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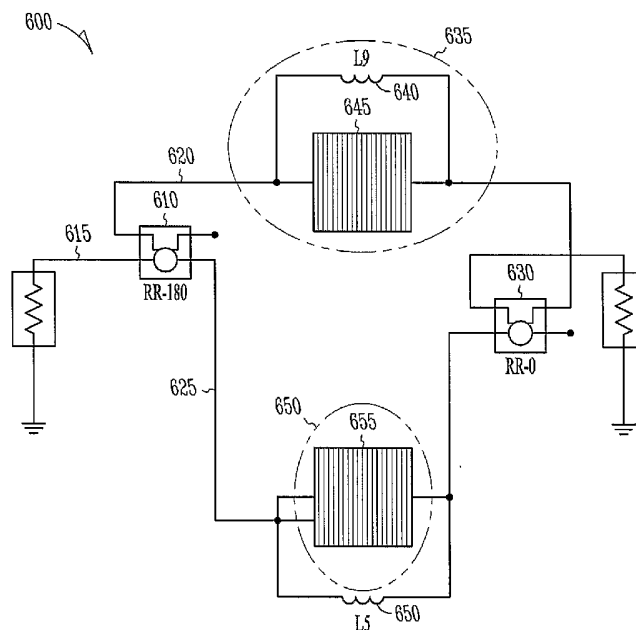
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(54) Title: PERMITTIVITY BASED SENSOR



(57) Abstract: A device includes a splitter that splits a time varying signal into two substantially equal power signals. A reference capacitor having a fluidic channel between capacitor plates is coupled to one of the equal power signals and a detection capacitor having a fluidic channel between capacitor plates is coupled to the other of the equal power signals. A detector is coupled to outputs of the reference capacitor and detection capacitor. The signals are shifted 180 degrees from each other in the absence of an analyte in the fluidic channel at or prior to the detector. In one embodiment the device is formed of microstrip circuit elements, or planar waveguide elements, and operates at microwave frequencies.

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PERMITTIVITY BASED SENSOR

Related Application

[0001] This application claims priority to United States Provisional Application serial number 60/668,272 (entitled METHODS AND APPARATUS FOR DETECTION OF PROTEINS, filed April 5, 2005) which is incorporated herein by reference.

Background

[0002] Various biological and disease detection techniques rely on the strong and specific binding of antibodies to antigens or similar immunobiological specific bindings. The most common approach is to immobilize probe molecule and detect the binding to the target molecule involving a label that signals occurrence of the binding event or amplifies such signal. Labels commonly used include enzymes, fluorescent molecules, nanoparticles and radiolabels. Label-free detection mechanisms, such as the electrical or electrochemical detection of probe-target binding, offer advantages in that they are often simpler, cheaper and can be more readily integrated into a compact apparatus.

[0003] Several of the methods and apparatus described in the prior art detect the probe-target binding via measuring the changes in electrical properties due to the binding occurring inside a capacitor-type structure. The target binds to the probe immobilized on an electrode surface, and this binding causes a change in the applied electric signal across the electrode plates. Often, the apparatus contains other circuitry to amplify the signal or differentiate it with respect to a reference capacitor structure. In other cases, the circuitry is designed for detecting changes in a resonant frequency. Although this type of impedance measurement relies on a change of the permittivity caused by the binding event, the physical mechanism giving rise to the permittivity change differs fundamentally as a function of frequency.

[0004] The immobilization of the probe on the electrode plates of the apparatus in the prior art has been accomplished, for example, via trapping in an electric field, covalent binding or immobilization on polyacrylamide gel pads. The selective population of the electrodes is often accomplished via means of micropatterning. Unrelated, there exists prior art on electrochemically induced reactions to link organic matter to semiconductor surfaces. The detailed chemistry can be application specific. However, none of the prior art immobilizes the probes via electrochemically induced covalent binding.

Summary

A device includes a splitter that splits a time varying signal into two substantially equal power signals. A reference capacitor having a fluidic channel between capacitor plates is coupled to one of the equal power signals and a detection capacitor having a fluidic channel between capacitor plates is coupled to the other of the equal power signals. A detector is coupled to outputs of the reference capacitor and detection capacitor. The signals are approximately 180 degrees out of phase with each other when combined before or at the detector, plus whatever shift may be introduced in the detection capacitor. In one embodiment the device is formed of microstrip circuit or co-planar waveguide circuits, and operates a microwave frequencies.

Brief Description of the Drawings

[0005] FIGs. 1A, 1B and 1C depict a conceptual schematic of the differential detection circuit with detection chambers containing covalently bound probes with and without the occurrence of a binding event.

[0006] FIG. 2 shows a cutaway view of an example arrangement of a reference chamber and a detection chamber.

[0007] FIGs. 3A, 3B and 3C detail top, cross-sectional and end-on views, respectively, of a fluidic channel passing through a capacitor (C_{ref}) of a reference chamber and the capacitor (C_{detect}) of a detection chamber aligned in a series.

[0008] FIGs. 3D, 3E and 3F detail top, cross-sectional and end-on views, respectively, of two fluidic channels arranged in parallel, each passing through a reference and a detection chamber.

[0009] FIG. 4A details a top view of an alternative arrangement where a fluidic channel passes through the capacitor (C_{ref}) of a reference chamber and the capacitor (C_{detect}) of a detection chamber placed across the fluidic channel in parallel so that both chambers are exposed to the sample at the same time.

[0010] FIG. 4B details a top view of an alternative arrangement where two separate fluidic channels pass through the capacitor (C_{ref}) of a reference chamber and the capacitor (C_{detect}) of a detection chamber.

[0011] FIGS. 4C and 4D detail top and cross-sectional views of an example embodiment that uses semiconducting or insulating pillars arranged between the chambers to regulate the flow speed of the sample through the channel.

[0012] FIGS. 5A and 5B show complex permittivity spectra of water and horseradish peroxidase solutions according to an example embodiment.

[0013] FIG. 6 is a block diagram of simulated microwave circuit according to an example embodiment.

[0014] FIG. 7 is a transmission profile as a function of frequency for the circuit of FIG. 6.

[0015] FIG. 8 is a transmission profile as a function of frequency for various configurations of ideal capacitors having various values according to an example embodiment.

[0016] FIGS. 9A, 9B, 9C and 9D illustrate a block circuit diagram with series inductance and corresponding transmission and frequency profiles according to an example embodiment.

[0017] FIGS. 10A, 10B, 10C and 10D illustrate a block circuit diagram with parallel inductance and corresponding transmission and frequency profiles according to an example embodiment.

[0018] FIG. 11 illustrates a corresponding output signal as a function of capacitance level according to an example embodiment.

[0019] FIG. 12 is a block diagram of a capacitive test cell formed according to an example embodiment.

[0020] FIG. 13 illustrates the phase behavior of impedances of test cells without cladding layers according to an example embodiment.

[0021] FIG. 14 illustrates the phase behavior of impedances of test cells with a parallel inductor and without cladding layers according to an example embodiment.

[0022] FIGs. 15A and 15B illustrate the phase behavior of impedances of test cells with a parallel inductor tuned to a resonance frequency of 7GHz according to an example embodiment.

[0023] FIG. 16 illustrates an impedance phase difference of test cells with a parallel inductor according to an example embodiment.

[0024] FIGs. 17A, 17B and 17C illustrate impedance magnitudes of parallel configurations for multiple example embodiments.

[0025] FIG. 18 illustrates changes in impedance magnitude for 90 degree contacting of water and solution capacitors according to an example embodiment.

[0026] FIG. 19 illustrates magnitudes of a final result of a fully simulated example circuit over a range of frequencies.

Detailed Description

[0027] In the following description, reference is made to the accompanying drawings that form a part hereof, and in which is shown by way of illustration specific embodiments which may be practiced. These embodiments are described in sufficient detail to enable those skilled in the art to practice the invention, and it is to be understood that other embodiments may be utilized and that structural, logical and electrical changes may be made without departing from the scope of the present invention. The following description is, therefore, not to be taken in a limited sense, and the scope of the present invention is defined by the appended claims.

[0028] A method and apparatus for detecting the difference in dielectric permittivity between two liquid media in a microfluidic system is described. The presence of the two liquid media with different permittivity may arise from (1) the separate introduction of two liquid media with inherent different permittivity such as arising, for example, from the different concentrations of solutes or (2) a different temperature of identical liquid media at the time of measurement causing different permittivity or (3) a temporal difference in permittivity

between the two liquid media at the time of measurement as can be achieved, for example, with capillary electrophoresis or (4) a localized difference in permittivity as can be achieved, for example, by localized chemical or biological reaction of targets with probes immobilized in the detection area.

[0029] The first part of the description of method and apparatus focuses on one embodiment for detecting the presence of targets that may or may not be indicative of disease. The presence of a target is identified by detecting a localized binding event that occurs between a target and a probe on electrode plates of a Capacitive Test Cell. The probe and target may be any antigen or any cell or any nucleic acid or any chemical or any antibody or any receptor (e.g., antigen presenting MHC Class 2 receptor). A design and simulation of microwave circuitry for carrying out the method and apparatus is then described, along with simulated results and discussion of current understanding of the operation of the design. The design and simulation in the second part relies only on the input of a difference in permittivity, and is thus representative of more than just the embodiment described for detecting the presence of targets that may or may not be indicative of disease.

I. Description of embodiment for detecting the presence of targets that may or may not be indicative of disease

[0030] The prior art uses low frequencies to detect the changes in dielectric properties (typically low Hz or KHz) range. At low frequencies, the rearrangement of small ions contained in the surrounding solution and their interaction with the electrode plates are responsible for signal changes. In the MHz frequency range, a change in the dielectric properties arises from the orientational relaxation of the protein dipoles. In the high MHz to low GHz frequency range, the change in dielectric properties appears primarily due to the changed presence of free water and the orientational relaxation of the hydration shell (strongly bound water), which surrounds the protein. In one embodiment, the dielectric properties include the orientational relaxation of biological molecules, moieties of biological molecules, hydration shells of molecules and free water. The advantage of using microwave frequency measurement is a

more direct measurement of target presence. In one embodiment, microwave frequencies are defined as in the range of approximately 300 MHz to 50GHz. In a further embodiment, the range of microwave frequencies of most interest include approximately 1GHz to 20 GHz, corresponding to significant microwave absorption by water.

[0031] Probes may comprise linker molecules that can be electrochemically attached to the surface while keeping the electrodes DC charged and using the same circuit as for the detection of high frequencies. Thus, in a first step, a linker molecule may be immobilized electrochemically on the surface of the electrode, and any number of prior or further chemical reactions may be performed in connection with the attachment of the probe. In a second step, the probe molecule may be reacted with the linker molecule. This invention is significant in that it allows the chip structure to be manufactured independently of the probe attachment (thus making it cheaper by using standard microfabrication technology as used, for example, in the context of semiconductor manufacturing). It is furthermore significant in that it allows for the selective immobilization of different probes in one apparatus, allowing multiple targets of multiple diseases, diseases of multiple targets, or combinations thereof, to be integrated in one apparatus using the same sample containing the multiple targets.

[0032] FIG. 1A depicts a conceptual schematic of a differential detection circuit 100 operating at microwave frequencies. The detection circuit in FIG 1A consists of a microwave signal source 110, the output of which is split into two equal signals via a splitter element 115 with one of the two signals being applied to a detection chamber or capacitor 120 and the other signal being applied to a reference chamber or capacitor 125 after being phase shifted 180 degrees by an inverter 130. In further embodiments, the signals are not phase shifted until after the respective chambers, but prior to or at recombination. The presence of an analyte in one channel may introduce a further phase shift from 180 degrees which may be detected. In other words, the phase shift is 180 degrees, plus any additional phase shift introduced by differences in dielectric in the two capacitors. The microwave signal source 110 may be a voltage controlled oscillator integrated with the device or a microwave signal coupled to the device

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from an external source via an antenna. The detection chamber 120, as well as the reference chamber 125 may comprise any identical sub-circuit, such as, for example, any resonant circuit, containing the detection and reference capacitor respectively.

[0033] On application of the signal and phase shifted signal to the detection and reference chambers (possibly comprising any identical sub-circuit) respectively, each signal undergoes a near identical phase and amplitude modification in the absence of target to probe binding in the detection chamber 120. In this case the combination of the two signals using a combiner 135 will result in the two signals canceling each other. This combined signal may be detected on-chip by detector 140, for example, after conversion by an RMS power detector as a near-zero DC signal, or transmitted to an external detector 140 with or without any kind of prior signal modification.

[0034] In one embodiment, the detection chamber 120 contains covalently bound probes as indicated at 150 in FIG. 1B without the occurrence of a binding event and the reference capacitor 125 with optional unreactive probes 155. FIG. 1C depicts the contrasting scenario to FIG. 1B, where the presence of a target triggers a binding event 160 in the detection capacitor. In this case, the detection capacitor will change its capacitance but the reference capacitor will not, so that the amplitude and phase modification of its signal will differ from that of the reference capacitor. In this instance the combination of the two signals will no longer cancel and the resultant combined signal will indicate the presence of the trigger.

[0035] The probes may be immobilized on one or both electrodes of the detection chamber 120 that forms the basis for measuring the capacitance of the content between the two electrodes. The detection chamber 120 may be a component of a microfluidic channel through which the sample, containing the target, is introduced. The microfluidic channel extends prior to and following the detection chamber 120 in a continuous manner. The binding event is detected by measuring the change in capacitance that occurs as a result of said binding event.

[0036] Multiple detection chambers may be used to detect binding events, and contain covalently bound probes. Each detection chamber may be

paired with one or more "reference chambers". A reference chamber differs from a detection chamber in that probes are not immobilized on either of the reference chamber's electrodes. Rather, "unreactive probes" may be immobilized on one or more of the reference chamber's electrodes. Unreactive probes are defined as being similar or identical in structure, and/or composition to probes, but without the correlative binding characteristics; ideally unreactive probes would encompass no correlative binding characteristics of the comparative probe. The circuit element of the microwave detection circuit, which is basis of the detection chamber, may be integrated on-chip with the fluidic channel and the surrounding capacitors.

[0037] FIG. 2 illustrates a cutaway view of an example embodiment 200 of a reference chamber and a detection chamber. The diagram shows an example arrangement of the capacitor and microfluidic channel in the on-chip integrated circuit for a given chamber. In this example, the capacitor 125 for the reference chamber and the capacitor 120 for the detection chamber are arranged in series in the fluidic channel. The separation of the capacitor electrodes of a given chamber is determined approximately by the height of the fluidic channel. The dimensions of the capacitor electrodes and the height of the fluidic channel, along with any additional layers on the electrode surfaces facing the fluidic channel, may be designed in accordance with the frequency used to give capacitance values which optimize the signal to noise ratio of the detection circuit and which may make use of a resonant circuit design. FIG. 2 also indicates a possible layout for connecting the capacitor electrodes to a coplanar wave guide 210. A different embodiment may use a microstrip layout (not shown in FIG. 2).

