SURFACE PLASMON RESONANCE AND QUARTZ CRYSTAL MICROBALANCE SENSORS ON MICROCHIP

Technical Field

This invention relates to a microchip which is adapted simultaneously to obtain complimentary signals from different techniques of detecting or monitoring molecular (especially biomolecular) adsorption. In particular, this invention relates to a microchip adapted to perform micro-gravimetric sensing (such as with a Quartz Crystal Microbalance (*QCM*)), together with another sensing technique using incident light.

Background of the Invention

There is a great need for reliable chemical sensors and biosensors in many areas. For instance, in environmental applications, there is a need for sensors which are able to detect trace quantities of toxic chemicals, organic molecules or biomolecules. Similar comments apply in relation to the need to accurately analyse biological samples. Sensors often need to be able to identify specific substituents and/or derivative chemical groups attached to the basic structure.

Numerous techniques have been developed to detect and/or monitor organic molecules or biomolecules in aqueous solutions. These techniques typically involve measuring the extent to which the relevant organic molecules or biomolecules are adsorbed onto a particular surface. In many situations, several of these techniques need to be performed separately in order to provide a greater degree of certainty in relation to the analytical results.

Some of the techniques which have been developed are mentioned below.

(a) Micro-gravimetric sensing

Micro-gravimetric sensing is mainly based on the use of Bulk Acoustic Wave (BAW)/Quartz Crystal Microbalance (QCM) devices and Surface

Acoustic Waves (SAW) devices. The molecules being analysed or monitored are adsorbed onto a surface electrode. The shift in the

resonance frequency on adsorption is usually measured and the mass loading before and after any processing is then determined.

A QCM is a quartz crystal wafer with two planar metal electrodes on the two surfaces of the wafer. The quartz crystal can be excited to mechanical resonance by an alternating electric field due to the inverse piezoelectric effect. The resonance frequency is dependent upon the mass of material adsorbed onto the surface electrode. For instance, resonance frequency decreases for mass accumulation and increases for mass reduction. The shift in frequency can be related to the adsorbed mass using analytical equations.

With an appropriate oscillation circuit, QCM devices are suitable for the detection of substances in either a gas, liquid or vacuum environment. A mass-loading in the order of about 1ng/cm^2 can be detected.

A QCM can be employed as a chemical sensor or biosensor by incorporating a suitable coating to the surface which can be selective for a particular substance.

Benefits of QCM devices include their low cost and the fact that they can be used as biosensors to detect interactions of biomolecules directly (ie. with no labelling of the analyte molecules).

(b) Surface Plasmon Resonance (SPR)

Surface Plasmon Resonance (SPR) is a general method for the detection of chemical changes occurring at the surface of a thin metal film. It measures changes in the optical thickness (i.e. refractive index) arising from molecular adsorption on the metal surface. The generality of SPR techniques has proven to be useful for the characterisation of a wide variety of chemistries. SPR techniques are used extensively in the biomedical area. For example one of the widely studied chemistries using SPR techniques is protein-protein interactions.

In SPR, an evanescent wave (which is an exponential-decaying wave) exists at the sensor surface. In what is known as the Kretchmann geometry, an evanescent wave is generated when total internal reflection of incident light occurs at the interface of a substance with a high

refractive index and a substance of low refractive index (i.e. a glass-air interface of a prism). SPR occurs under certain conditions when a thin film of metal (eg. gold or silver) is placed on the surface. When the incident light is monochromatic, the free electrons of the metal will oscillate (ie. a surface plasmon is excited) and absorb energy corresponding to a certain angle of incident light. The angle is called the SPR angle. The SPR signal is detected by measuring the intensity of the reflected light. At the SPR angle, a sharp decrease or "dip" in intensity is measured as the surface plasmon absorbs energy.

The position of the SPR angle depends on the refractive index of the sensing surface which changes upon the binding of molecules to the surface. Consequently, the SPR angle changes according to the amount of molecules bound to the surface.

The detection limitation of SPR is approximately 1ng/cm^2.

