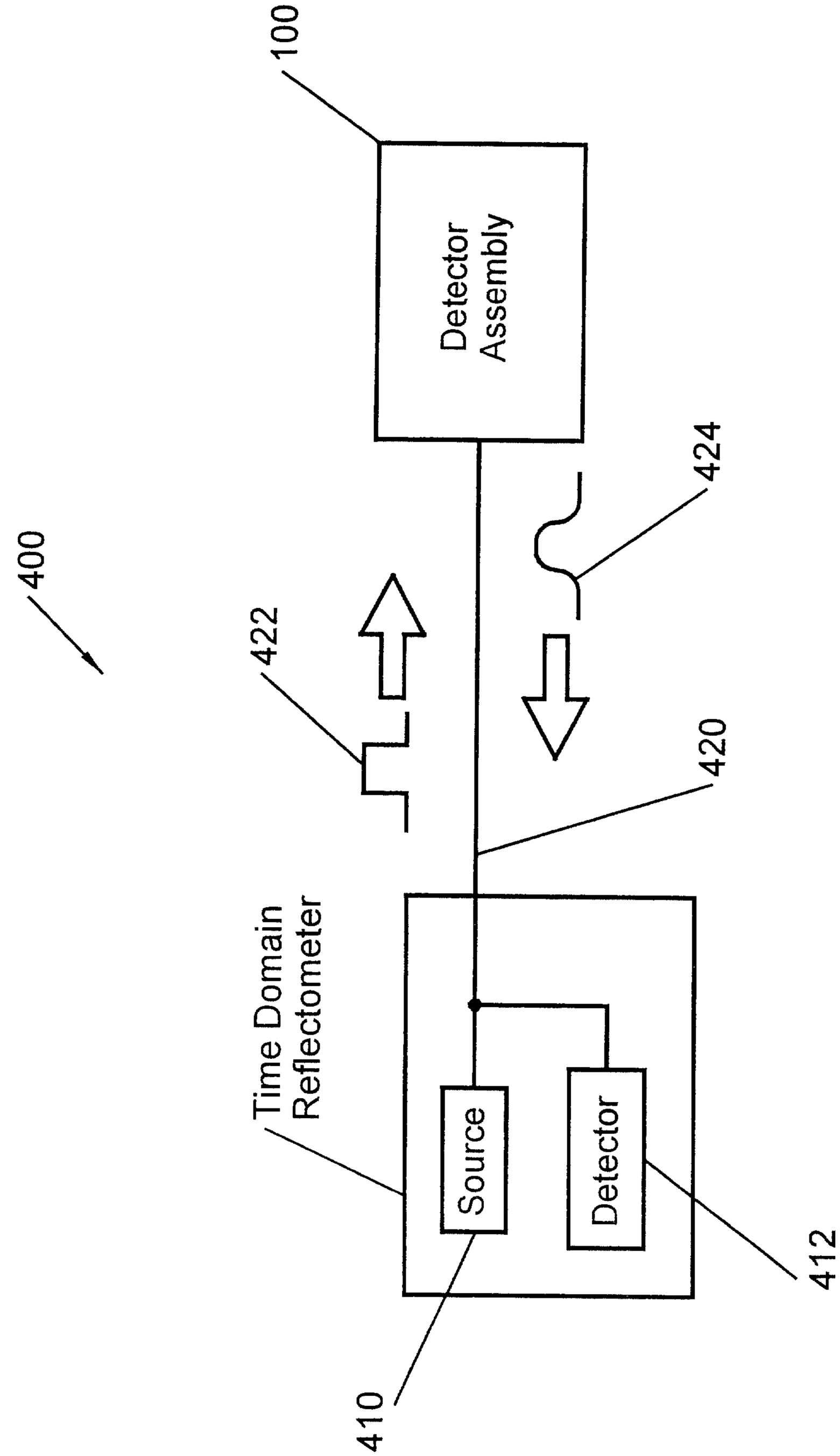

100
Detector Assembly
424
400
422
420
Time Domain Reflectometer
Source
Detector
410
412

FIG. 4A

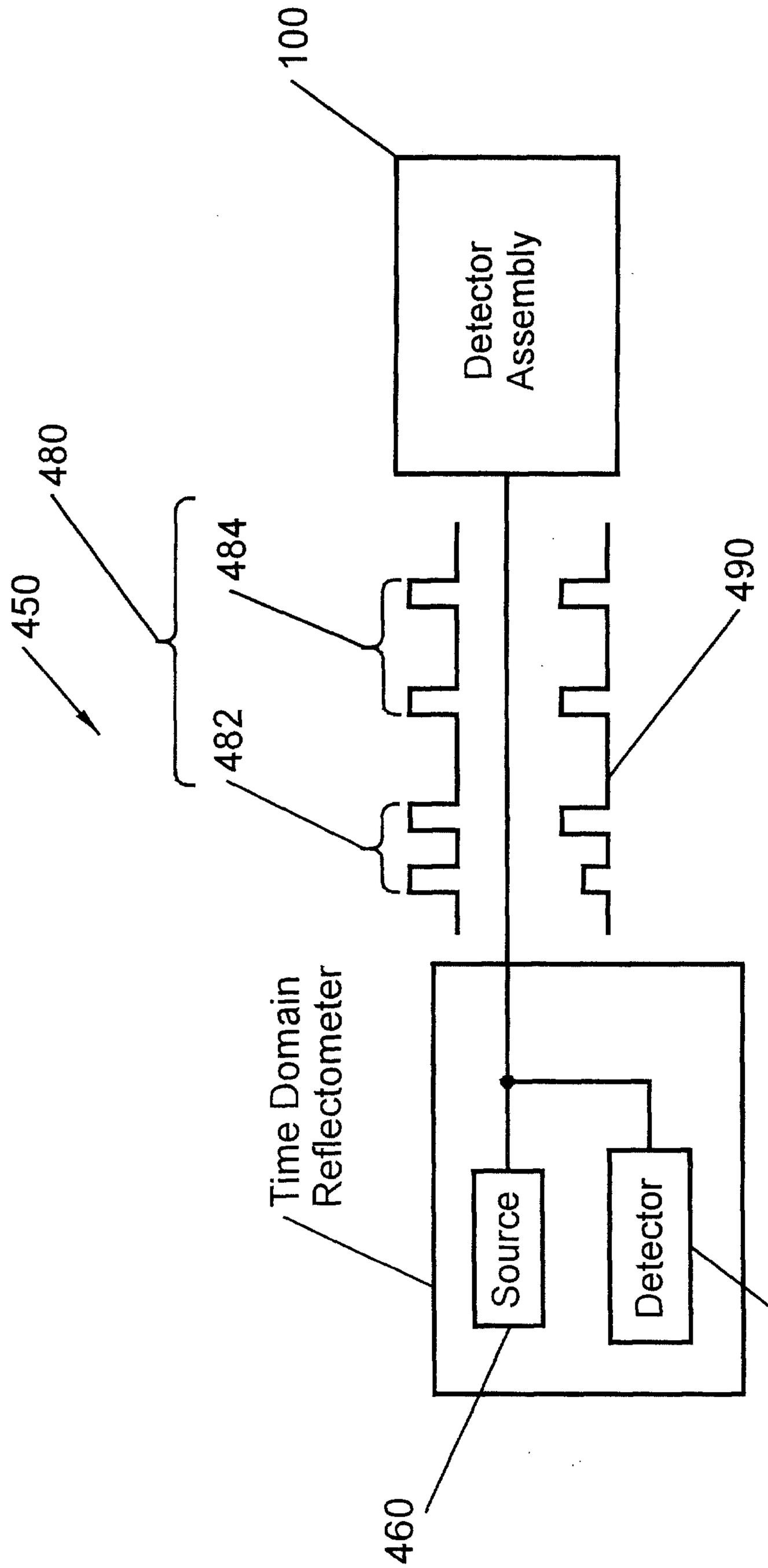

100
Detector Assembly
480
450
484
482
490
Time Domain Reflectometer
Source
Detector
460
462

FIG. 4B