[0038] FIGs. 3A, 3B and 3C detail top, cross-sectional and end-on views, respectively, of an example fluidic channel 310 passing through the capacitor (C_{ref}) of a reference chamber 125 and the capacitor (C_{detect}) of a detection chamber 120 respectively, aligned in a series.

[0039] FIGs. 3D, 3E and 3F detail top, cross-sectional and end-on views, respectively, of two fluidic channels 320, 330 arranged in parallel, each passing through reference chambers 335, 340 and corresponding detection chambers 345, 350. In this example, an upper fluidic channel 320 is detecting the presence

of a target C and therefore passes through the capacitor (C_{ref}) of reference chamber 335 and the capacitor (C_{detect}) of detection chamber 345. The lower fluidic channel 330 is detecting for the presence of target D and therefore passes through the capacitor (D_{ref}) of reference chamber 340 and the capacitor (D_{detect}) of detection chamber 350. It should be noted that detection chamber arrangements for detecting multiple different targets, of which FIGs. 3D to 3F are an example, does not rely on the chambers being arranged in parallel as they may also be arranged in series, and multiple further detection and reference chambers may be included.

[0040] The capacitor electrodes may consist of one or more conductive materials and the surface layer facing the channel may consist of either a metal, an insulator or a crystalline or polycrystalline or amorphous semiconductor.

[0041] FIG. 4A, details a top view of an alternative arrangement where a fluidic channel 410 passes through the capacitor (C_{ref}) of a reference chamber 415 and the capacitor (C_{detect}) of a detection chamber 420 placed across the fluidic channel in parallel so that both chambers are exposed to the sample at the same time. The detection capacitor is electrically isolated from the reference capacitor.

[0042] FIG 4B details a top view of an alternative arrangement where two separate fluidic channels 430, 435 pass through the capacitor (C_{ref}) of a reference chamber 415 and the capacitor (C_{detect}) of a detection chamber 420.

[0043] FIGs. 4C and 4D detail top and cross-sectional views respectively of a similar embodiment to that shown in FIGs. 3A and 3B but that uses semiconductor or insulator pillars 440 arranged between the chambers to regulate the flow speed of the sample through the channel.

Apparatus Manufacture

[0044] In one embodiment, the integrated circuit and microfluidic channel manufacturing process is separated from the process of selectively attaching the probes or unreactive probes to the capacitor electrodes of specific detection and reference chambers respectively. This separation provides the ability to customize the apparatus at the level of the individual detection or reference chamber while maintaining the economies of scale associated with

traditional semiconductor or microfluidic manufacturing methods. Furthermore, it allows for the reuse of the apparatus by refunctionalizing the detection and reference chambers with new probes and unreactive probes respectively. In doing so, the manufacturing cost associated with each detection is reduced. Finally, the ability to selectively attach different probes and unreactive probes to the capacitor electrodes of specific detection and reference chambers respectively, enables the apparatus to be configured to detect different targets in different detection chambers. This allows for the detection of multiple targets with a single sample. An application of this might enable the apparatus to use a single sample to detect multiple diseases and/or diseases which are diagnosed by the identification of multiple protein components.

Capacitor Electrode Funtionalization

[0045] The attachment of specific probes or unreactive probes to the capacitor electrodes of specific detection and reference chambers respectively occurs in three stages. The term probe is meant to include both probes and unreactive probes as the functionalization principle is the same for each.

[0046] A first stage involves the immobilization of a linker molecule on the surface of the capacitor electrode of the chamber to be functionalized. The linker molecule is designed such that it has a moiety that can react with the probe (e.g. an amino or carboxyl acid group) and that is initially protected and a ligand which is not protected. The linker molecule will bond via this unprotected ligand to a statically charged electrode surface. Therefore, the linker molecule is introduced into the fluidic channel while the capacitor of the chamber to be functionalized is charged at the appropriate potential for activating the surface reaction. The linker molecule will thus bind covalently to the capacitor of the addressed chamber. The DC voltage that is applied to the capacitor of the addressed chamber may utilize the same circuitry that is used for applying the detection signal.

[0047] The second stage involves unprotecting moiety that can react with the probe on the immobilized linker molecule.

[0048] The third stage involves the introduction of the probe to the channel. The probe will bind to moiety that can react with the probe, which is exposed on the immobilized linker molecule.

[0049] A fourth step may involve a coating of the probe to prevent denaturing.

[0050] The above reaction steps serve to illustrate the general electrochemical immobilization scheme, the details of which may of course involve any number or sequence of reactions within or outside the fluidic channel.

[0051] While the foregoing is directed to embodiments of the present invention, other and further embodiments of the invention may be devised without departing from the basic scope thereof, and the scope thereof is determined by the claims that follow.

II. Design and simulation of microwave circuitry for carrying out method and apparatus

[0052] A proof-of-principle and further embodiments for detecting the difference in dielectric permittivity between two liquid media will now be described, and has been done by simulation using an ADS software package from Agilent Technologies, the worldwide dominant design and simulation software for microwave circuits and components.

[0053] FIGs. 5A and 5B show the complex permittivity spectra of water and 34.8 mg/mL horseradishperoxidase solution. This data is taken from literature (Yokoyama et al., J.Phys.Chem. B, Vol. 105, No. 50, 2001) and the permittivity numbers used in the simulations are based on this data set. Similar tests may be performed to measure the same kind of data for any protein solution, or in fact any difference in permittivity of any two liquid media. The embodiments may be modified in a manner consistent with the following description for each such difference in permittivity of two liquid media. The permittivity spectra, such as that in FIGs. 5A and 5B, form an input to the design and simulation process in ADS. Then, masks may be generated for microfabrication directly from the design in ADS.

[0054] Based on the concept of detecting the permittivity change arising in one embodiment from the presence of additional protein with a phase comparing circuit as described above, similar circuits are designed and simulated on the chip level with Agilent's ADS software package. The circuits were designed as microstrip circuits for use at microwave frequencies, such as approximately 7 GHz. A core circuit 600 can be broken down into three levels of interacting functionality, as illustrated in FIG. 6. A comparator 610 splits the incoming signal 615 into two signals of equal power, but 180 degrees out-of-phase at 620, 625, and then combines them again at 630 in an equal-power configuration. In a further embodiment, the comparator 610 splits the incoming signal 615 into two signals of equal power, but in phase, or out of phase, and then at 630, the signals are placed 180 degrees out of phase and combined. Thus, in either embodiment, the signals are 180 degrees out of phase when combined. Material in the channel of the detection capacitor introduces further phase shift which can be measured.

[0055] A (parallel) resonance configuration 635 with an inductor 640 tuned approximately to be in resonance with an effective capacitance of a Capacitive Test Cell structure 645 at the operating frequency is provided at the first signal 620. A Capacitive Test Cell (detection chamber) is shown at 650. A physical layout of protein test-chambers is analogous to a microstrip thin-film on-chip capacitor 655, but with the microfluidic channel and dielectric cladding layers forming the filling between the two "capacitor plates". An inductor 660 is provided in parallel with the capacitor 655 and both are coupled to signal 625. The electromagnetic behavior in the circuit, is quite different from a simple capacitor.

[0056] In the following, each level is described in more detail, along with how they relate to produce a highly sensitive detector of small changes in permittivity.

[0057] The comparator 610, 630 is relatively straightforward in its function and design. The equal power split with 180 degree or other phase shift is accomplished in one embodiment with a Rat-Race-Coupler (RR-180), and the equal-power combining at 630 with a Rat-Race-Coupler without or with phase shift (RR-0) used in reverse such that the total phase shift is approximately 180

degrees. The Rat-Race-Couplers are designed for the target frequency (in the examples herein, 7 GHz) and the substrate properties using ADS. Both elements are passive, and their layout in reality is just a circular metallization pattern of well defined circumference, width and port positions. The designs may be easily modified for other frequencies. The difference between RR-180 at 610 and RR-0 at 630 lies in which ports are connected.

[0058] Connecting just these two elements with nothing else in either of the two arms in between produces the transmission profile as a function of frequency shown in FIG. 7. As expected from the 180 degree phase shift, practically no power is transmitted through this structure and the extinction of transmission with negative 100dB (output = input divided by 10 billion) is pretty much complete. Putting a random circuit with resistive, capacitive and inductive elements into the arms between the two Rat-Race-Couplers does not change the transmission spectrum as long as both arms are the same. This provides circuit stability and implies a self-calibrating feature of an ultimate biological test-chip, since any environmental change, contamination or change over time will always happen to both test-chambers equally, but will have no effect on the measurement.

[0059] Several advantages are apparent from having the 180 degree phase shift in the comparator regardless of the rest of the circuit design, as compared to having no phase shift. Let us suppose the protein presence would alter the transmission by 4% of the input power. Then the output/input power ratio would change from 1 to 0.96 for 0 degree phase shift, and it would change from 10^{-10} to 0.04 for 180 degree phase shift. Clearly, the signal difference and stability will always be better in the comparator configuration that has near total extinction as the self-calibrating reference.

The Resonator

[0060] Resonance in various forms is key to converting a small change in permittivity into a large differential signal. The resonance may be important in two ways: one is on the circuit level, which will be discussed in this section, and the other is on the level of the Capacitive Test Cell itself, which will be discussed in the next section. For illustration of the circuit level effect of

resonance, we shall consider the case of the Capacitive Test Cell being replaced with an ideal capacitor. Using the measurement data presented in FIG. 5, ϵ' would be about 68 for water and 66 for 34.8mg/ml horseradish peroxidase solution at 7 GHz. For an ideal parallel plate capacitor with the liquid as the dielectric, C_1 (solution) / C_2 (water) would therefore be 0.97. FIG. 8 plots the four transmission curves for (a) 1 pF ideal capacitors in both branches, (b) $C_1=0.97$ pF and $C_2=1$ pF (c) the same as in (b) but with series resonance at 7 GHz in both branches (identical ideal inductors in series) and (d) with the parallel resonance at 7GHz (identical ideal inductors in parallel). First, it may be observed that without resonance, the circuit translates a 3% change in relative capacitance to a signal change of 6 orders of magnitude (-104 dB vs. -39 dB). Series resonance improves this only slightly, while the parallel resonance configuration improves the signal by another factor of 30.

[0061] The results are quite different between series and parallel resonance. FIGs. 9A, 9B, 9C and 9D examine what is going on in detail for the series resonance case, and FIGs. 10A, 10B, 10C and 10D examine the parallel resonance. Both sets of figures have the response of the entire circuit as shown in FIG. 8 repeated for reference. FIGs. 9A and 10A show the measurement setup to produce the graphs in FIGs. 9B - 9D and 10B - 10D. The complex impedance of each branch is measured separately in an AC configuration, and the corresponding impedance magnitude and phase angle are plotted in the lower halves of the figures respectively.

[0062] As expected for any ideal series resonance, the circuit changes from capacitive to inductive at the resonance frequency with the abrupt 180 degree phase shift at the resonance frequency, and the magnitude of the impedance goes to zero. Due to the slightly different capacitance in the two branches, the resonance frequency shifts, and if one chooses the operating frequency (7GHz) of the circuit to be in between, as indicated in FIGs. 9A-9D, then one obtains a 180 degree phase shift in one branch, but not the other. Considering just the phase, this scenario would be expected to provide full transmission considering the previous 180 degree phase shift from the Rat-Race-Coupler. But in reality, the improvement due to simple series resonance is minimal as compared to no resonance. This is thought to be a result of the

magnitude of the impedance being very small and that the circuit is handling microwaves, and not low frequencies. For microwave transmission, the transmission lines and circuit elements should be impedance matched to avoid reflection. Both Rat-Race-Couplers have been designed for the standard 50 ohm impedance. Of course, they can be designed for different impedance. Thus, despite the perfect phase addition, most of the power is reflected at both branches resulting in little overall improvement. This, however, does not mean that some other useful scheme could be found to utilize a series resonance configuration. In fact, a series resonance within the Capacitive Test Cell will prove very useful.

[0063] In the case of parallel resonance, the circuit changes from inductive to capacitive at the resonance frequency, again with a 180 degree phase shift, but the circuit will now look more like an open circuit. This is illustrated by the simulation in FIGs. 10A-10D. Although the magnitude of the impedance with 1500 Ohm is high at 7 GHz as compared to the 50 Ohm, the reflection is less than that at near-zero Ohm of the series resonance case. Thus, the 180 degree phase shift now produces an overall improvement by a factor of 30, but due to the impedance mismatch, the transmission is still a factor of 200 (-23 dB) below ideal.

[0064] This characterizes the ideal, general scenarios on the circuit level. Any resonant circuit has an associated quality factor, which is a measure of how sharp the resonance peak is as a function of frequency. In the present embodiment, one would expect that the sharper the resonance peak is, the clearer the separation between the two unequal branches and the stronger the output signal. For a series resonance circuit, the quality factor $Q = 1/R * \sqrt{L/C} = 2 \pi * f * (1/R) * L$. For a parallel resonance circuit, $Q = R * \sqrt{C/L} = 2 \pi * f * R * C$. Here, R is the inherent resistance, L the inductance, C the capacitance and f the frequency. For the parallel resonance scenario then, one would expect to increase signal output if there is an increase in the frequency and/or the capacitance. Leaving the frequency fixed at 7 GHz, Table 1 shows the results from varying the capacitance in branch A by a factor of 2, while leaving the ratio between the two capacitances fixed. FIG. 11 shows the corresponding output

signal of the entire circuit, which behaves as expected as a function of capacitance level.

	Branch A Capacitance	Branch B Capacitance	Parallel Inductance (tuned to branch A)	Quality Factor	Output Signal (dB)
High C	2 pF	1.97 pF	0.2585 nH	0.088 R	-17.8
Medium C	1 pF	0.97 pF	0.517 nH	0.044 R	-23.8
Low C	0.5 pF	0.485 pF	1.034 nH	0.022 R	-29.8

Table 1.

[0065] Turning to the actual capacitive test-cell design, a nominal structure is illustrated at 1200 in FIG. 12. Structure 1200 is designed as microstrip circuits, which means that signal lines are separated from ground via a substrate. Three metal layers, GND 1210, Cond layer 1215 and Cond 2 layer 1220 are shown. Cond layer 1215 is the layer of the passive circuit, and Cond2 layer 1220 is the other plate of the capacitor. To imagine how the signal travels through the structure 1200, consider an electromagnetic wave arriving from the left. It travels between the Cond microstrip layer 1215 and the ground plane, GND 1210 through a 100 micron Si substrate 1225. When it gets to the capacitor structure, it sees an open transmission line, but a new transmission line between the Cond2 layer 1220 and GND 1210 becomes available as the wave travels to the right. So the wave “transitions” from the [Cond-GND] transmission line to the [Cond2-GND] transmission line via a capacitor filling 1230. Eventually, we bring the signal back down to the [Cond-GND] transmission line away from the capacitor. We called the 100 micron Si layer the “substrate” in the sense that it is the microstrip substrate for most of the circuit, but the thickest layer is the other Si layer. So in reality, the structure will probably be mounted up-side down from the way it is drawn in FIG. 12. The substrate and thick Si layer may be varied in thickness, and may in fact be any material at all that can be processed. If we can avoid the need for active components, there will be no need for a semiconductor. The “substrate” material can be different from the “carrier” material. SU-8 1255 here is the polymer for

wafer bonding, which also may be done in any number of ways depending on the materials chosen.