Advantages of SPR techniques in analysing/monitoring biomolecular interactions include real-time monitoring of binding events and label-free detection of macromolecular interactions. SPR techniques are also highly sensitive, easy to use and enable the study of adsorption kinetics in-situ.

(c) Optical detection

Optical detection techniques are also widely used in biomedical applications. The term "optical detection" is used in this specification to mean any generic method (excluding SPR technique) which uses light to measure the adsorption of molecules (especially biomolecules) on a surface.

In optical detection techniques, the optical signal (fluorescence, photoluminescence, Raman scattering, reflection absorption or transmission) of samples under different conditions are measured. Typically, within a given spectral band, each substance or molecule has a unique optical response or spectrum. The characteristic response or spectrum can be used to distinguish molecules adsorbed onto the substrate surface. Obviously, for absorption and transmission based

measurements (as in a spectrophotometer), the substrate used must be transparent.

One particular optical detection technique is Surface Enhanced Raman Scattering (SERS). This is quite a recent spectroscopy technique that has been developed for obtaining molecule-specific measurements on surfaces. In the SERS technique, a laser beam impinges onto a surface and either the back-scattered or the transmitted light is recorded on a Raman spectrometer. The key to obtaining a significant Raman signal from a surface is to enhance the electromagnetic interactions between the substrate and the adsorbed molecules. A major contribution to such enhancement of the local electric fields comes from the generation of surface plasmons, which can significantly increase the Raman emission. Coating the surface with metal, in the form of metal particles, colloids or microparticles coated in metal, roughened or porous metal surfaces etc also assists in the generation of strong surface plasmons.

The above optical detection methods tend to be robust and reliable.

Although the above analytical techniques are useful, they all have their limitations. There is a need for a device which enables more than one of these techniques to be conducted simultaneously so as to provide complementary results. These complementary results would improve the accuracy of the analytical data thereby obtained.

This invention is directed towards an improved device for micro-gravimetric sensing, together with at least one of the other types of sensing referred to above.

Summary of the Invention

According to a first aspect of this invention, there is provided a microchip, for simultaneous micro-gravimetric sensing and surface plasmon resonance (SPR) sensing, comprising:

- a transparent piezoelectric substrate having a first surface and a second surface;

- a thin film metal electrode formed on the first surface of the substrate, said metal being adapted to generate surface plasmon resonance upon excitation; and
- a second electrode, formed of a film of a conductive, substantially transparent material, formed on the second surface of the substrate, wherein the second electrode is formed of a transparent semiconductor metal oxide or of a transparent polymer.

The substrate can be any transparent piezoelectric crystal which is suitable for fabricating resonators. Typically, the substrate is formed of quartz or lithium niobate.

The metal electrode may be formed of any metal which is able to generate surface plasmon resonance upon excitation. Preferably, the electrode is formed of gold or silver. Alternatively, the metal electrode may be formed of platinum or copper.

The second electrode is formed of a transparent semi-conductor metal oxide or of a transparent polymer. The metal oxide is generally selected from indium tin oxide, indium zinc oxide and zinc oxide. Indium tin oxide is, however, generally preferred. Alternatively, the second electrode may be formed of a suitable transparent conductive polymer. Electrodes formed of these materials will have sufficiently high conductivity to function as electrodes for the above mentioned sensing devices. For example, when the electrode is formed of ITO, the conductivity will typically be between 10^{-3} to 10^{-5} ohm/cm^{-1}.

The optical transmission of the second electrode should be sufficiently high so as to enable a light beam to pass therethrough for optical detection and SPR applications. Generally, the optical transmission of the second electrode should be greater than about 70%.

The thickness and morphology of the electrodes are adapted so as to enable the excitation of surface plasmons. It is known that extremely thin metal electrodes are unsuitable for QCM operation. Therefore, in this aspect of the invention, the thickness of the metal electrode film is typically between about 60 nm and 100 nm.

It is preferred that the thickness of the film of the second electrode is between about 100 nm and about 1000 nm. The film of the second electrode should also exhibit high thermal stability and minimal drift at room temperature.