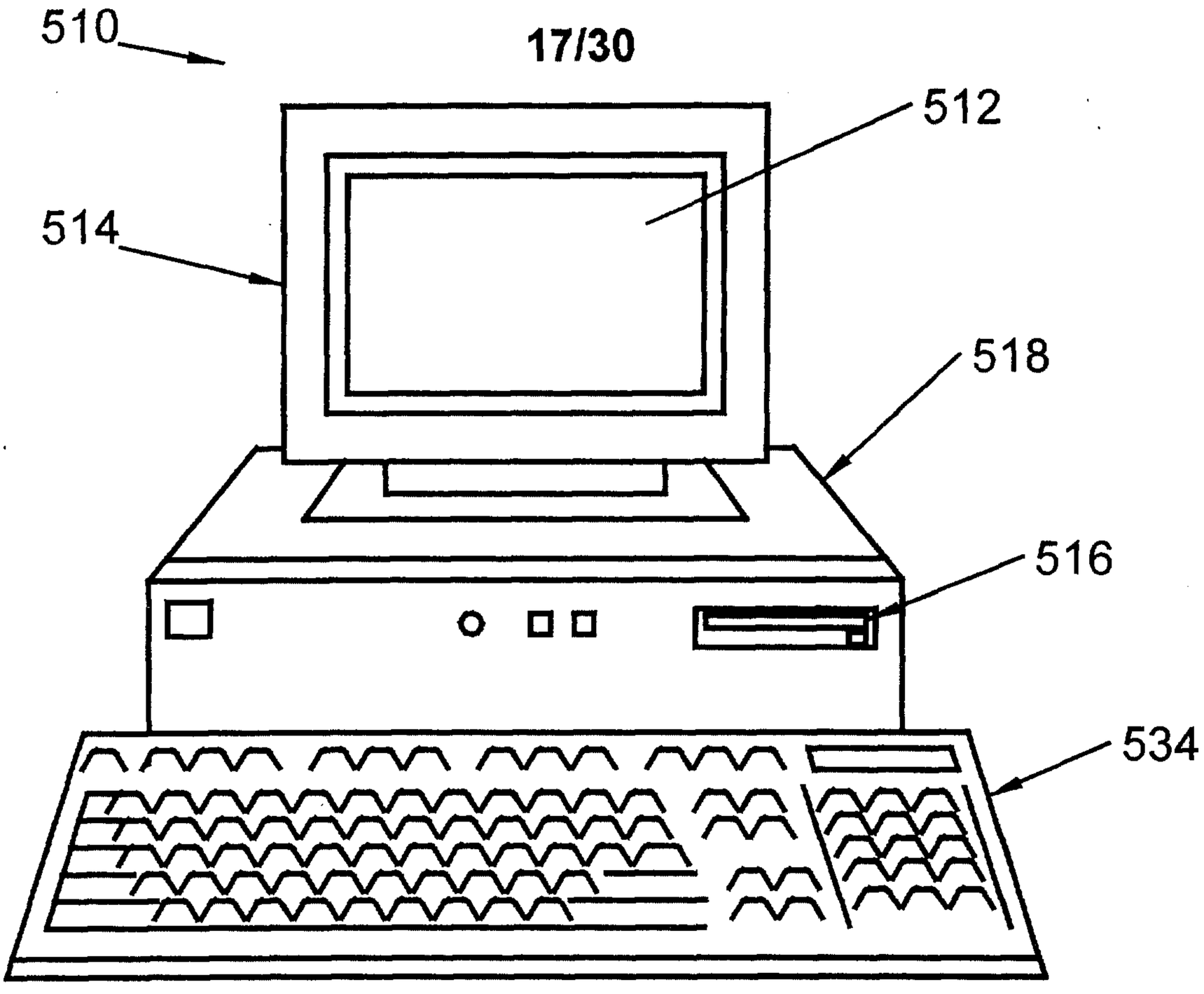

510
512
514
518
516
534

FIG. 5A

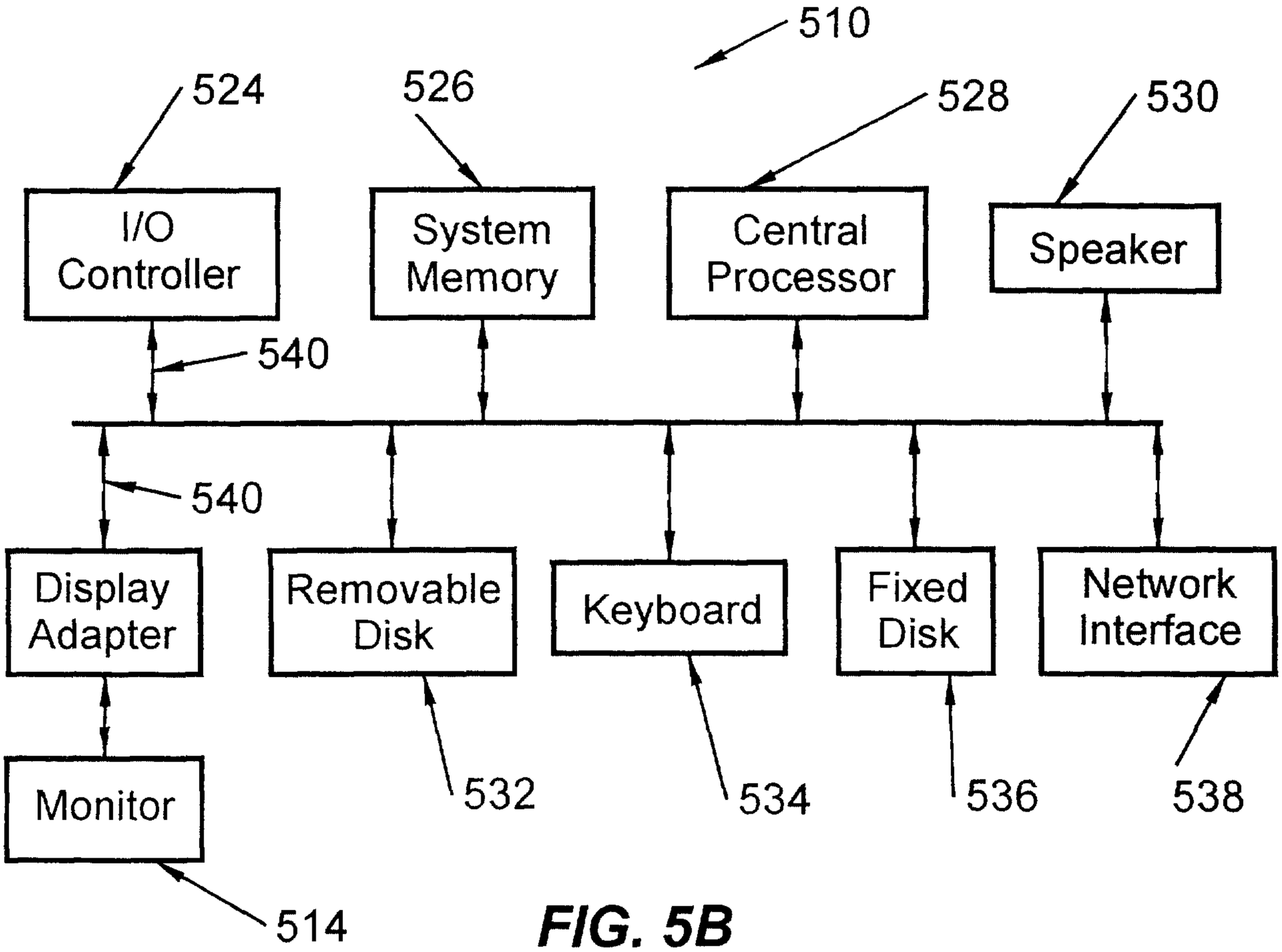

510
524
526
528
530
I/O Controller
System Memory
Central Processor
Speaker
540
540
Display Adapter
Removable Disk
Keyboard
Fixed Disk
Network Interface
Monitor
532
534
536
538
514