[0066] The capacitor filling 1230 is formed of a microchannel 1235 (liquid) that is surrounded by a dielectric cladding layer 1245 and 1250 on each side. The channel thickness is indicated as 1 micron, because shallower channels become nominally more difficult to fabricate, but it can certainly be accomplished. From an electrical signal point of view, a thin channel is good since it causes a high capacitance and results in a high volume fraction of the reacted proteins or other substance. However, there are of course many boundary conditions to be considered: (1) the product of capacitance and inductance determines the resonance frequency, and L cannot be made arbitrarily low nor is it entirely independent nor should the total frequency be too low; (2) fluidynamic considerations; (3) reaction chemical considerations etc. The dielectric cladding layers can be present or absent, and any material can be chosen that can be appropriately deposited. The cladding layers 1245, 1250 fulfill many functions: (1) they provide a surface other than metal for the electrochemical immobilization of the linker molecules, (2) they allow capacitance tuning independent of the channel, (3) they change the intrinsic inductance, (4) they change the intrinsic resistance, (5) they can be crucial for establishing an additional resonance, and probably many other.

[0067] Although the circuit and device behavior is conceptualized with discrete elements, this picture does not really hold at microwave frequencies. The reason is that, when electromagnetic wavelengths and circuit sizes become comparable, the cross terms of Maxwell's Equations can no longer be neglected. This is why a full electromagnetic simulation is desirable. Resistances, inductances and capacitances cannot be separated in reality. Literally everything has inductance, capacitance and resistance, and it all influences each other as may be seen in the following actual proof-of-principle.

[0068] Because of the above, everything may become a design parameter, and can act as a design freedom and restriction at the same time. Even the contact geometry of the capacitor test-cell matters significantly and there is no 100% clear distinction between the capacitor structure and the immediately surrounding circuit. That said, some of the primary design

parameters are: operating frequency, channel dimensions, cladding layer material, cladding layer dimensions, capacitor area, capacitor shape and capacitor contacting geometry.

[0069] In one embodiment, the depth or thickness of the microchannel varies from significantly thinner than 1 μm , such as 0.1 μm to approximately 10 μm . The cladding layer also may vary in thickness from approximately 0 to 1 μm . In one embodiment, the ratio of thicknesses of microchannel to cladding layer remains approximately 10 to one. In one embodiment, a 0.5 μm channel is used. Thicker microchannels result in a decrease in effective capacitance. Similarly the cladding layers may also be changed in thickness, but a large increase in thickness decreases effective capacitance due to the increased spacing of the capacitor plates and the lower dielectric constant of the cladding material, such as SiO_2 . Thicker cladding may also increase intrinsic inductance at microwave frequencies. Other materials may be used for the cladding as desired, such substantially insulating dielectrics such as oxides, nitrides or similar dielectrics. In one embodiment, the material is selected from the group consisting of silicon dioxide, silicon nitride, amorphous polysilicon, crystalline silicon, and polymers. Still further materials with varying dielectric constants may be used.

Behavior of Capacitive Test Cell Structure

[0070] FIG. 13 shows the phase behavior of the impedances of Capacitive Test Cells without cladding layers, a 1 micron channel, a 150 micron x 150 micron square design and a 180 degree contacting geometry. The curves correspond to the permittivity values indicated on the graph (they are not quite physical at these frequencies, but correspond to the lower frequency limit). The resonances are entirely inherent to the structures, as no additional inductances are introduced in the measurement circuit. This comparison reveals several aspects of the real behavior: 1) The inherent inductances and capacitances of a structure of the above type place the intrinsic resonance in the low GHz frequency range. 2) Without any absorption ($\epsilon''=0$), the resonance deviates from the ideal 180 degree phase shift and produces an “overshoot” in the vicinity of the resonance frequency. 3) The frequencies of self resonance for a realistic

$\Delta\epsilon' = 2$ are distinct and the separation on the order of 0.1 GHz. 4) When absorption is present, the phase shift “smears out” indicating a decreased quality factor of the resonance.

[0071] All this behavior is consistent with the Capacitive Test Cell structure acting as a series resistor (which increases with absorption), a series inductor and the series capacitor, which constitutes a series resonance circuit with f_0 around 5.5 GHz.

[0072] The phase of a signal from the no-absorption structures with an ideal inductor in parallel is shown in FIG. 14. The series self resonance remains, and the ideal inductor creates second resonance (parallel) with the capacitance of the test-structure, the frequency of which can be tuned with the inductor value.

[0073] These examples illustrate the general behavior of the test-cell structure as a series of resistance-inductance-capacitance. One can determine equivalent values for a specific design at a specific frequency. However, the values can change non-ideally with design parameters, and especially with frequency.

Behavior of Actual Capacitive Test Cell Structure at 7GHz

[0074] A “proof-of-principle” structure at 7 GHz is based on the protein spectra shown in FIG. 5. The structure is considered to be “locally optimized” for 7 GHz, but not necessarily “globally optimized”, and a modified design at another frequency may produce better results. Every design may be optimized in all its parameters depending on the input dielectric spectra. The structure here is the one shown in FIG. 12, which includes the two 0.1 micron silicon dioxide cladding layers 1245, 1250. The inclusion of the cladding layers lowers of course the overall capacitance of the structure, since it increases the plate separation while at the same time introducing material with a 15 times lower permittivity. At the same time, the cladding layers increase the effective series inductance significantly. All in all, the self-series-resonance shifts therefore to frequencies around 12 GHz. The total capacitance is really a series of individual capacitances due to the layers. Although one electrically sees primarily the total capacitance from outside, the individual capacitances still have a physically distinct existence.

[0075] FIGs. 15A and 15B illustrate what happens when a 0.1nH ideal inductor is placed in series with this structure to tune the resonance frequency down to around 7GHz. Each of the graphs show a series of 5 curves, which correspond to different square areas – from right to left in square microns: 140x140, 145x145, 150x150, 155x155, 160x160. FIG. 15A shows impedance phase shift data for water at 7 GHz ($\epsilon'=68$), and FIG. 15B for solution ($\epsilon'=66$). This allows some very meaningful insight: The Capacitive Test Cell with water exhibits a near ideal resonance for 145x145 and 150x150 capacitor areas, and slightly less for 140x140, but much less for 155x155 and 160x160. The resonance window is quite narrow and thus sensitive. The solution does not fall into this resonance window for any of the test areas. In still further embodiments, the capacitor square areas may be varied between 20x20 to 500x500. Yet further embodiments may utilize areas outside this range.

[0076] This has two significant consequences: 1) Despite the much, much higher absorption ($\epsilon''=24$), a high, and even near perfect resonance at higher frequencies, may be obtained. The higher absorption can easily be designed around. 2) The narrow resonance window allows further differentiation between the presence and absence of the proteins. This translates directly into additional passive amplification.

[0077] As to the explanation of this, recall the “sub-capacitances” in the structure with cladding layers. It is thought that the structure can exhibit a self resonance with a sub-capacitance, which under the right design can coincide with the resonance frequency of the (total capacitance) – (external inductor) tuning pair. The overall series resonance is not very useful for the ultimate goal due to the impedance mismatch for the impedance magnitude approaches zero in current embodiments. The parallel resonance occurs at approximately the same inductor value, however. So using a 0.102nH inductor in parallel instead with the 150x150 structure for both water and solution, we obtain the impedance phase *difference* between the two as shown in FIG. 16.

[0078] The series self resonance is 12 GHz, and the parallel tuned resonance at 7 GHz. Again, this is the phase difference between the water and

solution. Although the phase difference is only 40 degrees, the frequency distribution (width of peak) looks very good.

[0079] As earlier indicated, the impedance mismatch plays a crucial role in how much signal is transmitted through each branch. FIGs. 17A, 17B and 17C show the impedance magnitude of the parallel configuration for all the 5 structures, which vary in their capacitor area exactly as before (FIGs. 15A and 15B). FIG. 17A plots the impedance magnitude for water, FIG. 17B that of solution and FIG. 17C shows the difference between the two. First, a similar signature of the additional resonance is observed as discussed with respect to FIGs. 15A and 15B. Also, the impedance is around 50 Ohm, where for water it is above and for solution below this value. It turns out that it is best for signal amplification if the mismatched values are centered around the matching value (of 50 Ohm in this case). With the help of the self resonance tuned to the designed parallel resonance, an impedance magnitude difference of 25 Ohm is centered around the matching value of 50 Ohm, plus we have a 40 degree phase shift.

Proof-of-Principle

[0080] A 150x150 micron structure is used as laid out in FIG. 12 and as discussed above with one slight modification in the full microstrip circuit design. Instead of contacting the structure on opposite sides, it is contacted at 90 degrees to better fit the layout in one embodiment. This changes the parameters slightly, but allows design of the circuit from RR-180 to RR-0 with metal lines only with the tuning inductor being "created" by the metal trace pattern. FIG. 18 shows the changes in impedance magnitude for the 90 degree contacting geometry of water and solution Capacitive Test Cells. Both peak values are shifted down by about 15 Ohms, and the difference is slightly less.

[0081] FIG. 19 compares the final result of the fully simulated circuit for (a) no proteins in either Capacitive Test Cell (i.e. negative test-case) and (b) 34.8 mg/mL horseradishperoxidase present in one of the Capacitive Test Cells (positive test-case). The difference is signal 10^{10} at 7 GHz, and at least 10^7 over a frequency range of 0.2 GHz. This frequency stability is helpful, since an input signal has a finite bandwidth. Moreover, the positive test case returns 4% of the

input power, which may well be enough for not needing any active components, making it cheaper to manufacture. In further embodiments, the power transmitted can be transformed into a higher voltage signal to provide higher output signals if desired.

[0082] In various embodiments, the circuit structures, including microstrip circuitry, fluidic channels, and detection and data processing circuitry may be formed in or on a semiconductor substrate. Further circuitry may be included and packaged together on a chip, such as RFID circuitry for transmitting both measured results along with a patient and/or chip identifier. This makes tracking and properly correlating test data to a patient more reliable than current methods, especially given conditions in under-developed areas of the world, where good records may be difficult to create, manage and maintain.

[0083] The Abstract is provided to comply with 37 C.F.R. §1.72(b) to allow the reader to quickly ascertain the nature and gist of the technical disclosure. The Abstract is submitted with the understanding that it will not be used to interpret or limit the scope or meaning of the claims.

CLAIMS

1. A device comprising:
 - a splitter that splits a time varying signal into two substantially equal power signals;
 - a reference capacitor having a fluidic channel between capacitor plates coupled to one of the equal power signals;
 - a detection capacitor having a fluidic channel between capacitor plates coupled to the other of the equal power signals; and
 - a detector coupled to outputs of the reference capacitor and detection capacitor such that the two time varying signals are approximately 180 degrees out of phase in the absence of an analyte in the channels, and that detects a change in dielectric relaxation that occurs in the fluidic channel of the detection capacitor.
2. The device of claim 1 wherein the dielectric properties comprise the orientational relaxation of biological molecules, moieties of biological molecules, hydration shells of molecules and free water.
3. The device of claim 1 wherein the detector includes a combiner that combines the outputs of the reference capacitor and detection capacitor substantially equally, such that the total phase shift of the time varying signals is approximately 180 degrees in the absence of the analyte.
4. The device of claim 3 wherein the splitter, combiner and capacitors form elements of microstrip circuits or co-planar waveguide circuits for use at microwave frequencies.
5. The device of claim 4 wherein the splitter, combiner and capacitors are adapted to operate at a frequency within a range of approximately 1-20 GHz.
6. The device of claim 5 wherein the splitter and combiner are formed with Rat-Race-Couplers.

7. The device of claim 1 wherein the fluidic channels comprise microfluidic channels defined by dielectric layers coupled between the capacitor plates.
8. The device of claim 7 wherein the dielectric layers are formed of substantially insulating dielectrics such as oxides or nitrides.
9. The device of claim 7 wherein the dielectric layers are formed of a material from the group consisting of amorphous or polycrystalline or crystalline semiconductors, and polymers.
10. The device of claim 1 and further comprising covalently bound probes coupled to one or more detector capacitor plates.
11. The device of claim 10 wherein the permittivity of the detection capacitor is a function of binding events to the bound probes.
12. A device comprising:
 - a splitter that splits a time varying signal into two substantially equal power signals on a respective first branch and second branch;
 - a reference microwave resonator disposed in the first branch having a capacitor having a fluidic channel between capacitor plates coupled to one of the equal power signals;
 - a detection microwave resonator disposed in the second branch having a capacitor having a fluidic channel between capacitor plates coupled to the other of the equal power signals; and
 - a detector coupled to outputs of the reference resonator and detection resonator such that the two time varying signals are approximately 180 degrees out of phase in the absence of an analyte in the fluidic channels.
13. The device of claim 12 wherein the reference and detection microwave resonators further comprise an inductance coupled to the capacitor.

14. The device of claim 13 wherein the inductance and capacitor of each microwave resonator are coupled to behave like in a parallel LC circuit.
15. The device of claim 14 wherein the detector includes a combiner that combines the outputs of the microwave resonators in each branch substantially equally, such that the total phase shift of the time varying signals is approximately 180 degrees in the absence of an analyte in the fluidic channels.
16. The device of claim 15 wherein the splitter, combiner and resonators form elements of microstrip circuits or co-planar waveguide circuits for use at microwave frequencies.
17. The device of claim 16 wherein the splitter, combiner and resonators are adapted to operate at a frequency within a range of approximately 1-20 GHz..
18. The device of claim 17 wherein the splitter and combiner are formed with Rat-Race-Couplers.
19. The device of claim 12 wherein the fluidic channels comprise microfluidic channels defined by dielectric layers coupled between the capacitor plates, wherein the dielectric layers are formed of substantially insulating dielectrics such as oxides or nitrides.
20. A method of detecting the presence of a target in a liquid sample, wherein
the target binds to a probe, said method comprising:
 adding a sample to a detection chamber having probes bound to at least one plate of a capacitor forming the detection chamber; and
 measuring the change in capacitance that occurs between capacitor plates due to binding events between the target and the probe, thereby detecting the presence of a target, wherein the change in capacitance is measured at a selected microwave frequency.

21. The method of claim 20 wherein measuring the change in capacitance comprises:

- dividing a sinusoidal signal into two equal power signals;
- providing one of the signals to a reference chamber;
- providing the other signal to the detection chamber; and
- phase shifting the signals such that they are approximately 180 degrees out of phase in the absence of an analyte in the chambers and combining such shifted signals.