According to a second aspect of this invention, there is provided a microchip, for simultaneous micro-gravimetric sensing and optical sensing, comprising:

- a transparent piezoelectric substrate having a first surface and a second surface; and
- first and second thin film electrodes formed of a conductive, substantially transparent material, said electrodes being formed on the first and second surfaces of the substrate,

wherein said electrodes have a thickness of less than about 1500 nm, and wherein the conductive, substantially transparent material is formed of a semiconductor metal oxide.

The thickness of the electrodes will largely be determined by their intended uses. If the electrodes are too thin, they may not adequately conduct electricity. On the other hand, if they are too thick, their substantial transparency will be compromised. It is generally preferred that the thickness of the electrodes is between about 100 nm and 1000 nm.

Again, the substrate may be formed of any transparent piezoelectric crystal which is suitable for fabricating resonators. Typically, the substrate is formed of quartz or lithium niobate.

The conductive, substantially transparent material is formed of a semi-conductor metal oxide. This metal oxide is preferably selected from indium tin oxide, indium zinc oxide and zinc oxide. Indium tin oxide is, however, generally preferred. As mentioned above, electrodes formed of these materials will have sufficiently high conductivity (eg. 10^{-3} to 10^{-5} ohm/cm^{-1} for ITO) to function as electrodes in the above sensing devices.

In a preferred embodiment, the microchip further includes a SERS active surface deposited on the first thin film electrode. This is so as to enable SERS sensing to be conducted. Preferably the SERS active surface is formed of a material which can generate surface plasmons under excitation. Preferably, the

SERS active surface is a thin film formed of gold or silver. Alternatively, it may be a thin film formed of platinum or copper.

In a preferred embodiment, the microchip may be formed with an array of electrodes for performing multiple analysis. That is, the electrode formed on the first surface may, in fact, comprise a plurality of separate sub-electrodes. These sub-electrodes are typically formed as a grid of sub-electrodes, separated by gaps, on the substrate. These sub-electrodes may be in series or parallel modes. The arrangement/configuration of an array of such sub-electrodes may, however, vary widely.

According to a third aspect of this invention, there is provided bio-analysis sensing apparatus comprising at least one microchip as described above. The bio-analysis sensing apparatus may comprise an array or plurality of such microchips.

In general, each sub-electrode (sensor) in the array is able to perform the same functions as an individual sensor. So a single sub-electrode (sensor) of the array may be used. Alternatively, any number of such sub-electrodes (or sensors) of the array may be used to provide multiple information.

As a first (non-limiting) example, for the array to perform both QCM and SPR analysis, the elements in the same chip can be coated with different coatings as the sensing materials. These coatings may be chosen for their specificity in being able to sense or detect different kinds of target materials or molecules. This coated chip then can possibly be used as an “electronic nose” for detecting different gases in the atmosphere or it can be used as a biosensor which can discriminate different target molecules in a liquid. The mass loading information of each element can thereby be obtained together with the variation of SPR angles.

As a second (non-limiting) example, for the array to perform both QCM and optical / fluorescence detection, the same coating process as mentioned above can be adopted. The chip can then be used to qualitatively detect, by optical means, various target molecules or materials, or it can be used quantitatively as a mass loading sensor (QCM). The optical detection function can be used to judge the kind or species of the materials since each species

may have different characteristic spectrum. Then, the corresponding QCM signal can be used to measure the mass adsorbed. This can be very useful in a number of fields of genetics engineering, pharmacology, food industry, etc.

A method of fabricating a microchip, according to the first aspect of this invention, comprises the following steps:

- depositing on a first surface of a transparent piezoelectric crystal substrate a thin metal film formed of a material capable of generating surface plasmon resonance under excitation, said material being selected from gold, silver, platinum and copper;
- patterning the metal film into an electrode shape;
- depositing on a second surface of the crystal substrate a thin film of a transparent conductive semiconductor metal oxide; and
- patterning the metal oxide film into an electrode shape.

In one embodiment of this method, the metal film and the metal oxide film are deposited and coated with photoresists and the electrode patterning is effected by photolithographic techniques. After the patterning has been done, the photoresists are removed.