FIG. 5B

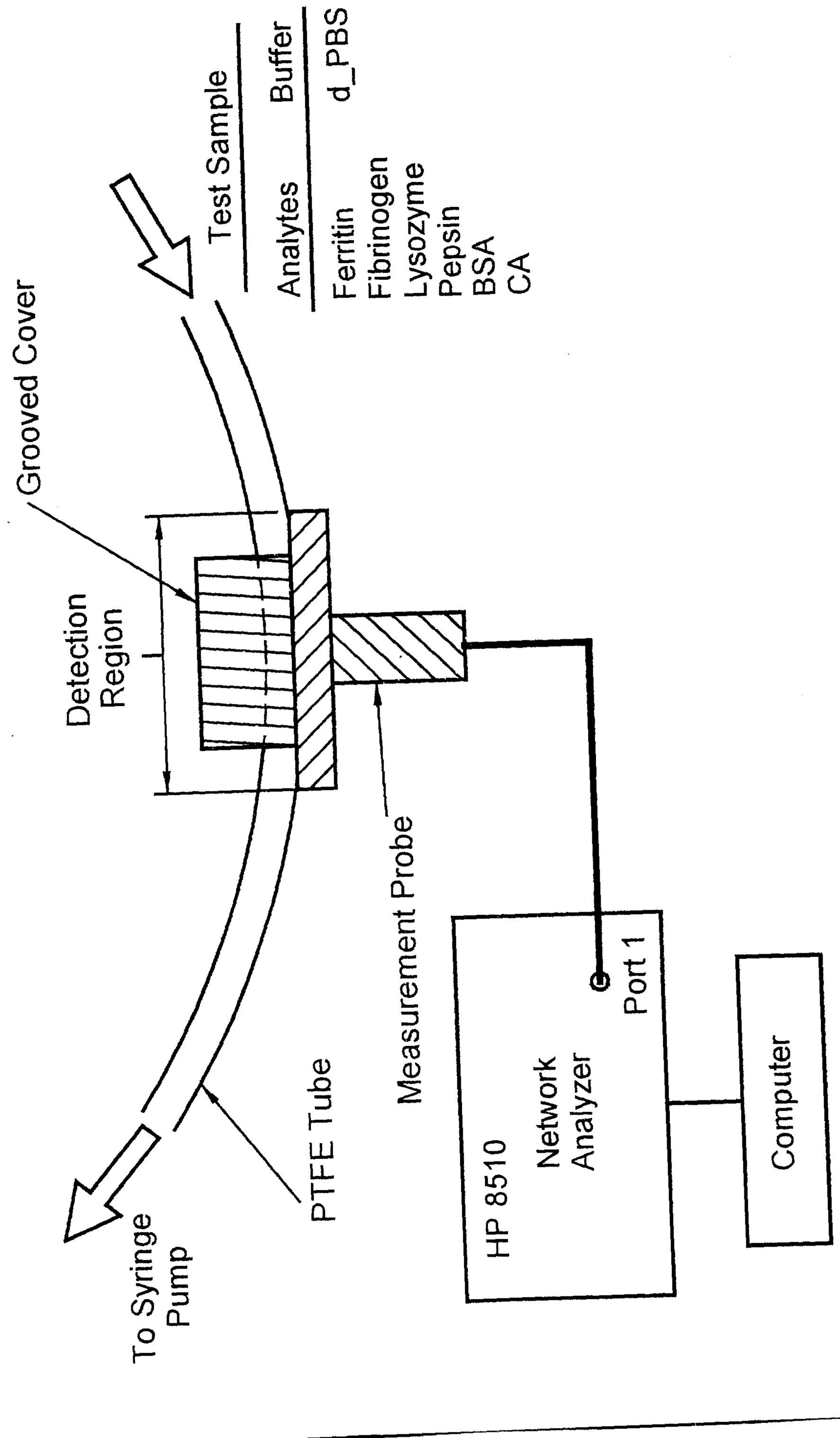

Buffer
d_PBS
Test Sample
Analytes
Ferritin
Fibrinogen
Lysozyme
Pepsin
BSA
CA
Grooved Cover
Detection Region
Measurement Probe
PTFE Tube
To Syringe Pump
HP 8510
Network Analyzer
Port 1
Computer

FIG. 6A

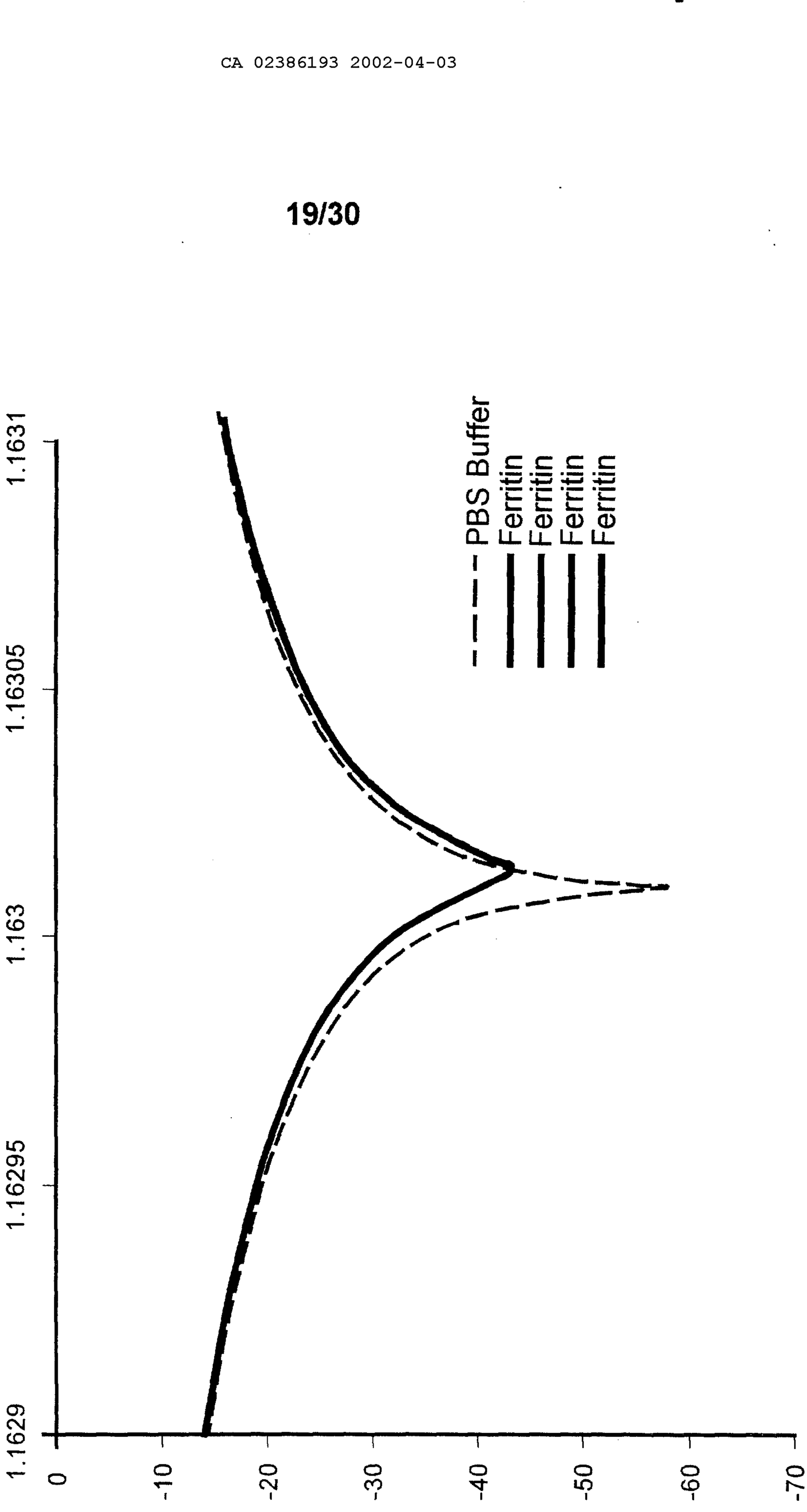

PBS Buffer
Ferritin
Ferritin
Ferritin
Ferritin
Frequency (GHz)
1.16315
1.16305
1.163
1.16295
1.1629
0
-10
-20
-30
-40
-50
-60
-70
Power (dB)

FIG. 6B

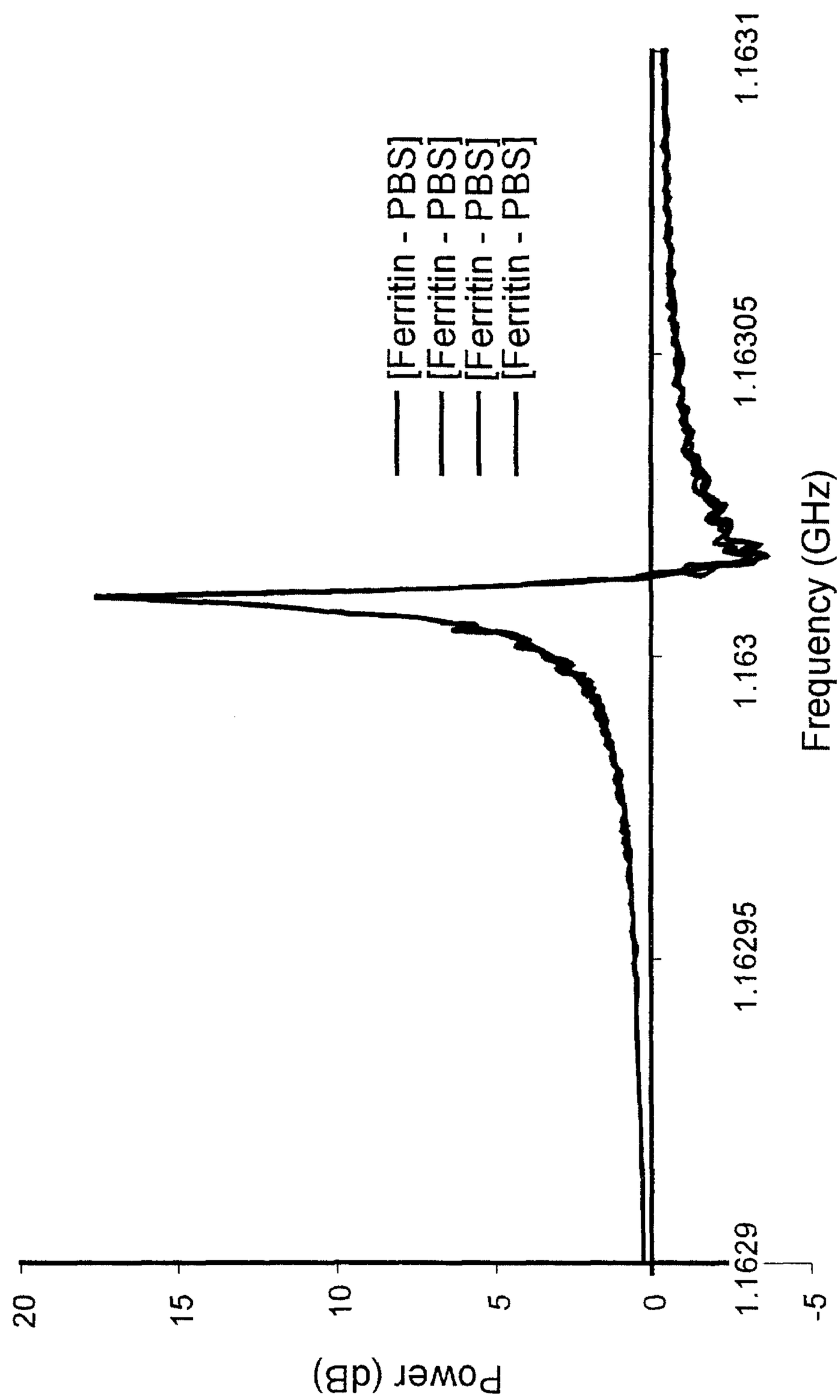

[Ferritin - PBS]
[Ferritin - PBS]
[Ferritin - PBS]
[Ferritin - PBS]
1.16295
1.163
1.16305
1.1631
Frequency (GHz)
20
15
10
5
0
-5
1.1629
Power (dB)