22. A method of covalently binding probes selectively to capacitor plates of a detection chamber, the method comprising:

- adding a linker molecule to the detection chamber that covalently binds to the capacitor plate surface via an electrochemically activated reaction; and
- charging capacitor plates of the detection chamber by applying a DC voltage such that the electrochemically activated reaction will occur.

23. The method of claim 22 wherein multiple detection chambers are disposed about one or more channels and wherein selective capacitor plates are charged in the presence of different linker molecules passed through the channel or channels.

24. A device comprising:

- a splitter that splits a time varying signal into two substantially equal power signals on a respective first branch and second branch;
- a reference resonator disposed in the first branch having a capacitor having a fluidic channel between capacitor plates coupled to one of the equal power signals;
- a detection resonator disposed in the second branch having a capacitor having a fluidic channel between capacitor plates coupled to the other of the equal power signals; and

a detector coupled to outputs of the reference resonator and detection resonator that detects a difference in dielectric properties of fluid in the fluidic channels at microwaves frequencies.

25. The device of claim 24 wherein the dielectric properties comprise the orientational relaxation of biological molecules, moieties of biological molecules, hydration shells of molecules and free water.

26. The device of claim 24 wherein the equal power signals are phase shifted approximately 180 degrees from each other, in the absence of an analyte in the fluidic channels, prior to detecting the difference in dielectric properties.

27. A capacitive test cell structure for a microwave based substance detection device, the capacitive test cell structure comprising:

a first metal layer having a first capacitor plate formed therein;

a second metal layer spaced from the first metal layer and having a second capacitor plate formed therein; and

a capacitor filling disposed between the first and second capacitor plates, wherein the filling includes a fluidic microchannel, wherein the dielectric properties of the filling may be measured by the use of microwaves.

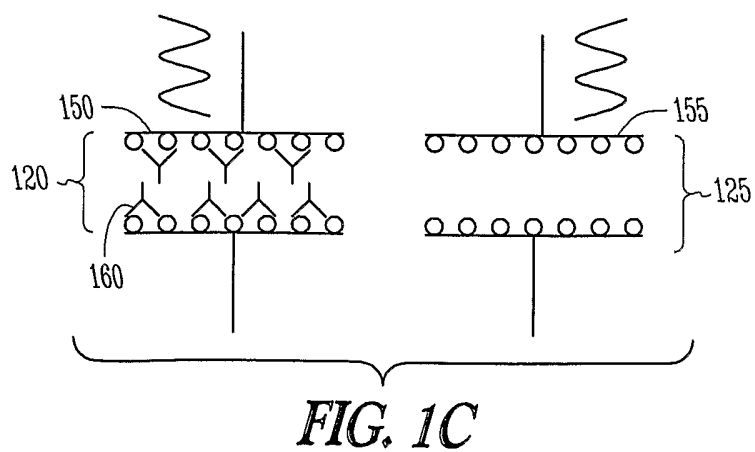
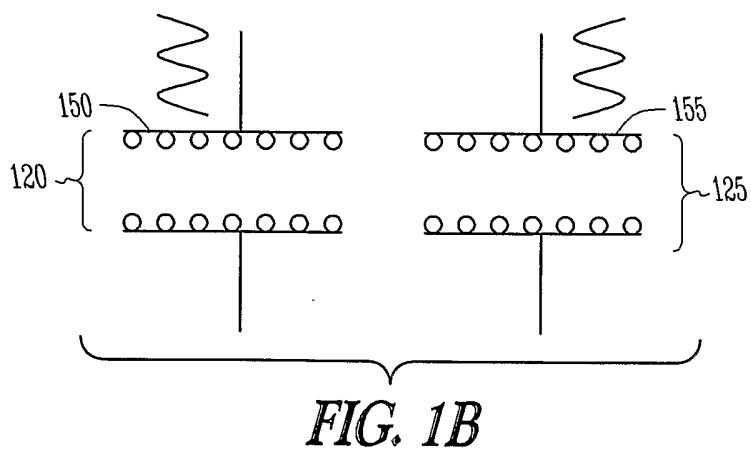
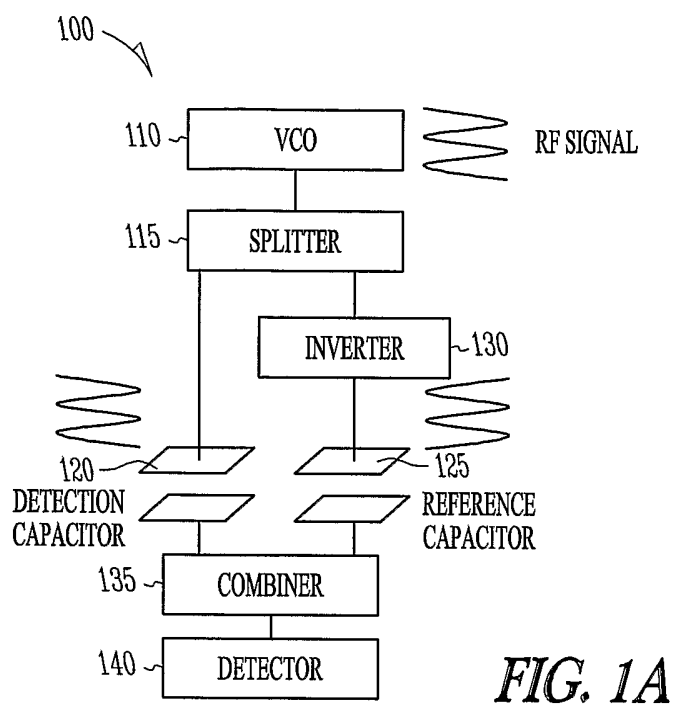
28. The capacitive test cell structure of claim 27 and further comprising a metal ground layer spaced apart from the first metal layer to form a microstrip circuit.

29. The capacitive test cell structure of claim 27 wherein the capacitor filling further comprises one or more dielectric cladding layers on each capacitor plate with the fluidic microchannel disposed between them.

30. The capacitive test cell structure of claim 29 wherein the fluidic microchannel is approximately 0.1 to 10 μm thick and the dielectric cladding layers are approximately 0.0 to 1 μm thick.

31. The capacitive test cell structure of claim 29 wherein the dielectric cladding layers are formed of substantially insulating dielectrics such as oxides or nitrides.
32. The capacitive test cell structure of claim 29 wherein the dielectric layers are formed of a material from the group consisting of amorphous or polycrystalline or crystalline semiconductors, and polymers.
33. The capacitive test cell structure of claim 29 wherein the capacitor plates overlap each other by approximately between 20 μm x 20 μm to 500 μm x 500 μm .
34. The capacitive test cell structure of claim 29 wherein parameters, such as dimensions and spacing of the elements are selected to contribute to one or more resonances between approximately 1 and 20 GHz.
35. The capacitive test cell structure of claim 34 wherein the multiple layers and plates contribute to resonances, and wherein parameters are selected to tune one or more such resonances to approximately a selected operating frequency.
36. The capacitive test cell structure of claim 35 wherein the parameters comprise operating frequency, external tuning inductances and capacitances, channel dimensions, cladding layer material, cladding layer dimensions, capacitor area, capacitor shape and capacitor contacting geometry.

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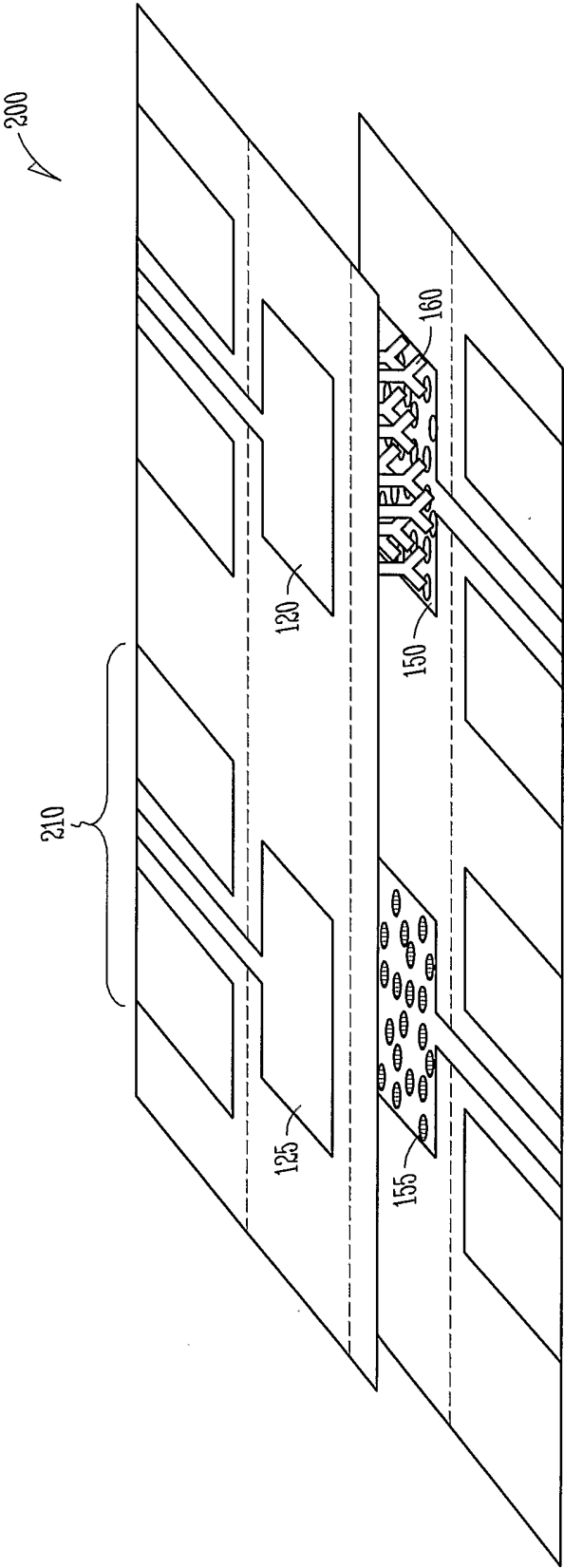
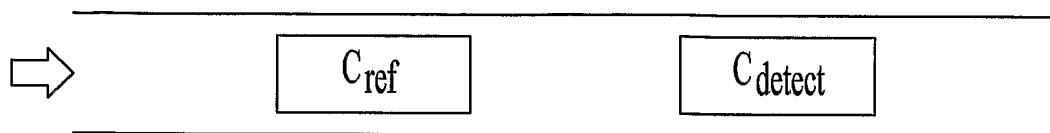
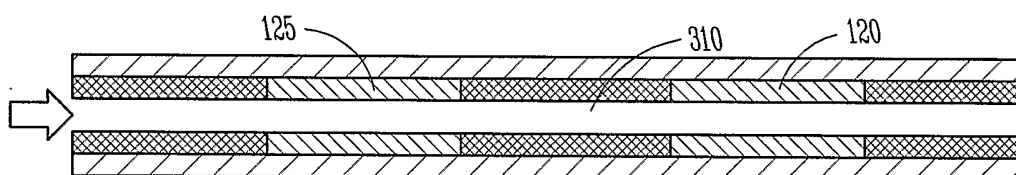
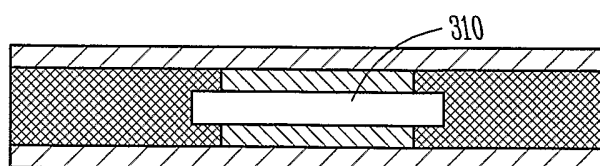


FIG. 2

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*FIG. 3A**FIG. 3B**FIG. 3C*

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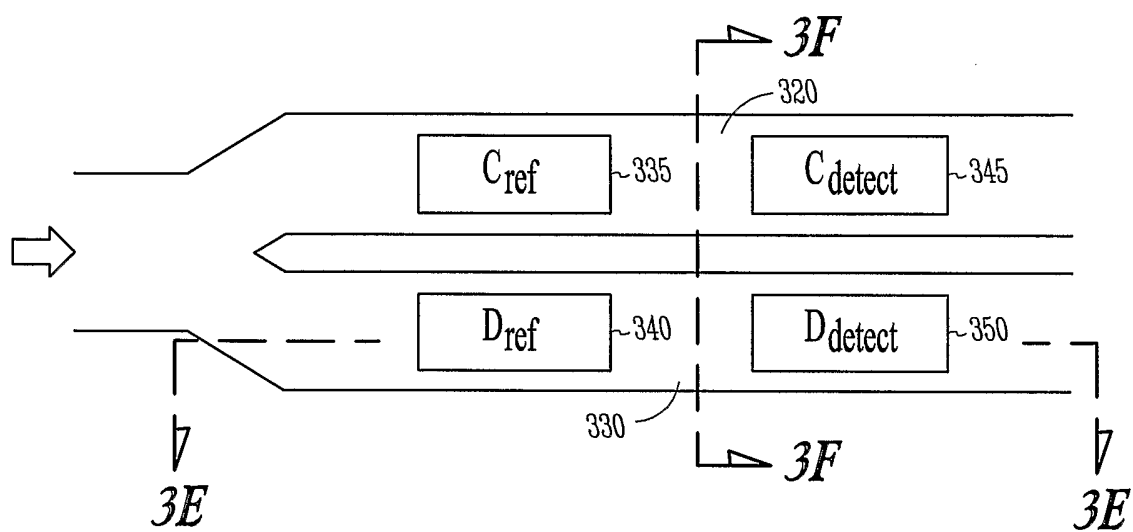


FIG. 3D

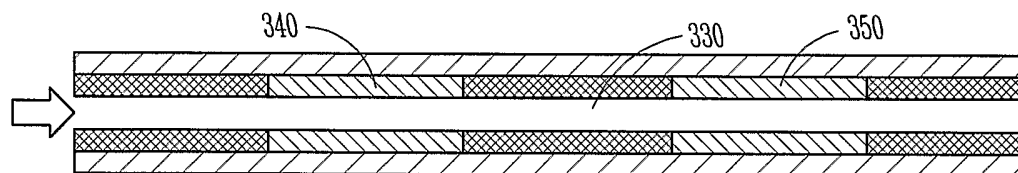


FIG. 3E

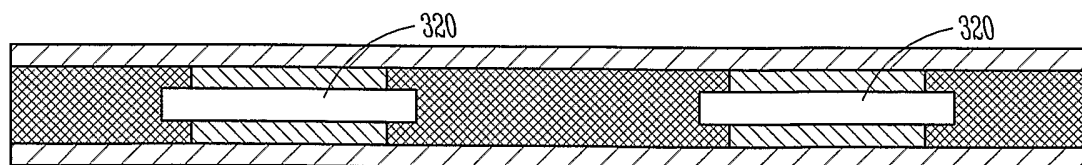


FIG. 3F

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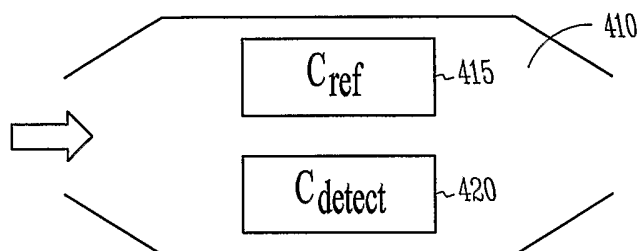