A method of fabricating a microchip, according to the second aspect of this invention, comprises the following steps:

- depositing on a first surface of a transparent piezoelectric crystal substrate a first thin film of a transparent conductive semiconductor metal oxide;
- patterning the first metal oxide film into an electrode shape;
- depositing on a second surface of the crystal substrate a second thin film of a transparent conductive semiconductor metal oxide; and
- patterning the second metal oxide film into an electrode shape.

In the above methods, the substrate is preferably formed of quartz or lithium niobate. In one embodiment in which the substrate is a quartz crystal wafer, the wafer typically has a substantially rectangular shape having dimensions (for example) of about 10mm x 10mm x 0.1mm. Alternatively the quartz crystal wafer may have a substantially circular shape having, for example, a diameter of about 14mm and a thickness of about 0.167mm. It will, however, be appreciated that the size and/or dimensions of the quartz crystal wafer is not critical and any shapes or dimensions, which are suitable for the intended application, may be adopted.

The method of depositing the first thin film of electrode material or the second thin film of electrode material (or both) is preferably selected from thermal evaporation, electron-beam evaporation and RF sputtering at low temperature.

The deposition temperature of the electrodes should be below any piezoelectric phase transition or de-poling temperature of the substrate. For example, ITO can be deposited on quartz at about 200ºC, the α-β phase transition for quartz being about 573ºC.

In one embodiment of this method, the metal film and the metal oxide film are coated with photoresist and the electrode patterning is effected by photolithographic techniques. After the patterning has been done, the photoresist is removed.

Preferably the patterned electrodes are aligned using double-sided alignment on the surfaces of the substrate.

Films formed according to the above methods exhibit high thermal stability and minimal thermal drift at room temperature. The films can be fabricated by microfabrication processes to ensure good uniformity and repeatability. It follows that such electrode configurations and manufacturing processes are suitable for creating array structures.

Throughout this specification, unless the context requires otherwise, the word “comprise” or variations such as “comprises” or “comprising”, will be understood to imply the inclusion of a stated element, integer or step, or group of

elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Any discussion of documents, acts, materials, devices, articles or the like, which has been included in the present specification, is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

In order that the present invention may be more clearly understood, the prior art and preferred forms of the invention will be described with reference to the following drawings and examples.

Brief Description of the Drawings

Figure 1 is a simplified diagram of a simple QCM device using a conventional tuned oscillation circuit.

Figure 2A is a simplified schematic view of a microchip according to the first aspect of this invention.

Figure 2B is a simplified schematic view of a microchip according to the second aspect of this invention.

Figure 3 is a simplified schematic view of a microchip according to the first aspect of this invention located beneath an analyte solution and showing a beam of incident light passing there through.

Figure 4 is a simplified schematic view of a microchip according to the second aspect of this invention showing an optical light source and optical detectors.

Figure 5 is a simplified schematic view of a microchip according to the second aspect of this invention adapted for SERS sensing.

Figure 6A is a simplified schematic view of an array of microchip on a single substrate.

Figure 6B is a simplified plan view of the array of Figure 6A.

Figure 7 is a graph showing resonance frequency against measurement time of a microchip according to a preferred embodiment of this invention.

Figures 8A and 8B are graphs showing the frequency response, before (A) and after (B) mass loading with an antibody, of a microchip according to a preferred embodiment of invention.

Figure 9 is a photograph of a microchip, according to a preferred embodiment of this invention, under a fluorescent microscope.

Figure 10A is a graph showing transmittance spectra of a microchip, according to a preferred embodiment of this invention, under different conditions.

Figure 10B is a graph showing differentials of the data curves of Figure 10A, for the different conditions shown in Figure 10A.

Detailed Description of the Invention

A more detailed description of preferred embodiments of the invention is now provided with reference to the above drawings.

Figure 1 is a schematic diagram of a simple quartz crystal microbalance (*QCM*) using a conventional tuned oscillation circuit, which is commonly used for micro-gravimetric analysis. The QCM device consists of a quartz substrate 1 and two thin film metal electrodes 2 which are in double-sided alignment on the surfaces of the substrate. The electrodes 2 of the QCM device are in electrical communication. Within the circuitry there is an oscillation circuit 30 and a frequency counter 40.