FIG. 6C

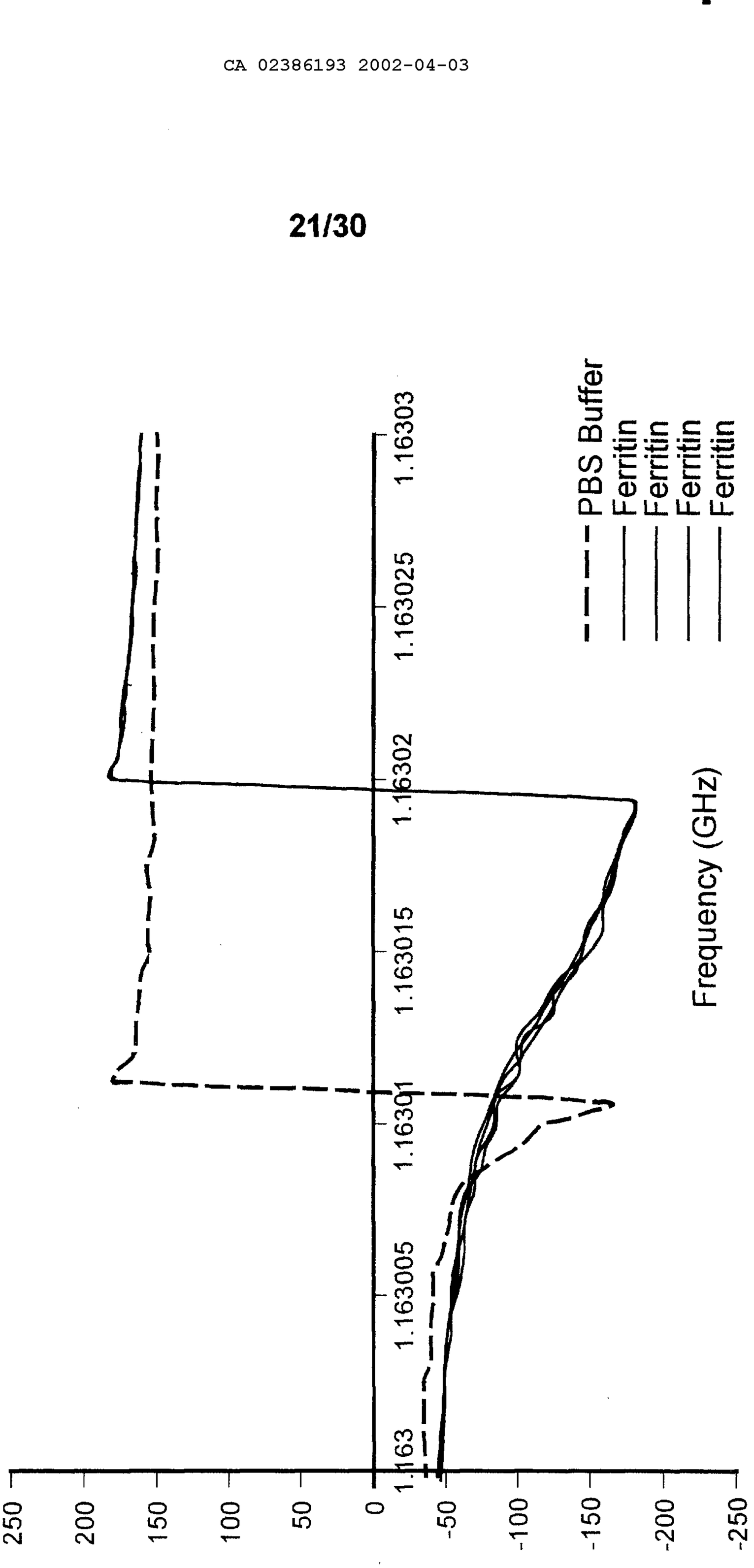

PBS Buffer
Ferritin
Ferritin
Ferritin
Ferritin
Frequency (GHz)
1.163025
1.16302
1.163015
1.16301
1.163005
1.163
250
200
150
100
50
0
-50
-100
-150
-200
-250
Phase (Degrees)

FIG. 6D

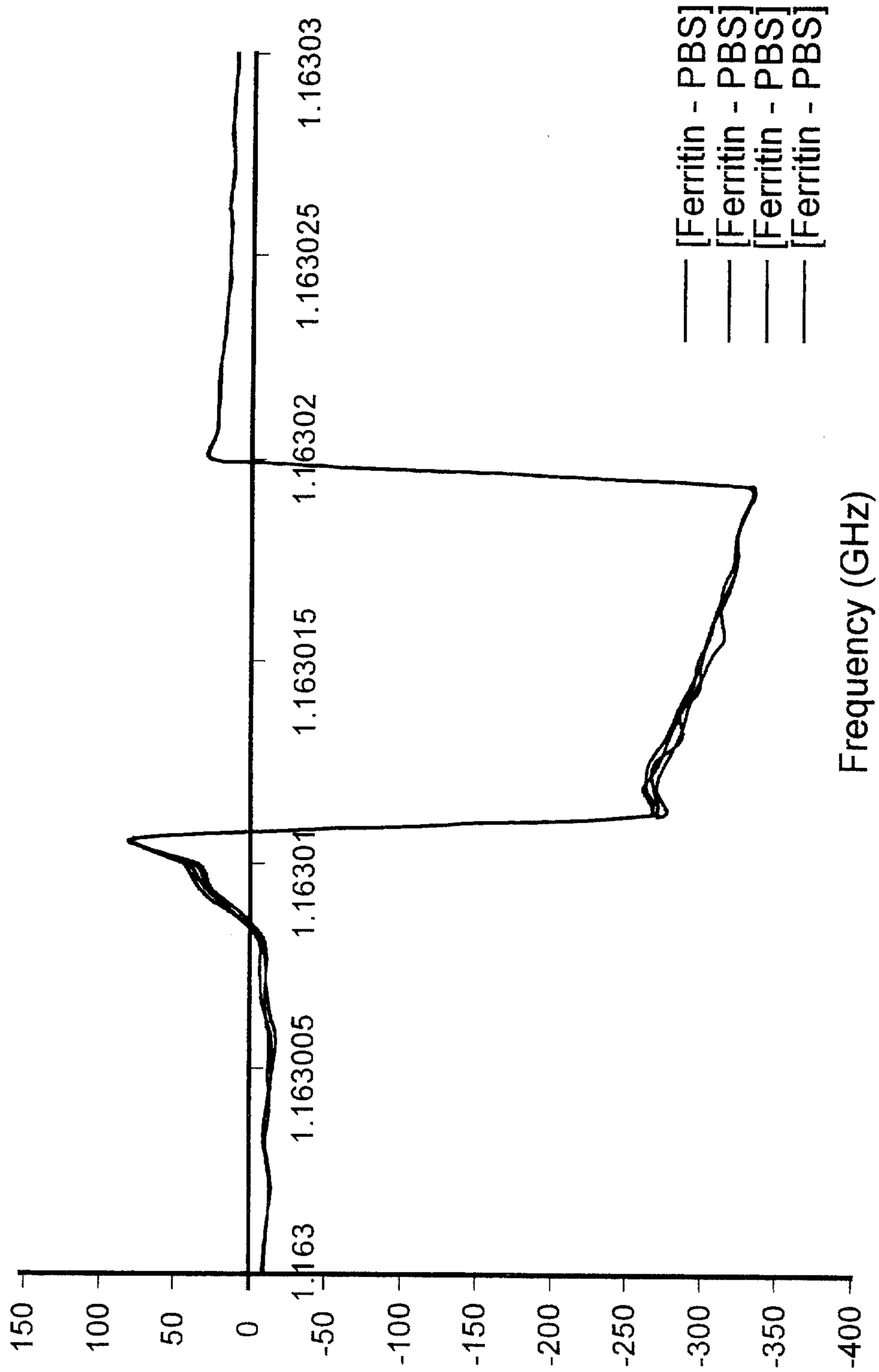

[Ferritin - PBS
[Ferritin - PBS
[Ferritin - PBS
[Ferritin - PBS
1.163025
1.163015
1.163005
1.162995
Frequency (GHz)
150
100
50
0
-50
-100
-150
-200
-250
-300
-350
-400
Phase (Degrees)