FIG. 4A

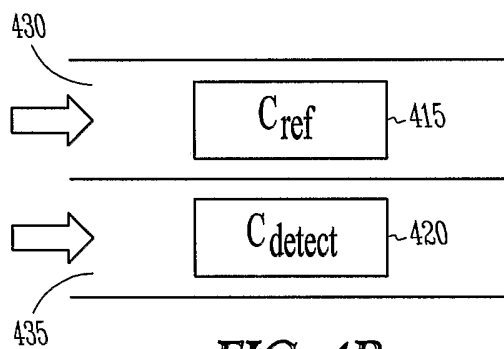


FIG. 4B

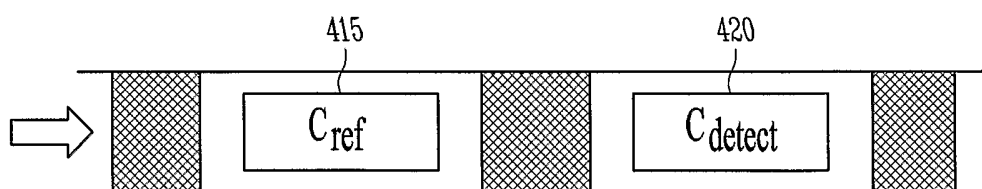


FIG. 4C

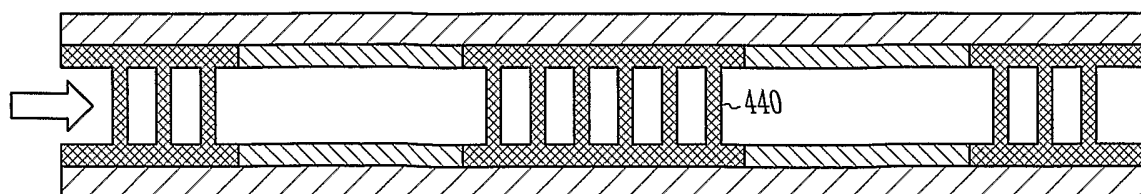


FIG. 4D

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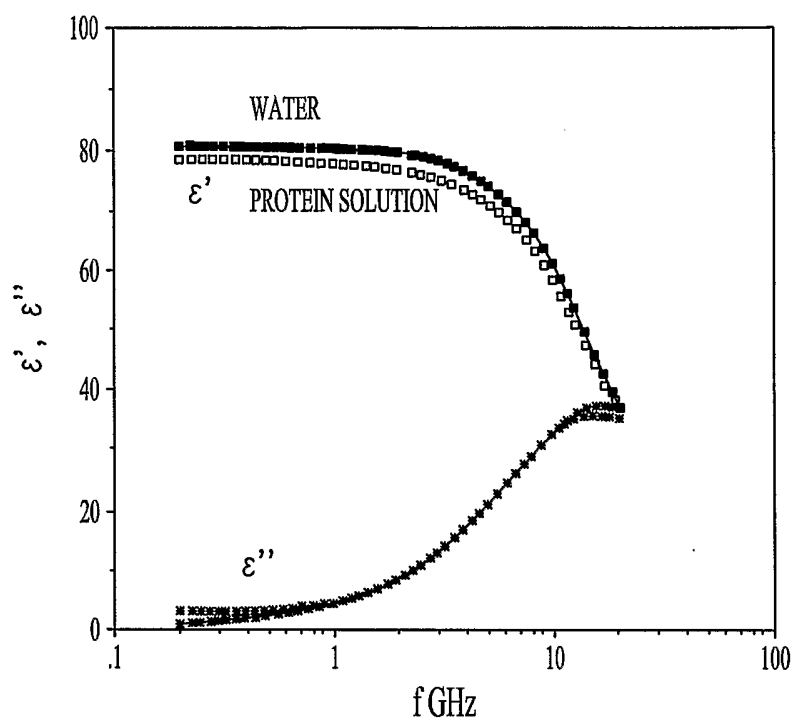


FIG. 5A

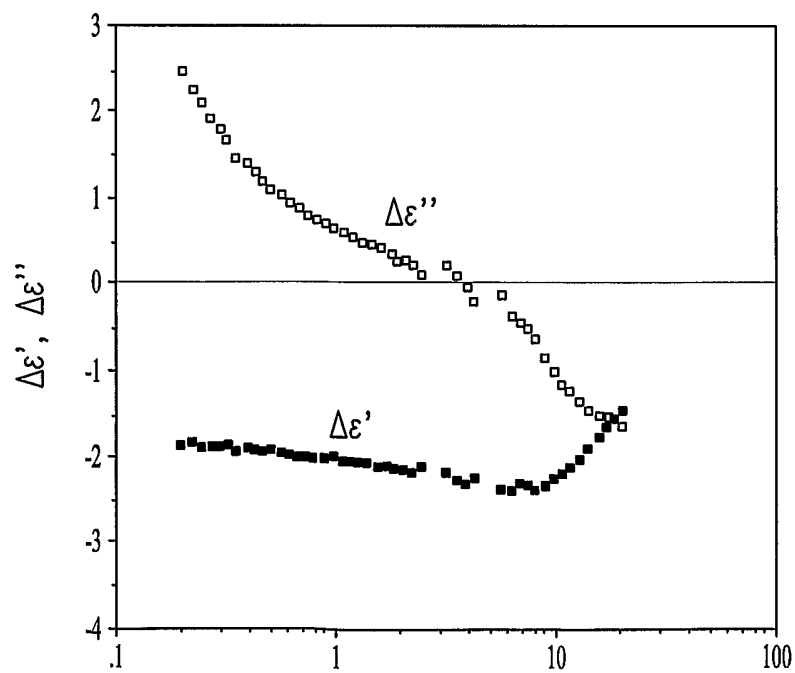
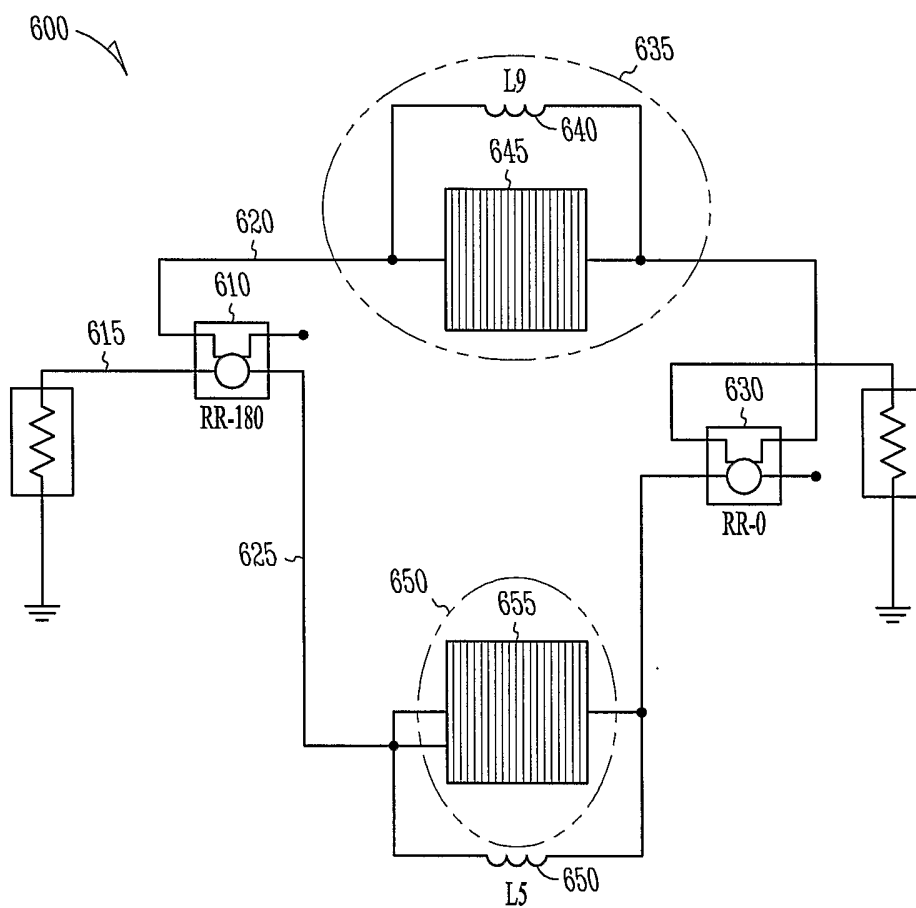