Figure 2A is a simplified schematic view of a microchip according to the first aspect of this invention. The sensor consists of a quartz crystal substrate 1. A first electrode 2 is formed of metal and the other electrode 3 is formed of a suitable transparent material. The electrodes 2, 3 and the crystal substrate 1 enable micro-gravimetric analysis to be performed. Having the second electrode 3 being transparent, this also enables SPR analysis to be conducted by enabling a beam of incident light to pass through the second electrode 3 and through the substrate 1 so that, at the relevant angle, the light beam is reflected causing surface plasmon resonance to occur in the metal electrode 2.

This is more clearly exemplified in Figure 3, where the microchip is located under an analyte solution 10. Figure 3 shows a beam of incident light 5 passing through the second electrode 3 and through the substrate 1. A reflected beam of light 6 passes back out through the substrate 1 and the second electrode 3. The evanescent wave is represented by the curved line 25. At appropriate incident light angle surface plasmon resonance (*SPR*) occurs in the second electrode 2. The SPR signal is affected by any compounds deposited on the second electrode 2. Therefore, by comparing the SPR when no compounds are deposited on the electrode 2 against the SPR when compounds are deposited on the electrode, appropriate determinations in relation to the compounds can thereby be made.

Figure 2B is a simplified schematic drawing of a microchip according to a second aspect of the present invention. According to this aspect, the microchip incorporates a quartz crystal substrate 1, a first transparent electrode 3 and a second transparent electrode 12. This microchip is adapted for use in gravimetric sensing and optical sensing.

Figure 4 is a schematic diagram showing the microchip of this invention, set up for use for gravimetric sensing and optical sensing. As can be seen the microchip is connected to an electrical circuit 40. A light source 14 directs a beam of light 5 onto the microchip. The beam of light may be reflected and this beam of reflected light may be analysed by a first optical detector 15. Alternatively, the beam of light may be refracted and transmitted through the microchip so that the refracted/transmitted beam of light may be detected by a second optical detector 16.

Figure 5 is a simplified schematic diagram of the microchip of the second aspect of this invention having an SERS active layer positioned on the sensor. As can be seen, in this embodiment, the microchip consists of a quartz crystal substrate 1, transparent electrodes 3, 12 and an SERS active layer 20. This SERS layer is typically a metal such as gold or silver. Alternatively, the metal may be platinum or copper. A beam of incident light 5 passes through the transparent electrodes 3, 12 and the substrate 1 and is reflected at or near the surface of the SERS active layer 20. The reflected beam of light 6 exhibits Raman scattering and this can be detected by an appropriate optical detector.

Figure 6 shows a possible configuration for an array of combined QCM and SPR sensors for QCM and SPR sensing. The portion of the array shown in Figure 6 incorporates a quartz crystal substrate 1 on which is located a number of metal electrodes 2 (or sub-electrodes). In the array of Figure 6, there are a number of sensors 38, which incorporate the electrodes 2, located on the upper surface of the substrate. The sensors 38 are associated with a common transparent electrode 3. The region 37 surrounding each sensor has no electrode. The size of each QCM metal electrode 2 defines one pixel element. The metal electrodes 2 are preferably shaped as a grid. Beams of incident light 30, 31 travel through the transparent electrode 3. These beams 30, 31 undergo reflection at the upper surface of the substrate 1. SPR or SERS signals will only be excited on the metal coated regions thus producing a spatially dependent (i.e. array) measurement.

The graph of Figure 7 shows the frequency stability of a microchip of the present invention driven at resonance. The measurement was carried out at room temperature using a conventional oscillation circuit. The frequency drift was about 150 Hz over a period of about 10 hours. The graph of Figure 7 demonstrates the stability of electrodes formed of the transparent conductive semiconductive oxide–ITO. The relevant microchip was made of AT-cut quartz crystal with one side being coated with a gold electrode and the other side coated with ITO as the counter electrode. The microchip was then packaged in a tray for testing. The microchip was measured with a conventional oscillation circuit with an excitation voltage of about 5V. The circuit oscillates the quartz at its fundamental resonant frequency. In the testing process, the temperature was kept constant at about 25ºC. The graph of Figure 7 shows that the stability for the ITO coated quartz is good and acceptable for practical applications.