*FIG. 6E*

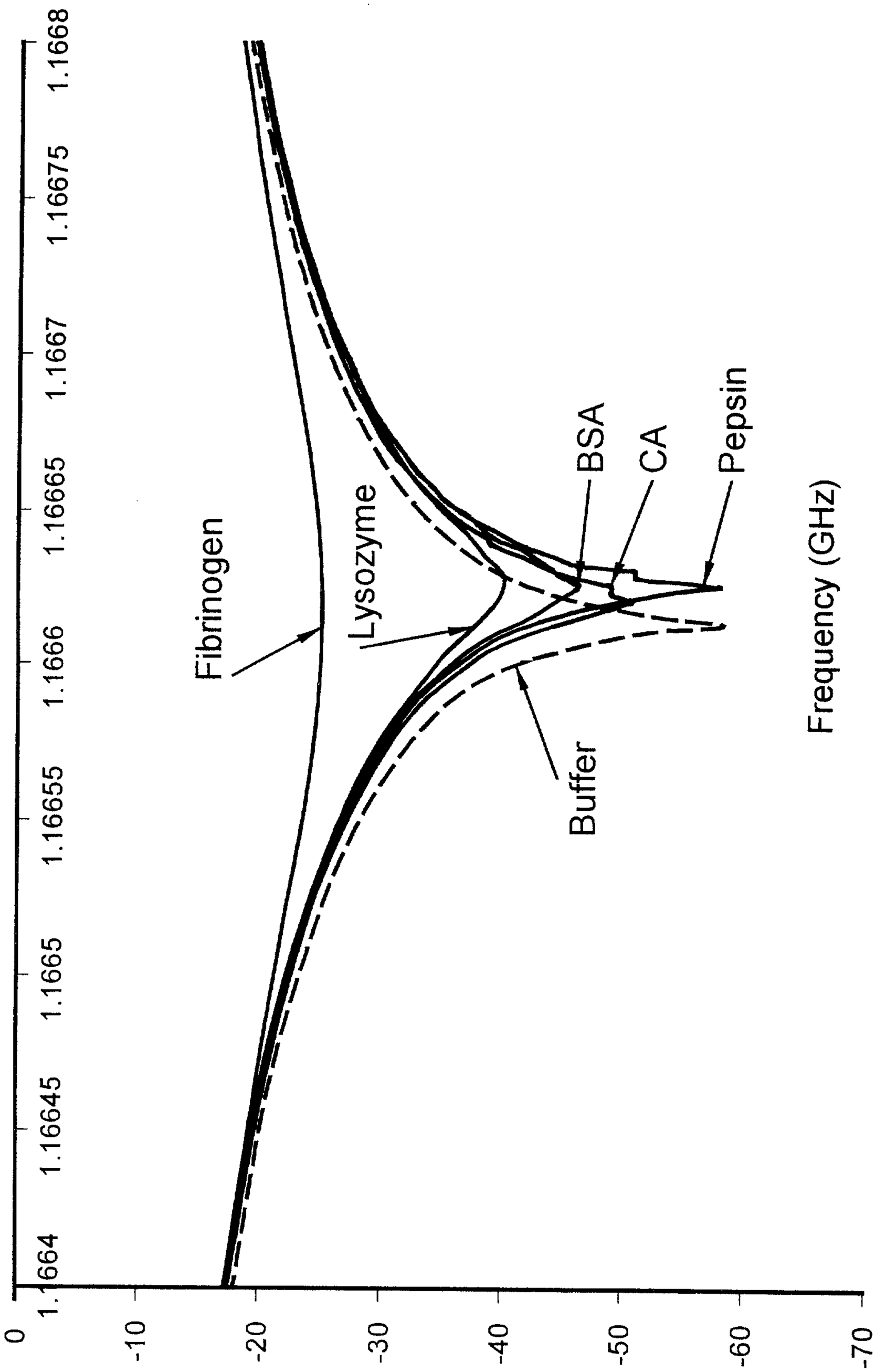

1.1668
1.16675
1.1667
1.16665
1.1666
1.16655
1.1665
1.16645
1.164
Fibrinogen
Lysozyme
BSA
CA
Pepsin
Buffer
Frequency (GHz)
0
-10
-20
-30
-40
-50
-60
-70
Power (dB)

***FIG. 6F***

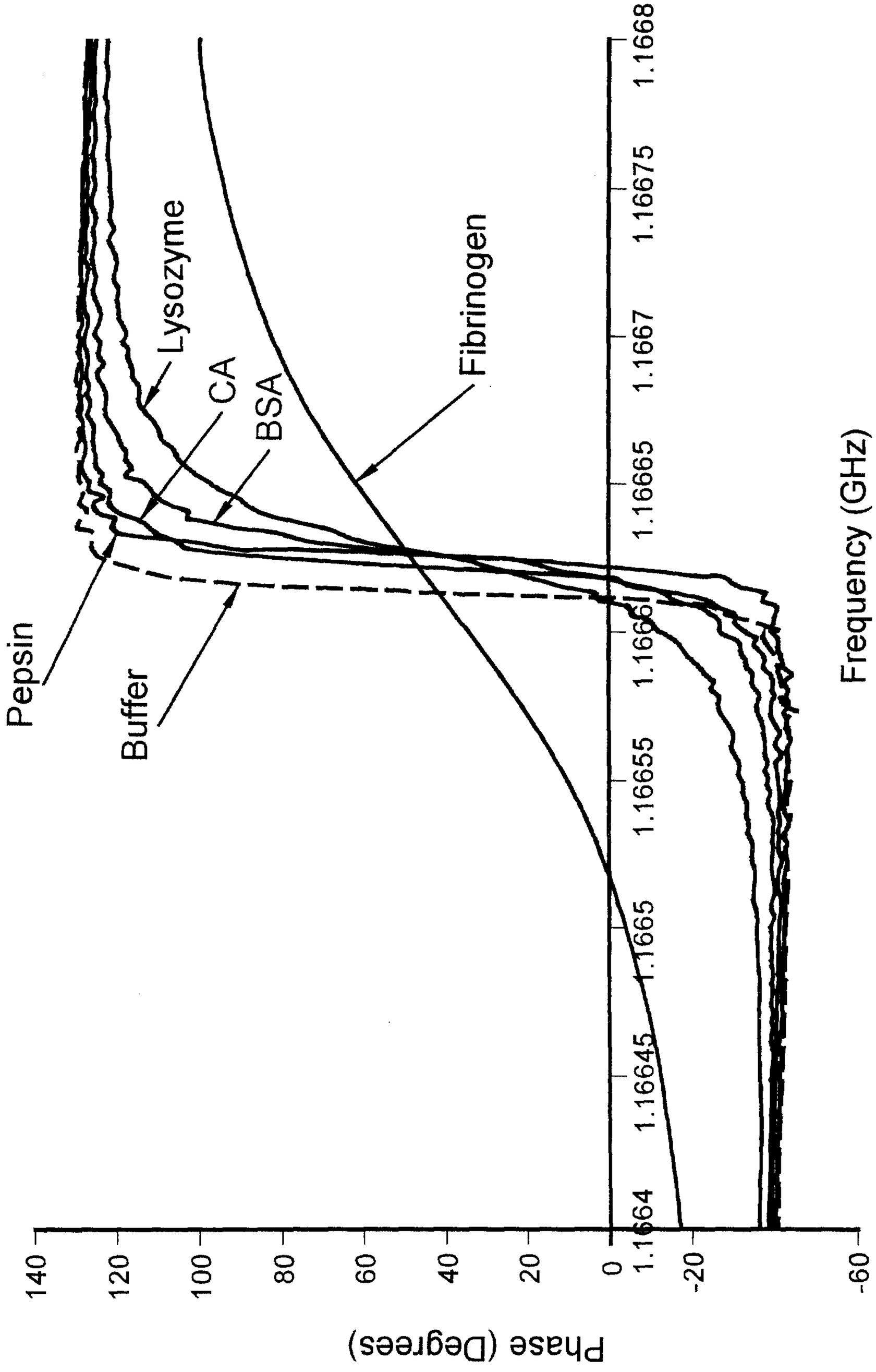

Phase (Degrees)
140
120
100
80
60
40
20
0
-20
-60
1.1664
1.16645
1.1665
1.16655
1.1666
1.16665
1.1667
1.16675
1.1668
Frequency (GHz)
Pepsin
Buffer
Lysozyme
CA
BSA
Fibrinogen