FIG. 5B

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**FIG. 6**

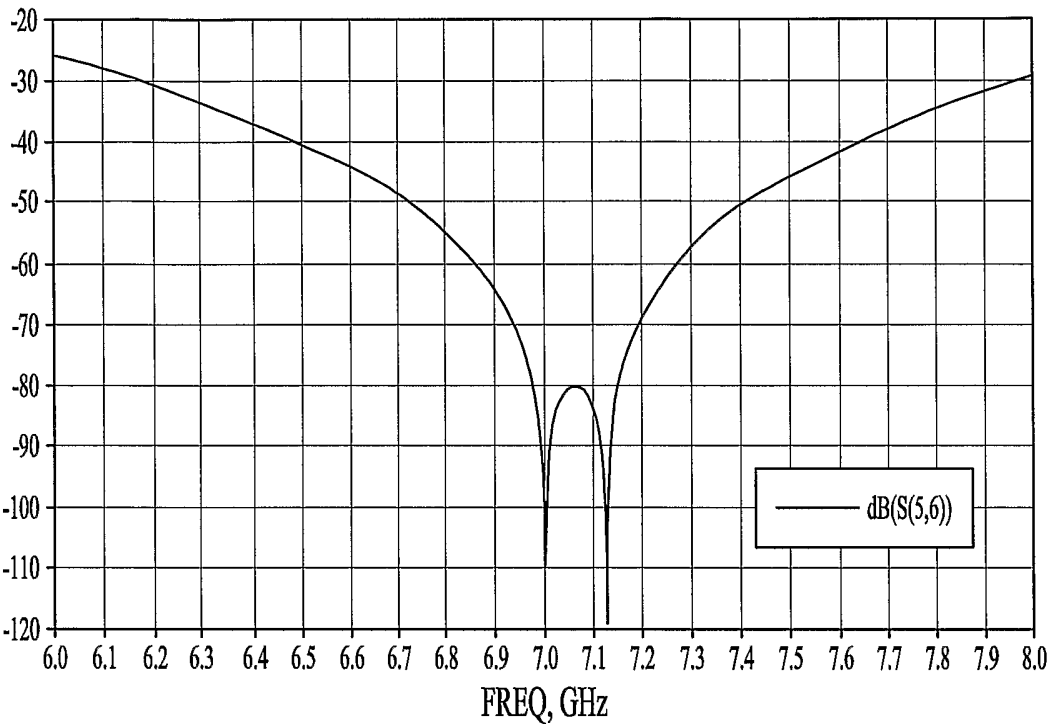


FIG. 7

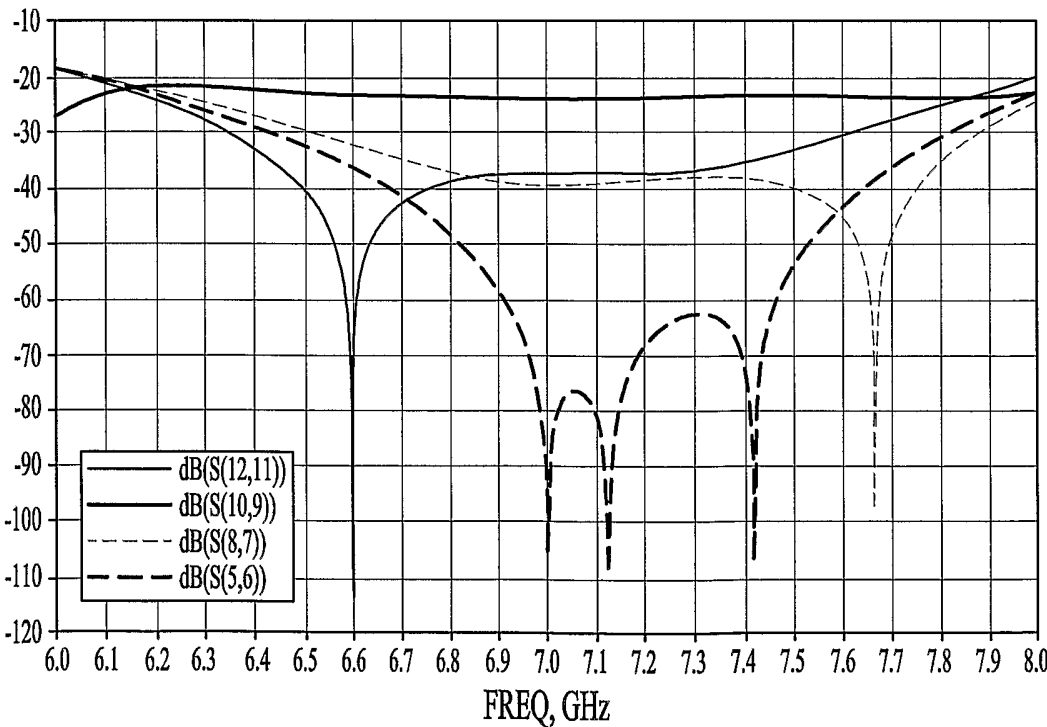


FIG. 8

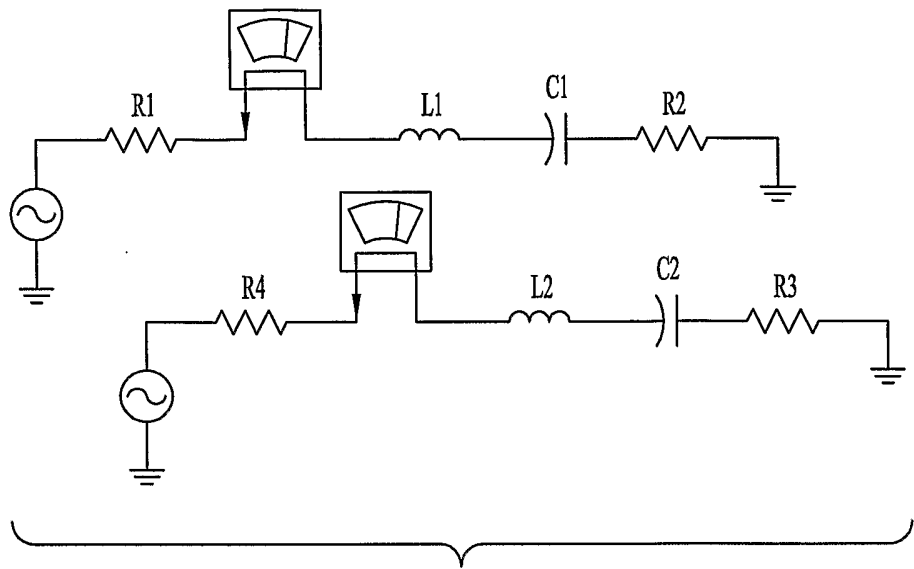


FIG. 9A

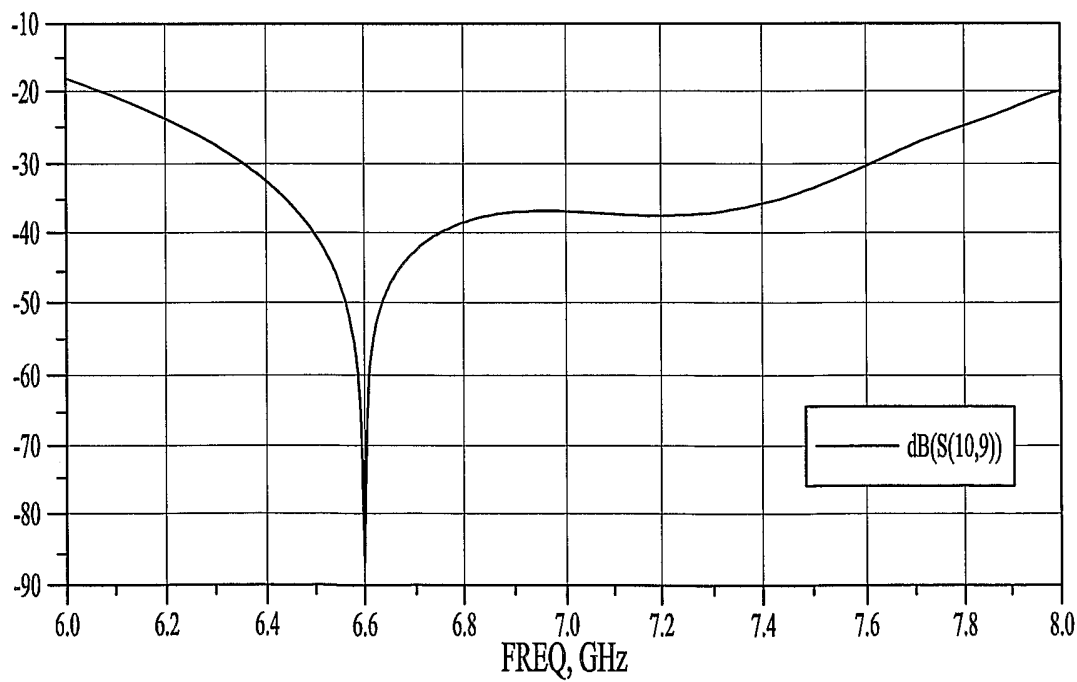


FIG. 9B

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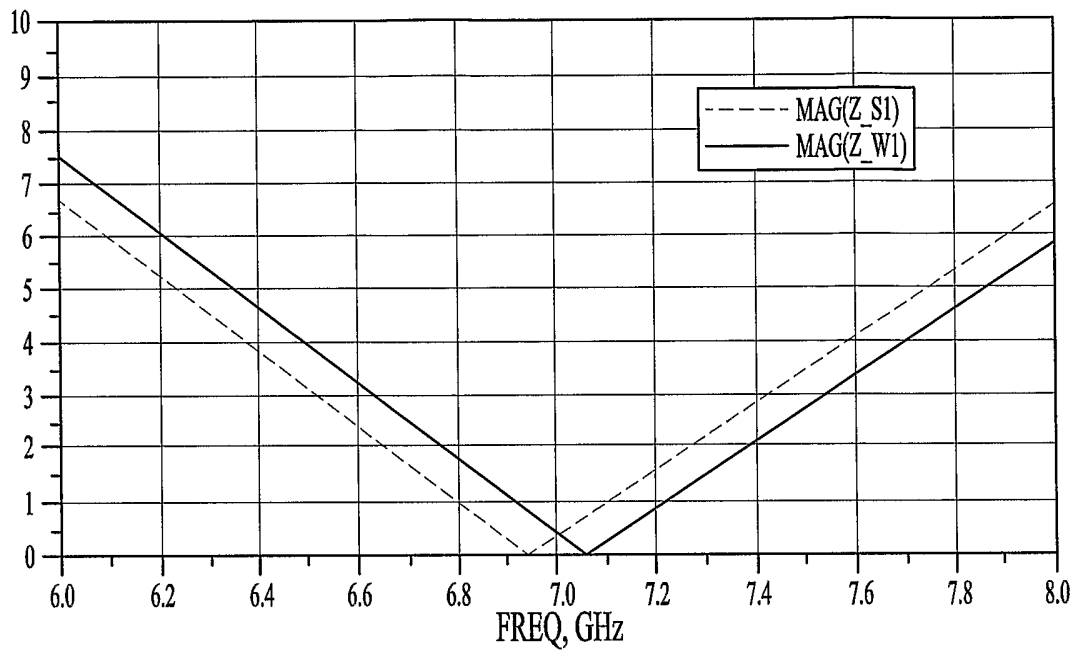


FIG. 9C

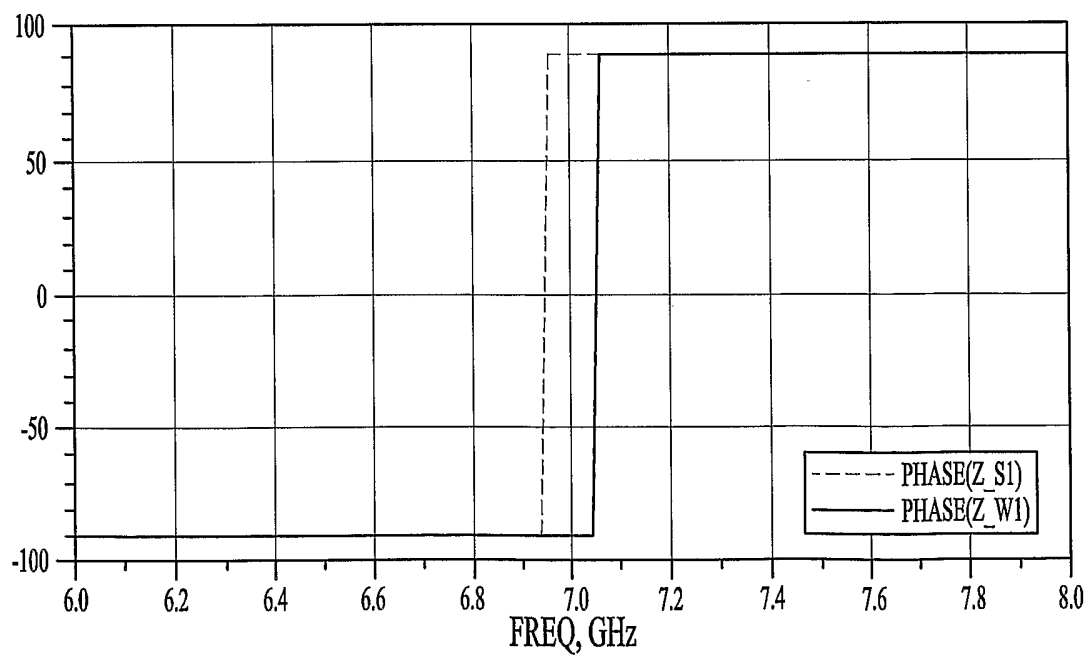


FIG. 9D

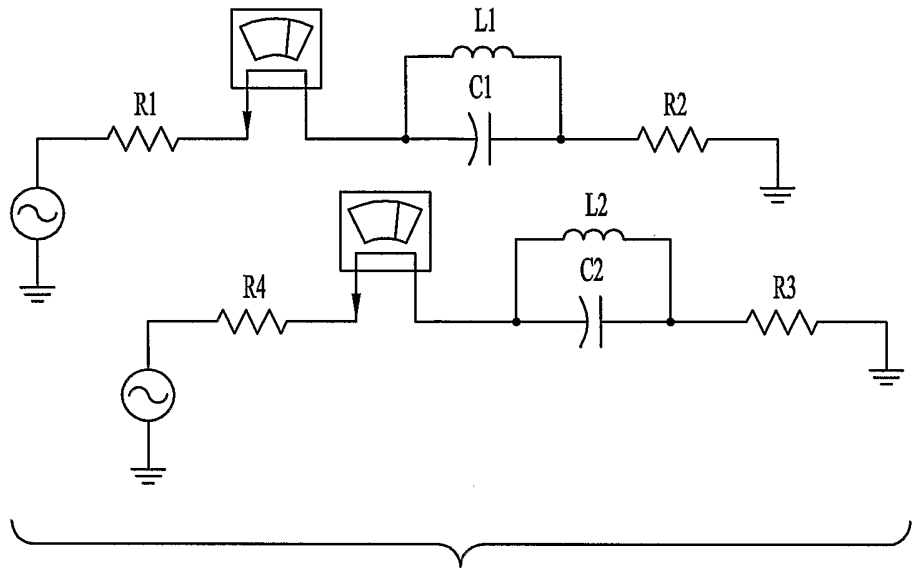


FIG. 10A

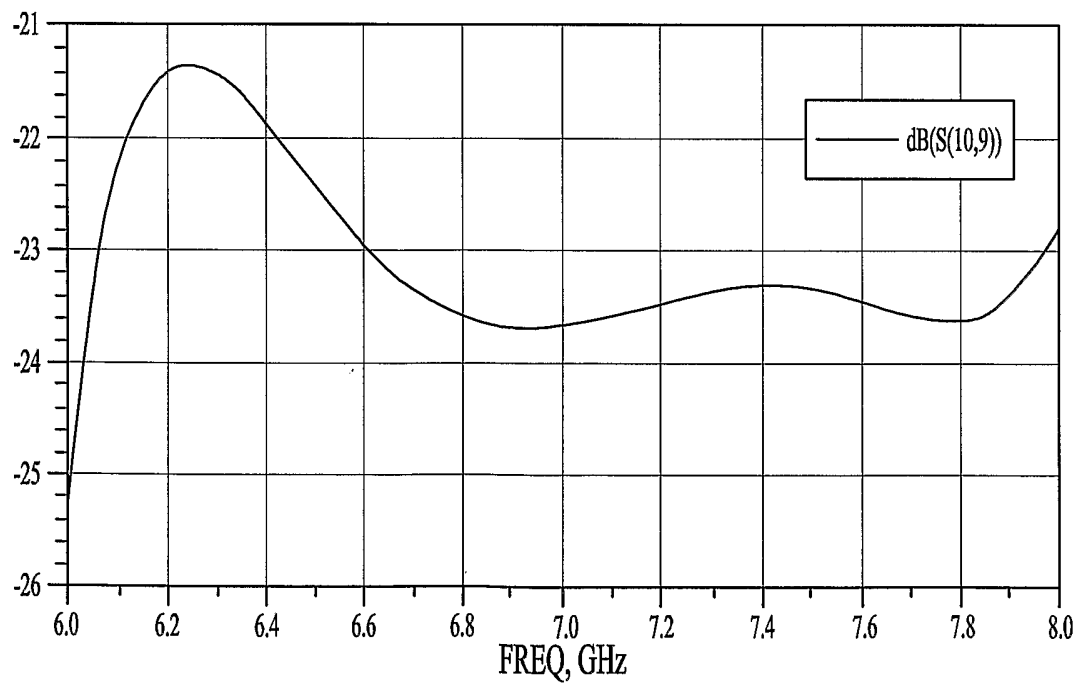
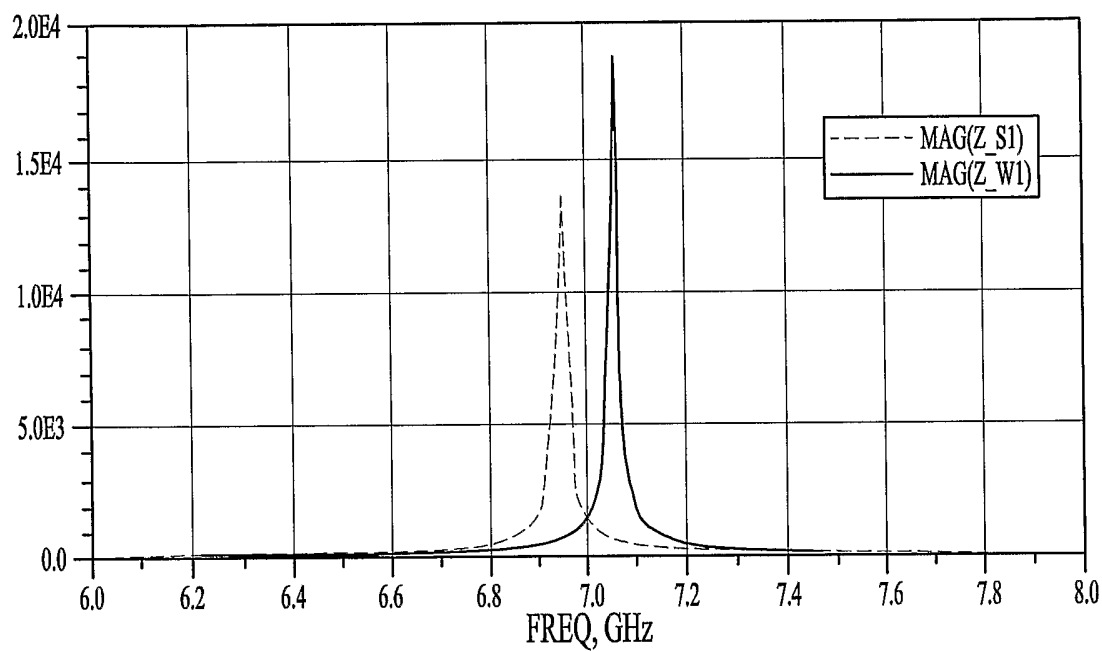
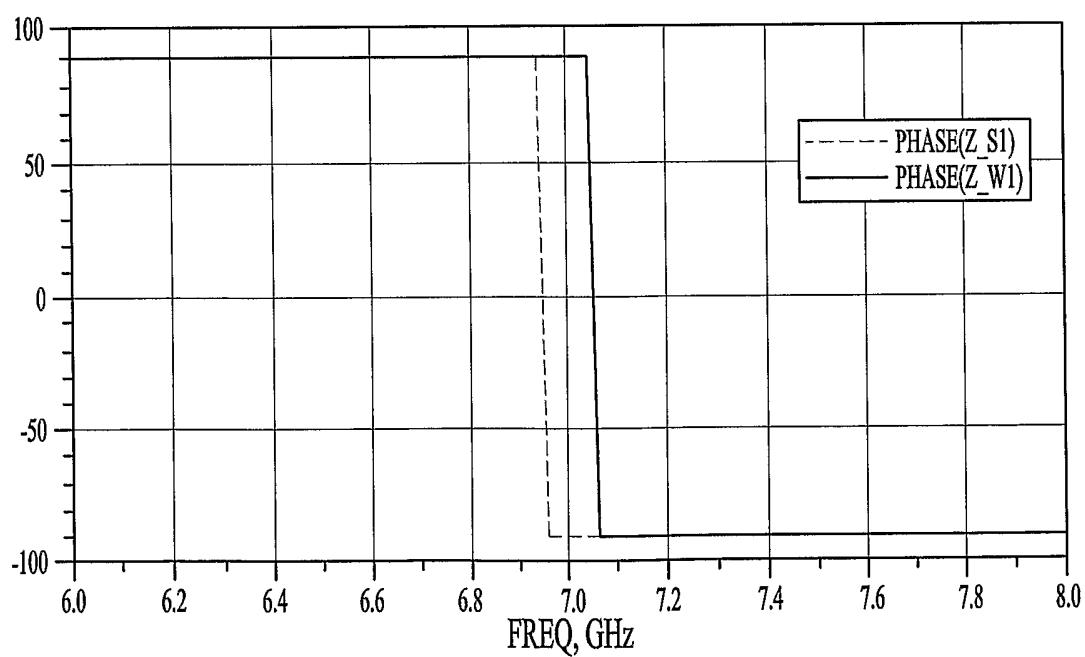