Figures 8A and 8B show graphs of frequency response of another microchip before and after mass loading with an antibody. Figure 8A shows the frequency response before the mass loading. As can be seen, the resonant frequency is about 17,447,000 Hz. As shown in Figure 8B, after the mass loading, the resonant frequency is about 17,446,200 Hz. This shows that, upon adsorption of the antibody, a negative frequency shift of about 800 Hz occurs. In this example, the resonant frequency is determined from the impedance

minimum in the curve. The mass loading (mentioned above in relation to Figure 8B) was effected by dipping the chip in a fluorescent-tagged IgG protein solution and drying with nitrogen. As can be seen, the chip had a large negative frequency shift of about 800Hz, indicating that mass (namely lgG proteins) had attached onto the chip surface. The microchip used in determining the frequency response was made from AT-cut quartz crystal, on both sides of which semiconductor oxide-ITO was coated and patterned forming the electrodes. In this case, although the ITO was of high conductivity, it is difficult to excite the sensor with a conventional oscillation circuit. Accordingly, the sensor response was analysed with a network analyser. The figure shows that the device can be used as a mass sensitive sensor.

In Figure 9 (being the photograph of a microchip of this invention under the fluorescent microscope), the excitation UV light is projected from the backside of the microchip and the observation is undertaken from the front side. The fluorescently-tagged antibody can then be observed adsorbed onto the ITO surface. Figure 9 shows that the use of transmission optical arrangements is viable for the device.

Figures 10A and 10B are graphs of transmittance spectra for a microchip according to this invention with 2 ITO surface electrodes under different conditions. The graphs of Figures 10A and 10B demonstrate the optical application for a chip with two-sided ITO as the electrodes. The chip fabrication process is the same as mentioned in relation to Figure 8. Then, the chip, treated with different protein-contained solutions was tested with an UV-vis photospectrometer which can be used to record the transmittance or absorbance properties.

Curve 1 in Figure 10A shows the transmittance spectra of a bulk solution of FITC-Ab. This data is included for reference. Curve 2 of Figure 10A shows the transmittance spectra following FITC-Ab adsorption onto an ITO surface (modified with aminosilane APTES). Curve 3 of Figure 10A shows the transmittance spectra following FITC-Ab adsorption onto an ITO surface (modified with silane HDMS).

Figure 10B shows differentials of the data curves contained in Figure 10A. The peaks can be more easily distinguished in this figure. This figure shows that optical detection of biomedical adsorption is possible on a QCM with ITO electrodes.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as described herein without departing from the spirit or scope of the invention. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive.

CLAIMS:

The claims defining this invention are as follows:

1. A microchip, for simultaneous micro-gravimetric sensing and surface plasmon resonance (SPR) sensing, comprising:
   - a transparent piezoelectric substrate having a first surface and a second surface;
   - a thin film metal electrode formed on the first surface of the substrate, said metal being adapted to generate surface plasmon resonance upon excitation; and
   - a second electrode, formed of a film of a conductive, substantially transparent material, formed on the second surface of the substrate, wherein the second electrode is formed of a transparent semiconductor metal oxide or of a transparent polymer.
2. A microchip according to claim 1, wherein the substrate is formed of a transparent piezoelectric crystal which is suitable for fabricating resonators.
3. A microchip according to claim 2, wherein the transparent piezoelectric crystal is quartz or lithium niobate.
4. A microchip according to any one of claims 1 to 3, wherein the metal electrode is formed of gold, silver, platinum or copper.
5. A microchip according to claim 1, wherein the transparent semiconductor metal oxide is selected from indium tin oxide, indium zinc oxide and zinc oxide.
6. A microchip according to claim 5, wherein the transparent semiconductor metal oxide is indium tin oxide.
7. A microchip according to any one of claims 1 to 6, wherein the optical transmission of the second electrode is sufficiently high so as to enable a light beam to pass there-through for optical detection and SPR applications.

8. A microchip according to claim 7, wherein the optical transmission of the second electrode is greater than 70%.

9. A microchip according to any one of claims 1 to 8, wherein the film of the second electrode exhibits high thermal stability and minimal drift at room temperature.