FIG. 6G

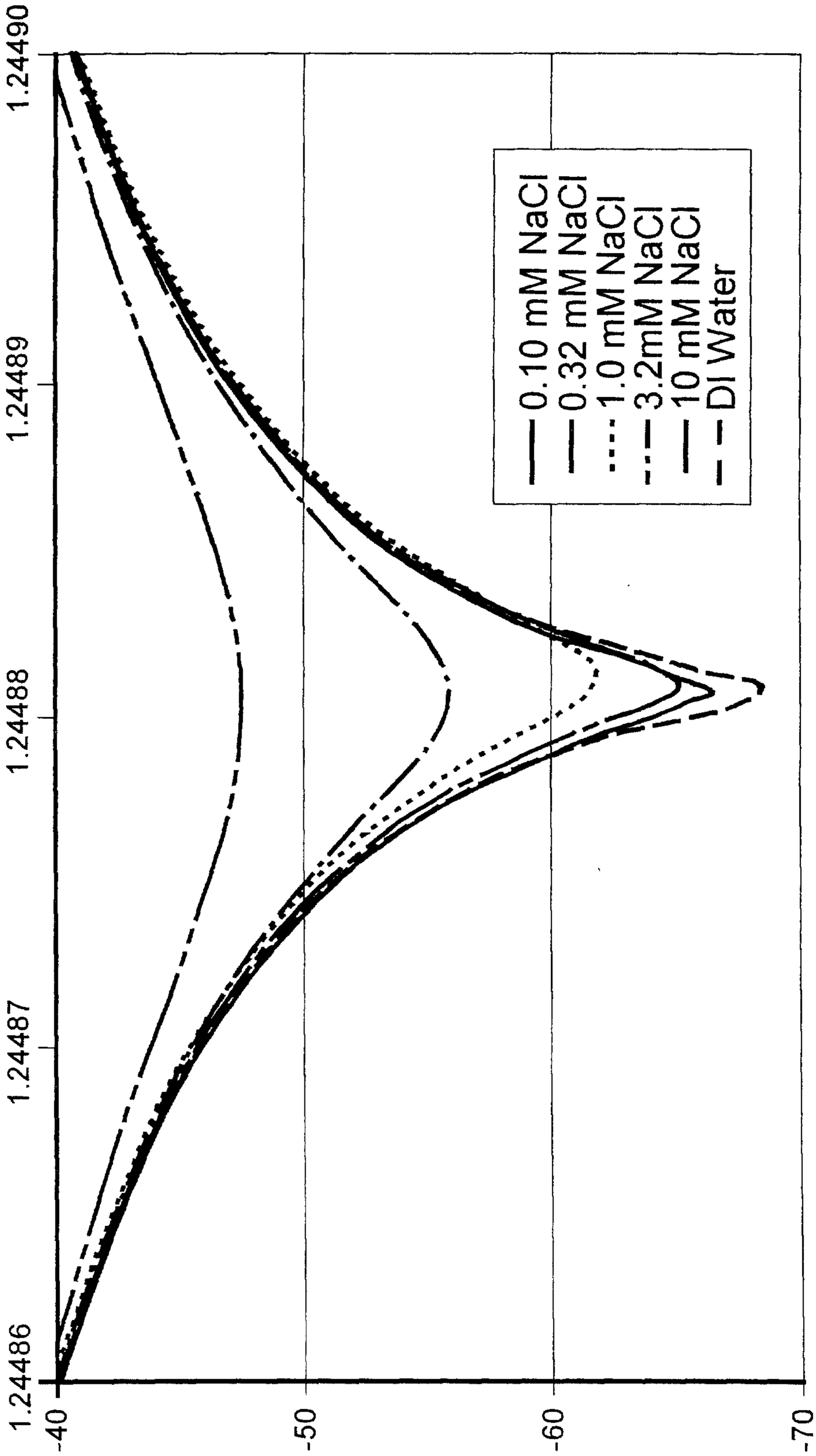

Concentration Titration of Sodium Chloride
in the Coaxial Resonating Fixture
1.24490
1.24489
1.24488
1.24487
1.24486
-40
-50
-60
-70
Magnitude (dB)
Frequency (GHz)
0.10 mM NaCl
0.32 mM NaCl
1.0 mM NaCl
3.2mM NaCl
10 mM NaCl
DI Water

***FIG. 6H***

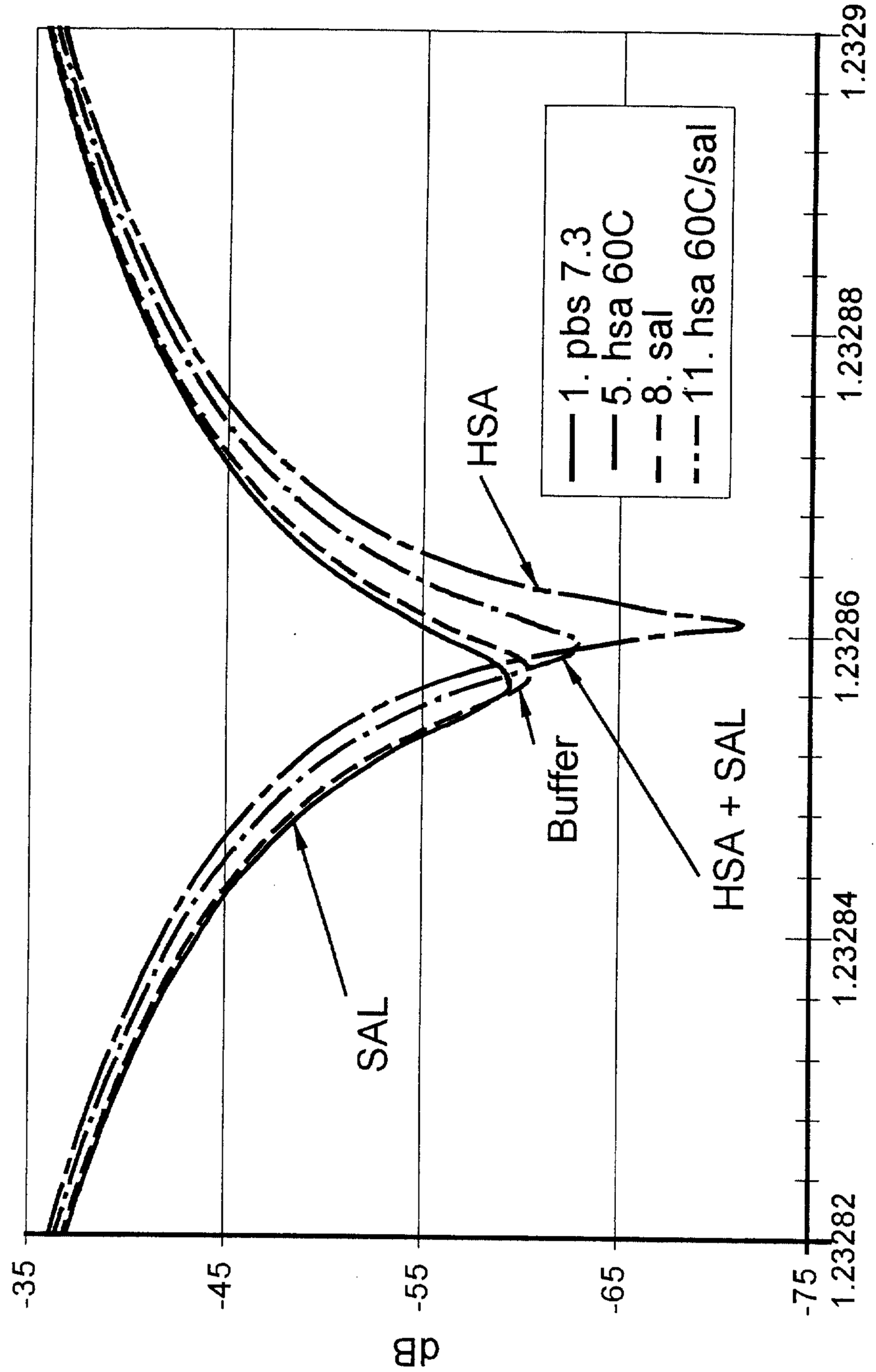

Denatured Human Serum Albumin
Not Binding To Salbutamol
1. pbs 7.3
5. hsa 60C
8. sal
11. hsa 60C/sal
HSA
SAL
Buffer
HSA + SAL
dB
-35
-45
-55
-65
-75
1.23288
1.23286
1.23284
1.23282
Frequency (GHz)