FIG. 10B

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*FIG. 10C**FIG. 10D*

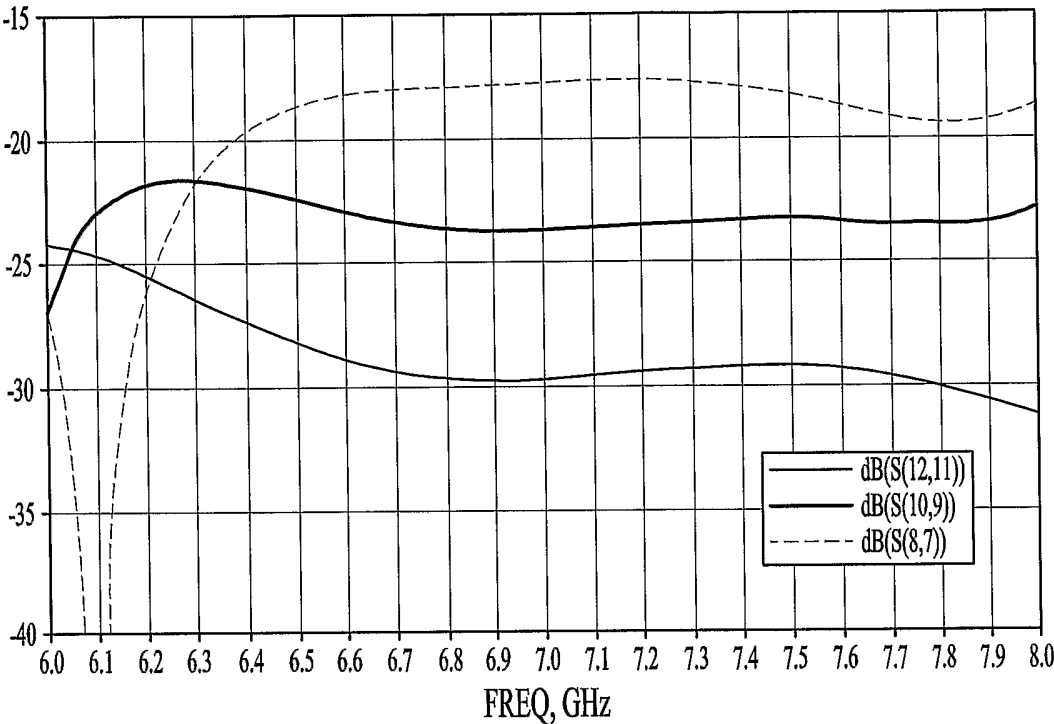


FIG. 11

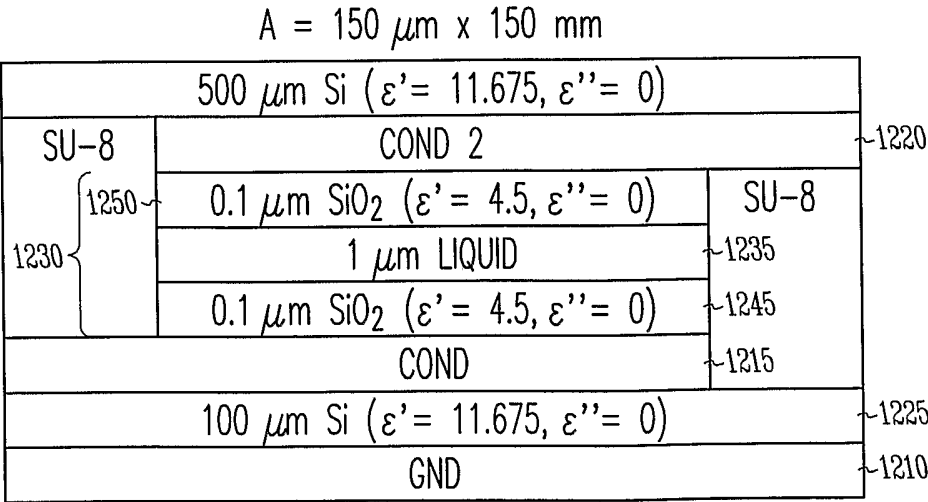
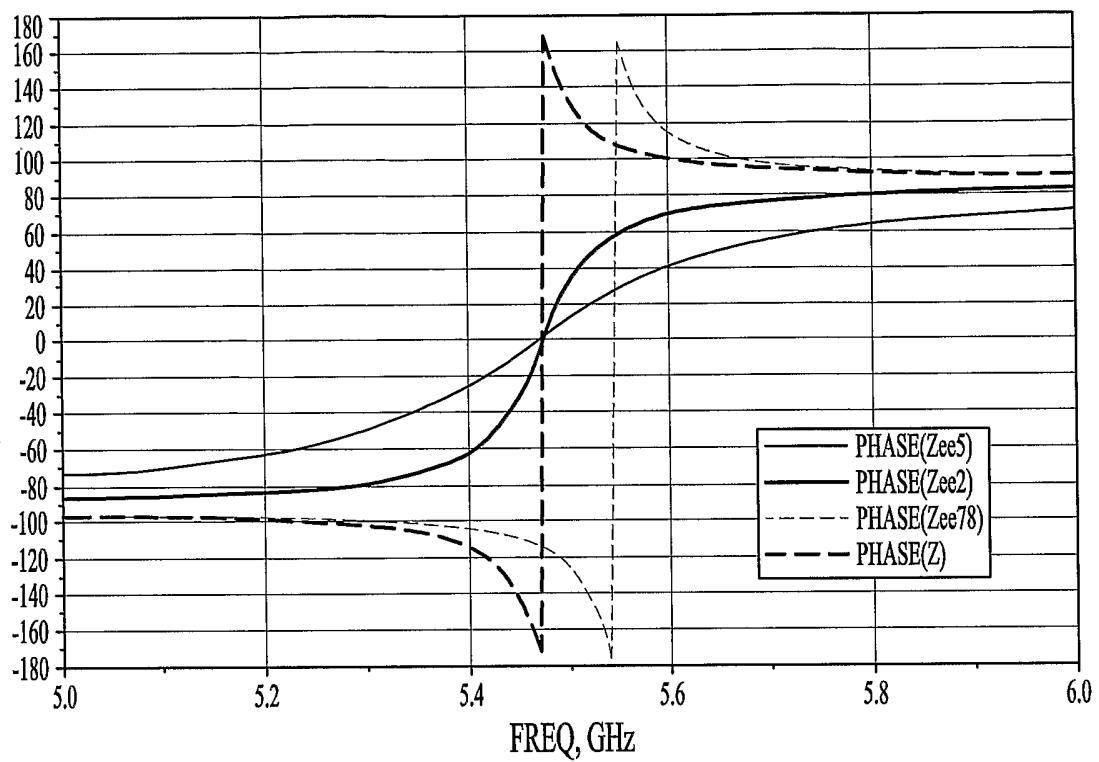
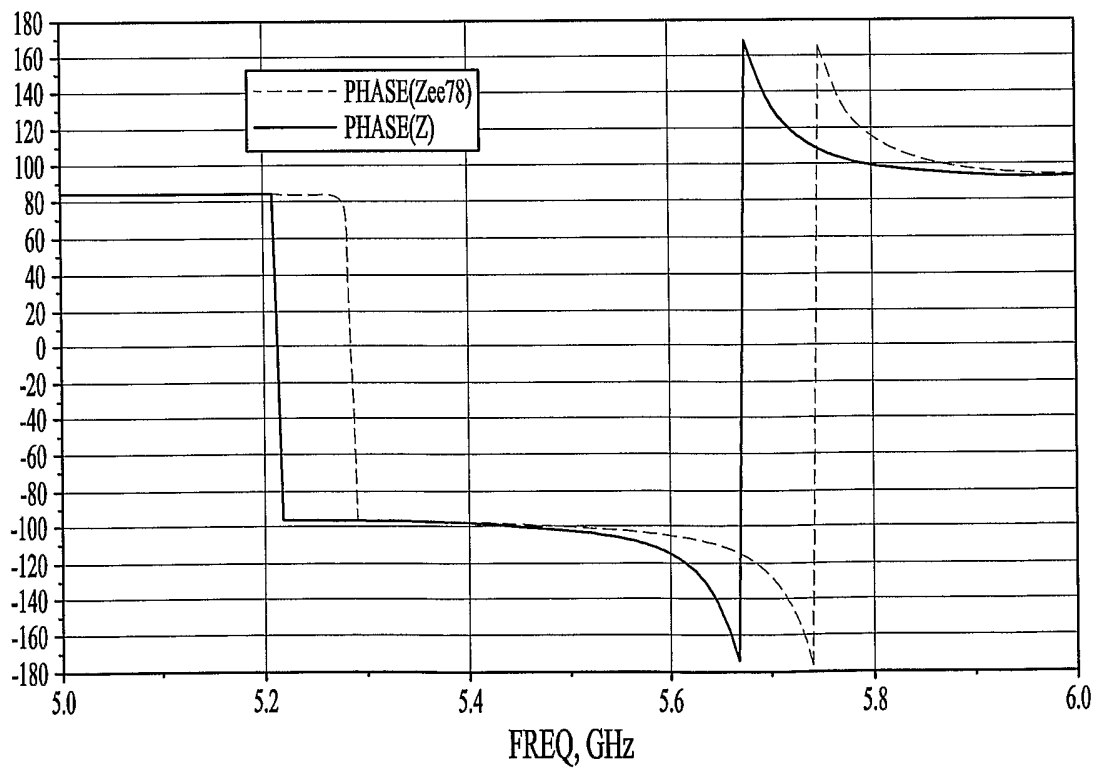
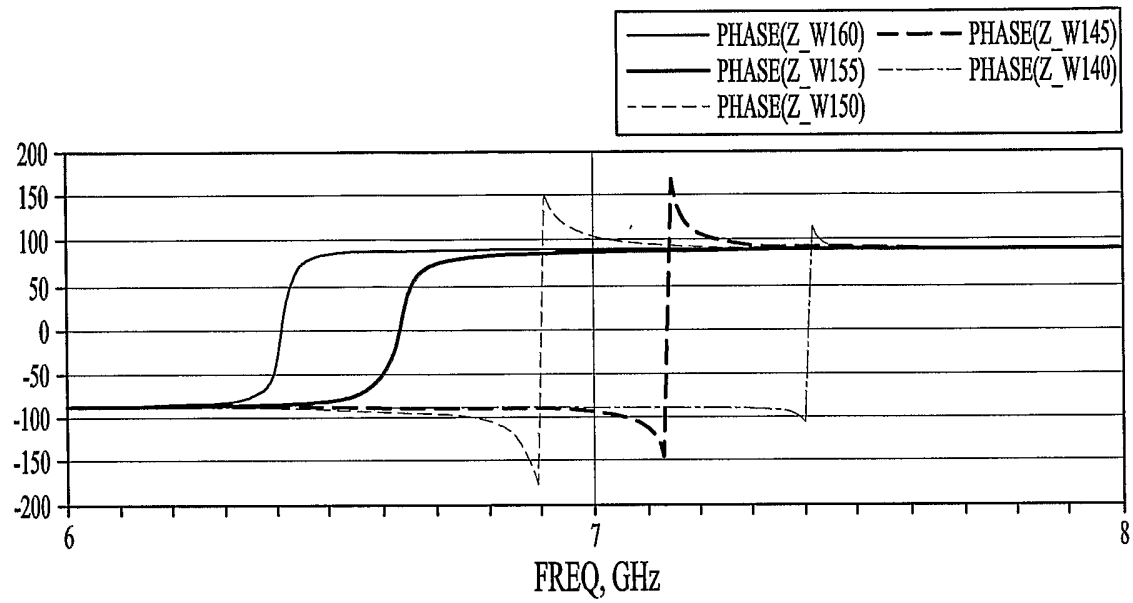
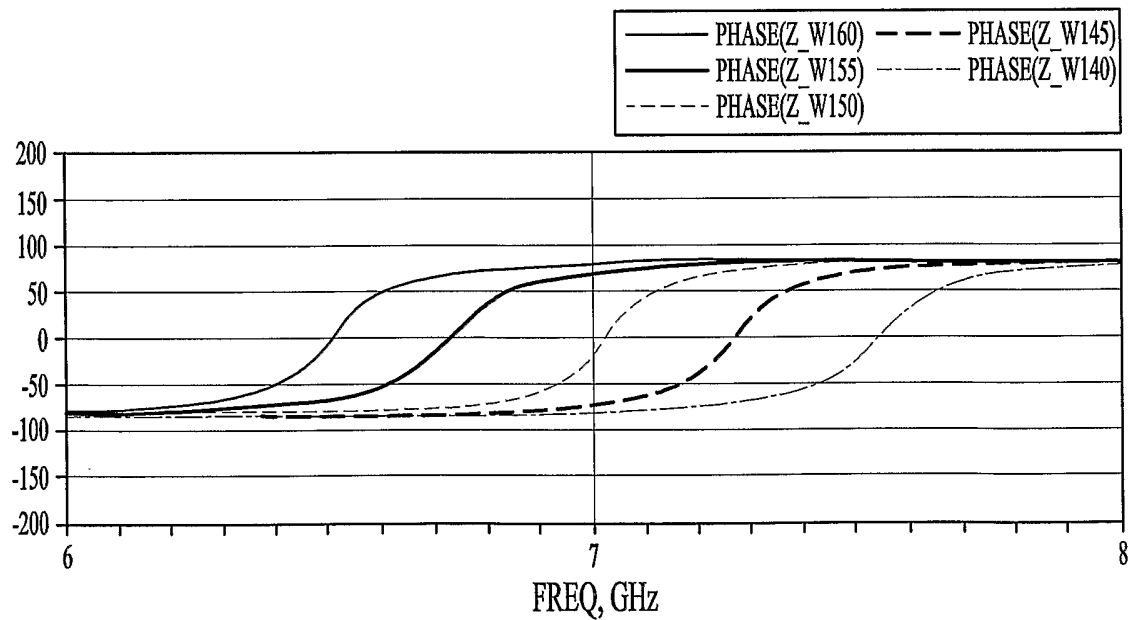