FIG. 6I

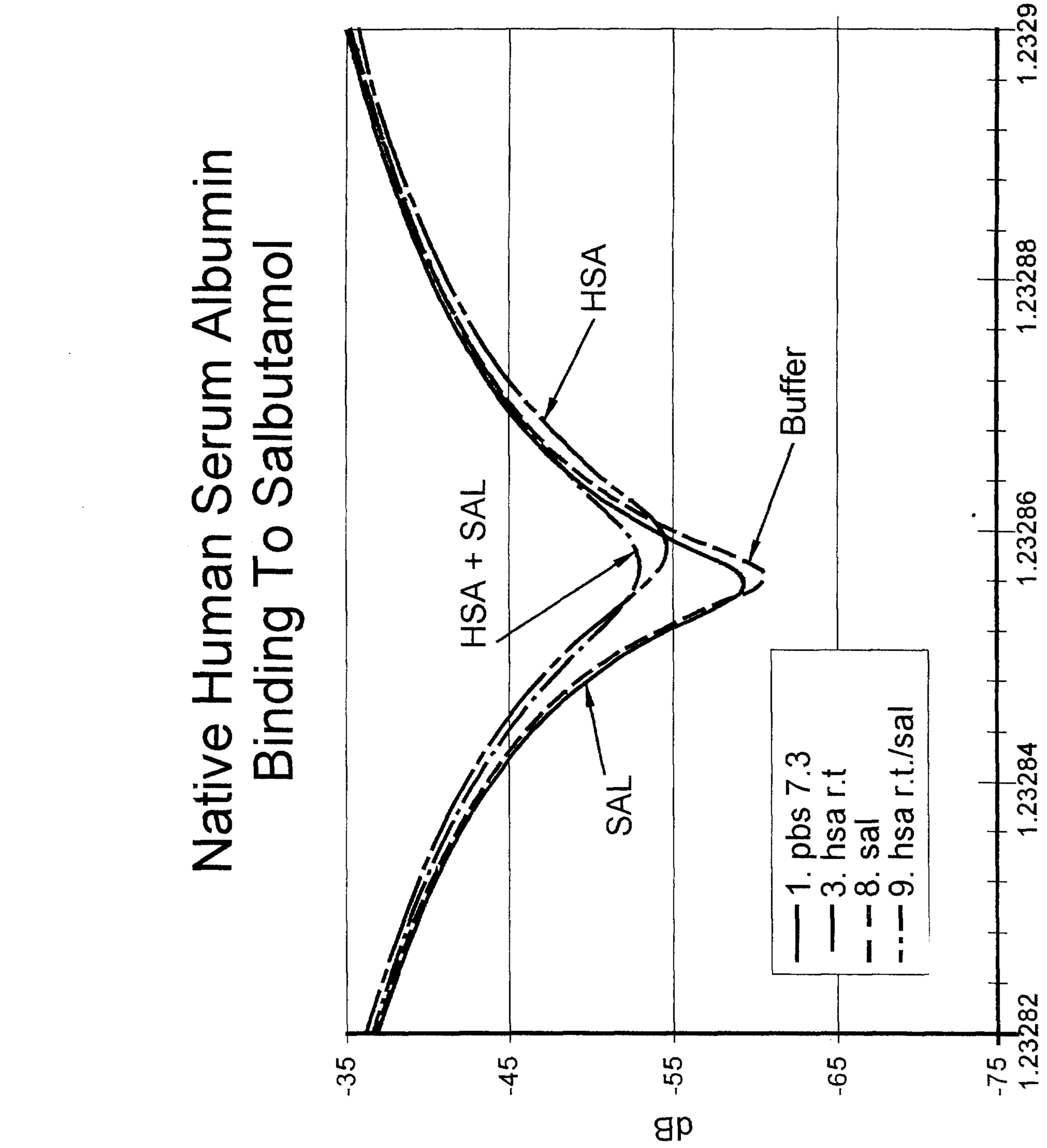

Native Human Serum Albumin
Binding To Salbutamol
HSA
HSA + SAL
SAL
Buffer
1. pbs 7.3
3. hsa r.t
8. sal
9. hsa r.t./sal
dB
-35
-45
-55
-65
-75
1.23288
1.23286
1.23284
1.23282
Frequency (GHz)

FIG. 6J

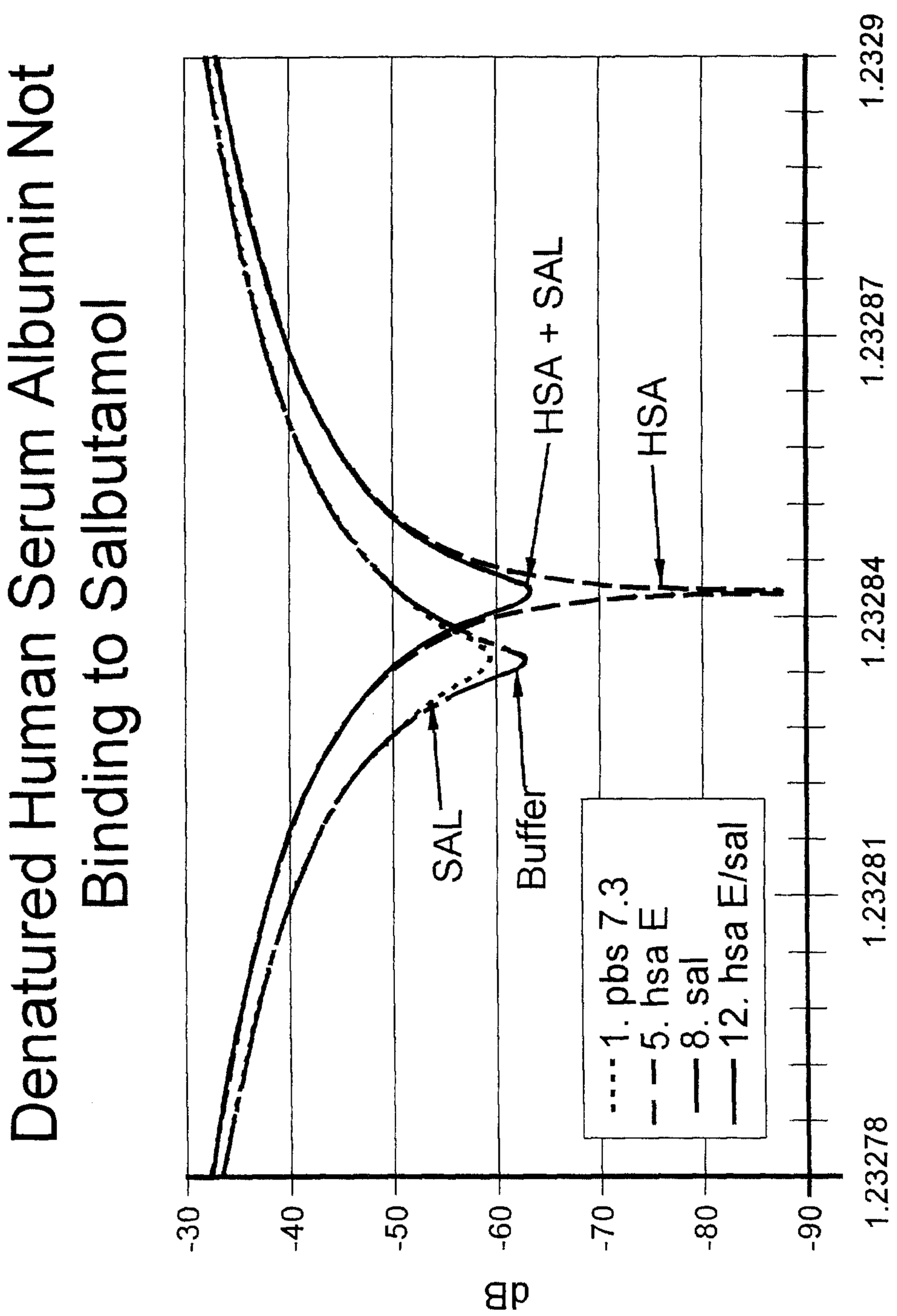

Denatured Human Serum Albumin Not Binding to Salbutamol
HSA + SAL
HSA
SAL
Buffer
1. pbs 7.3
5. hsa E
8. sal
12. hsa E/sal
dB
-30
-40
-50
-60
-70
-80
-90
1.23278
1.23281
1.23284
1.23287
1.2329
Frequency (GHz)

FIG. 6K

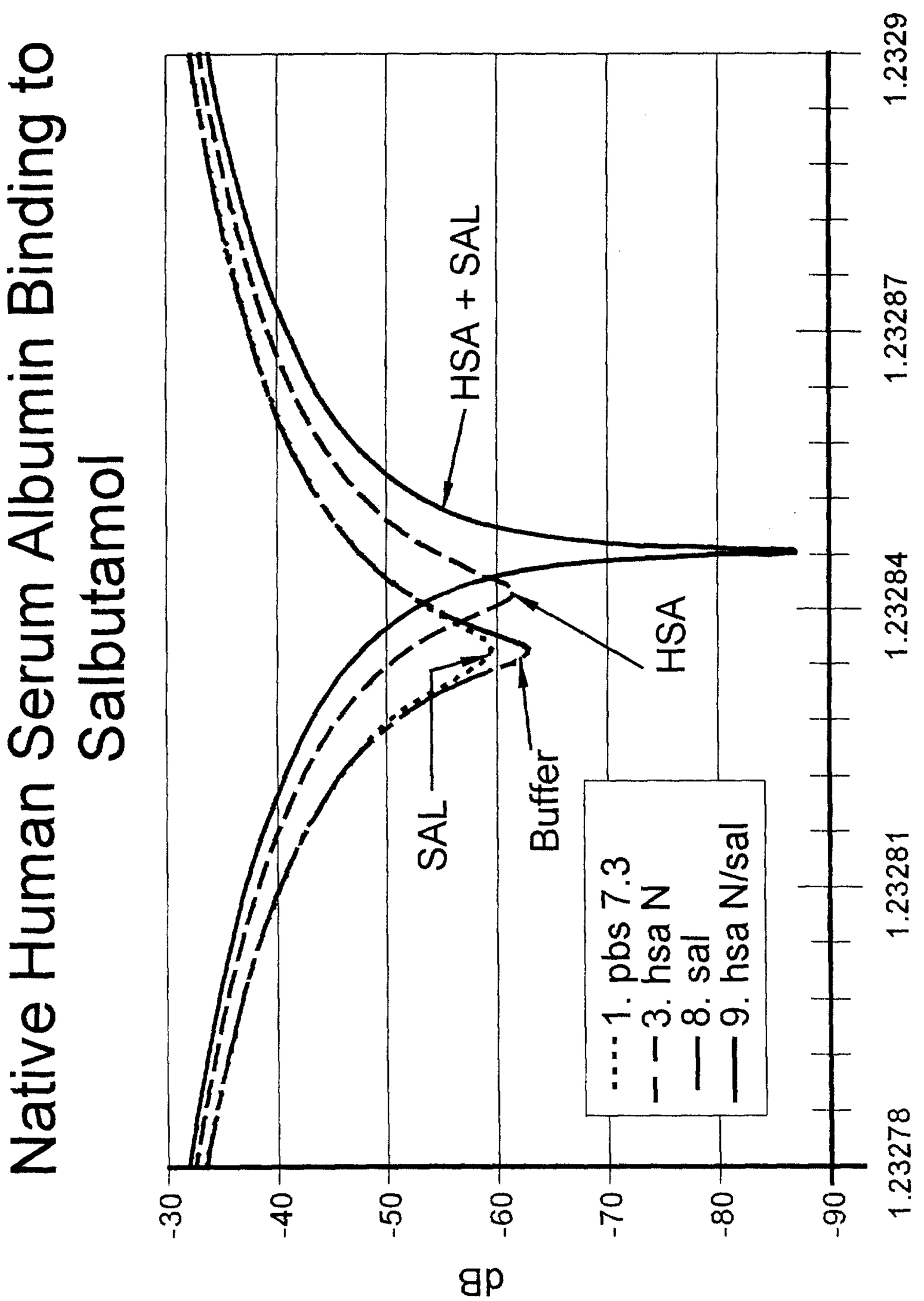

Native Human Serum Albumin Binding to Salbutamol
HSA + SAL
SAL
Buffer
HSA
1. pbs 7.3
3. hsa N
8. sal
9. hsa N/sal
dB
-30
-40
-50
-60
-70
-80
-90
1.23278
1.23281
1.23284
1.23287
1.2329
Frequency (GHz)

*FIG. 6L*

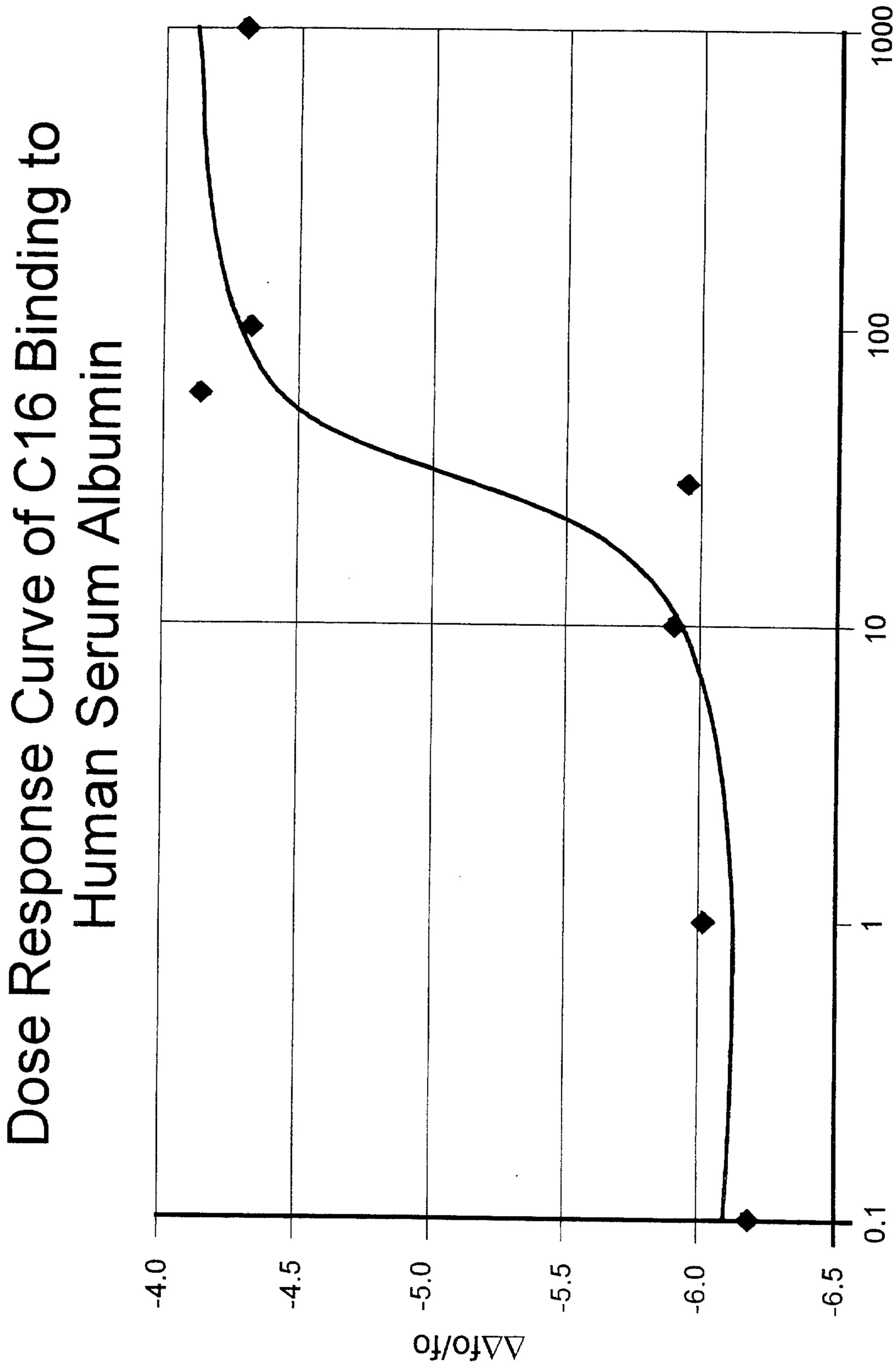
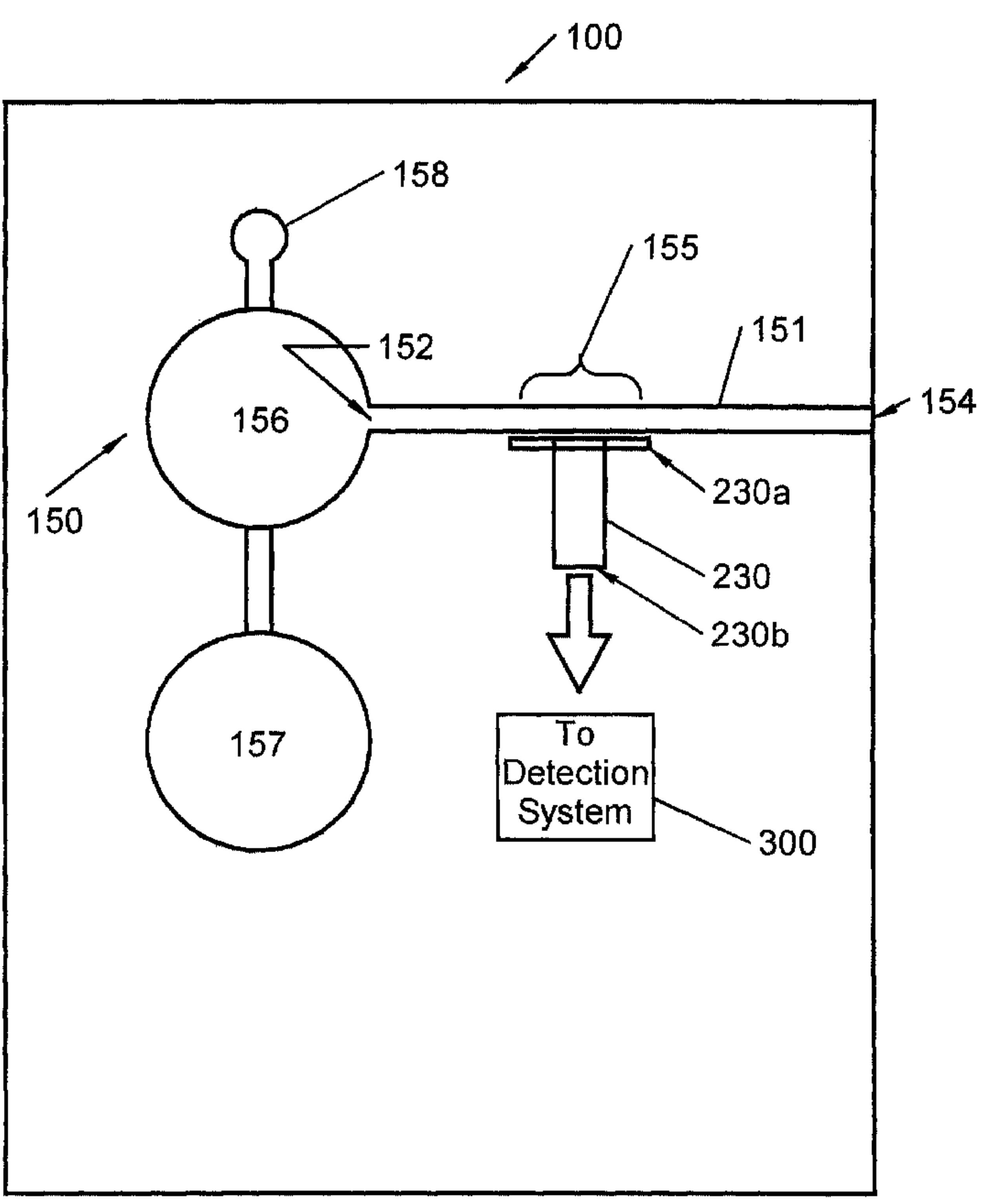

Dose Response Curve of C16 Binding to Human Serum Albumin
ΔΔfo/fo
-4.0
-4.5
-5.0
-5.5
-6.0
-6.5
0.1
1
10
100
1000
C16 (μM)

***FIG. 6M***


100
158
155
151
152
154
156
150
230a
230
230b
157
To
Detection
System
300