FIG. 12

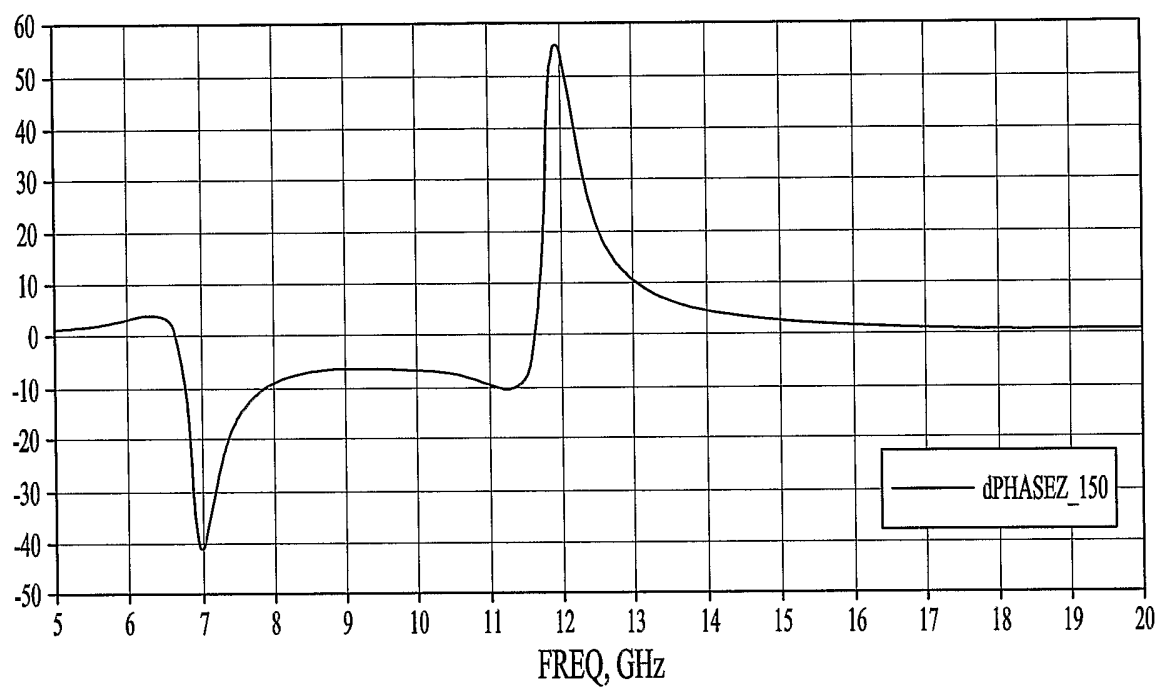
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*FIG. 13**FIG. 14*

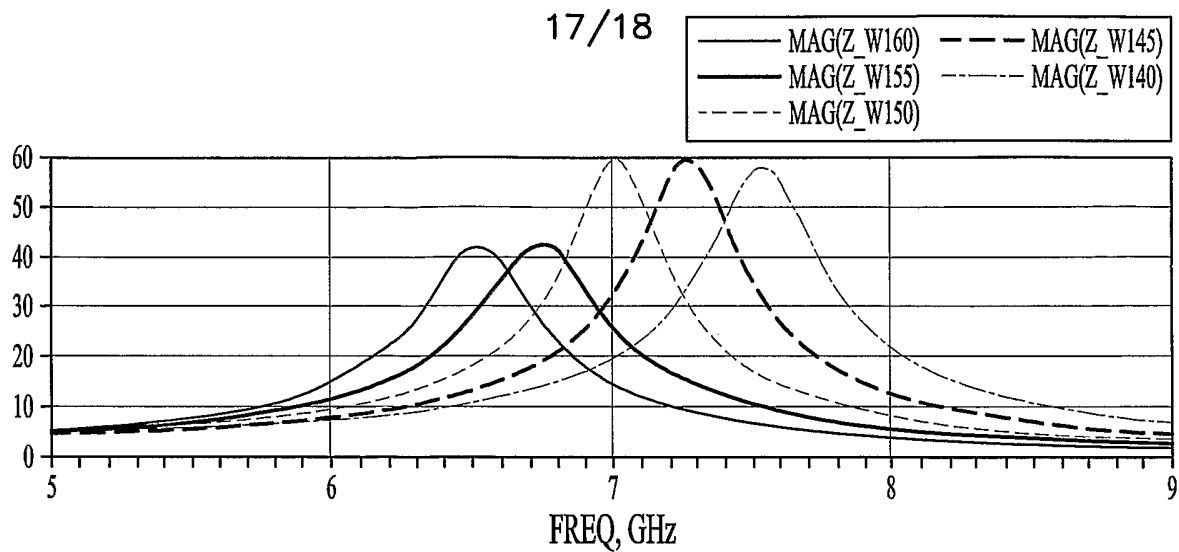
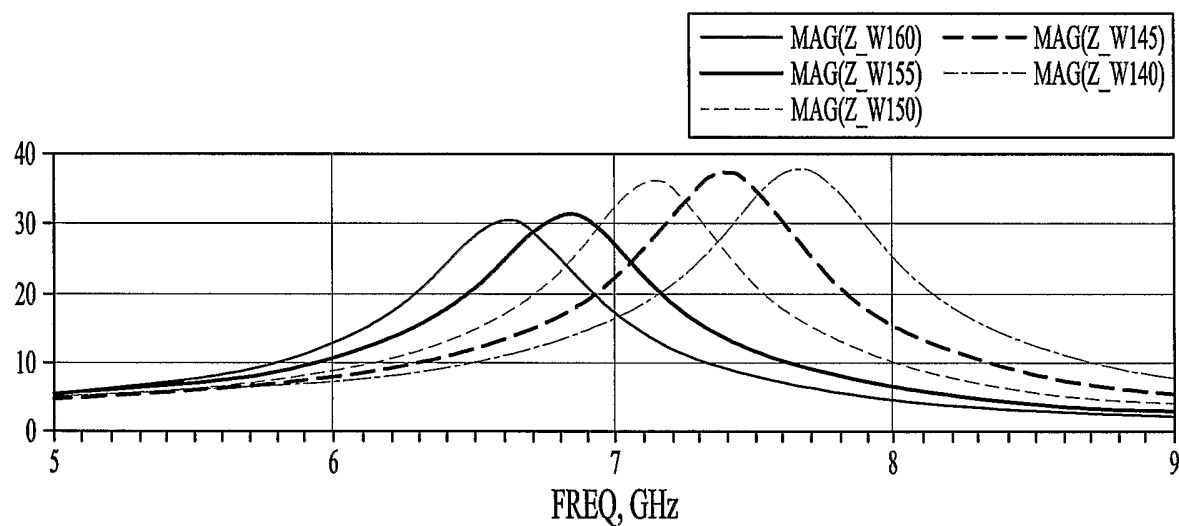
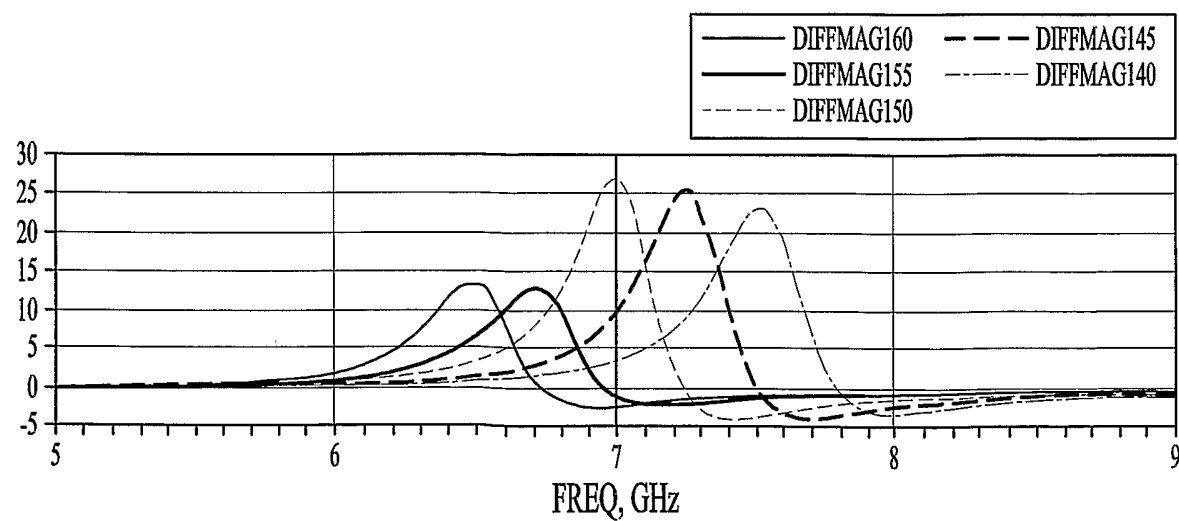
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*FIG. 15A**FIG. 15B*

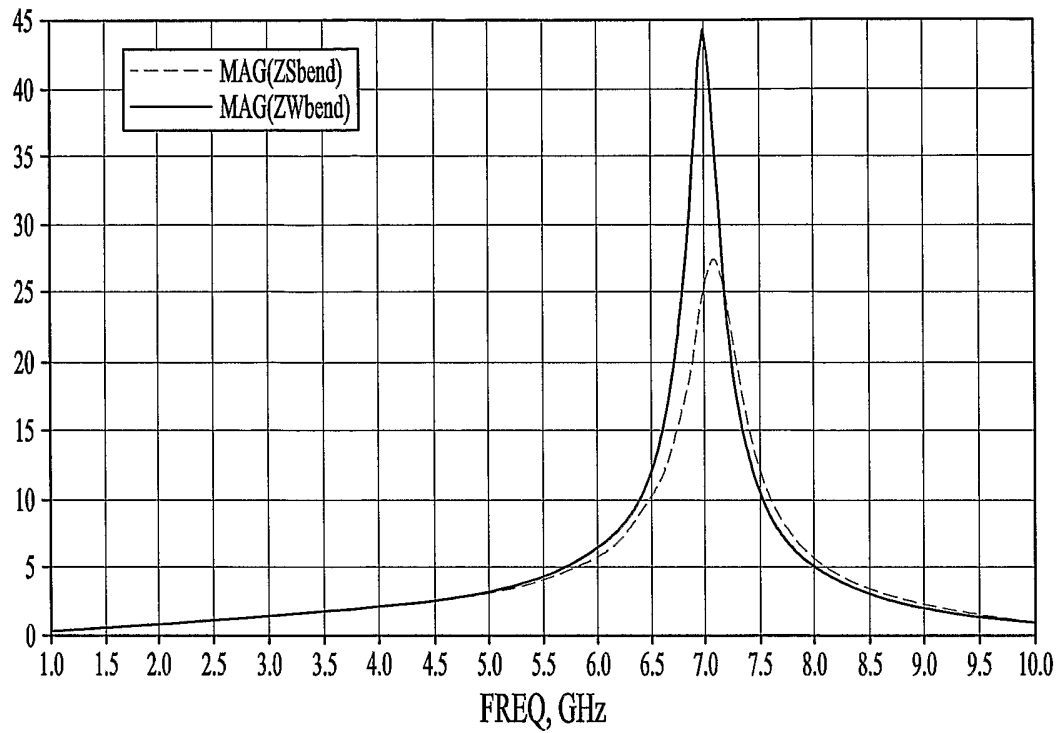
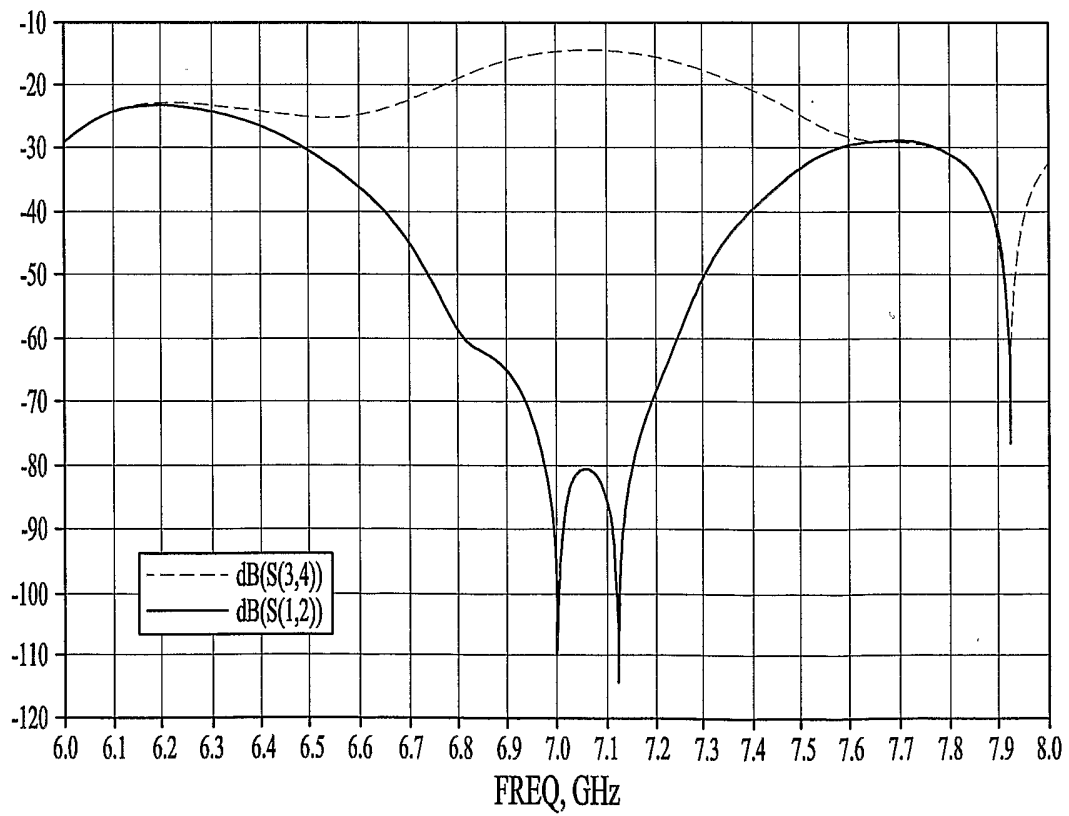
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**FIG. 16**

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*FIG. 17A**FIG. 17B**FIG. 17C*

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*FIG. 18**FIG. 19*