10. A microchip according to any one of claims 1 to 9, wherein the thickness and morphology of the electrodes are adapted so as to enable the excitation of surface plasmons.

11. A microchip according to claim 10, wherein the film of the second electrode has a thickness of from about 100 nm to about 1000 nm.

12. A microchip according to any one of claims 1 to 11, wherein the metal electrode film has a thickness of from about 60 nm to about 100 nm.

13. A microchip according to any one of claims 1 to 12, wherein the conductivity of the electrodes is sufficiently high so as to enable the sensor to perform quartz crystal microbalance (QCM) sensing or surface acoustic wave (SAW) sensing.

14. A microchip, for simultaneous micro-gravimetric sensing and optical sensing, comprising:

- a transparent piezoelectric substrate having a first surface and a second surface; and
- first and second thin film electrodes formed of a conductive, substantially transparent material, said electrodes being formed on the first and second surfaces of the substrate,

wherein said electrodes have a thickness of less than about 1500 nm and wherein the conductive, substantially transparent material is formed of a semiconductor metal oxide.

15. A microchip according to claim 14, wherein the thickness of the electrodes is between about 100 nm and 1000 nm.

16. A microchip according to claim 14 or claim 15, wherein the substrate is formed of a transparent piezoelectric crystal which is suitable for fabricating resonators.

17. A microchip according to claim 16, wherein the transparent piezoelectric crystal is quartz or lithium niobate.

18. A microchip according to claim 14, wherein the semiconductor metal oxide is selected from indium tin oxide, indium zinc oxide and zinc oxide.

19. A microchip according to claim 18, wherein the semiconductor metal oxide is indium tin oxide.

20. A microchip according to any one of claims 1 to 19, further including a SERS active surface deposited on the first thin film electrode.

21. A microchip according to claim 20, wherein the SERS active surface is a thin film formed of gold, silver, platinum or copper.

22. A microchip according to claim 21, wherein the SERS active surface is a thin film formed of gold or silver.

23. A microchip according to any one of claims 1 to 22, wherein the electrode formed on the first surface of the substrate comprises a plurality of sub-electrodes.

24. A microchip according to claim 23, wherein the plurality of sub-electrodes is in the form of a grid.

25. Bio-analysis sensing apparatus comprising at least one microchip according to any one of claims 1 to 24.

26. Bio-analysis sensing apparatus according to claim 25 comprising multiple microchips according to any one of claims 1 to 24.

27. A method of fabricating a microchip according to any one of claims 1 to 13, 23 or 24, comprising the following steps:

- depositing on a first surface of a transparent piezoelectric crystal substrate a thin metal film selected from gold, silver, platinum and copper;
- patterning the metal film into an electrode shape;

- depositing on a second surface of the transparent piezoelectric substrate a thin film of a transparent conductive semiconductor metal oxide; and
- patterning the metal oxide film into an electrode shape.

28. A method of fabricating a microchip according to any one of claims 14 to 22, comprising the following steps:

- depositing on a first surface of a transparent piezoelectric crystal substrate a first thin film of a transparent conductive semiconductor metal oxide;
- patterning the first metal oxide film into an electrode shape;
- depositing on a second surface of the transparent piezoelectric crystal substrate a second thin film of a transparent conductive semiconductor metal oxide; and
- patterning the second metal oxide film into an electrode shape.

29. A method according to claim 27 or claim 28, wherein the transparent piezoelectric substrate is a quartz crystal wafer.

30. A method according to claim 29, wherein the quartz crystal wafer has a substantially rectangular shape having dimensions of about 10 mm x 10 mm x 0.1 mm.

31. A method according to claim 29, wherein the quartz crystal wafer has a substantially circular shape having a diameter of about 14 mm and thickness of about 0.1 mm.

32. A method according to any one of claims 27 to 31, wherein the method of depositing the first thin film of electrode material or the second thin film of electrode material (or both) onto the relevant surface is selected from thermal evaporation, electron-beam evaporation and RF sputtering at a low temperature.

33. A method according to claim 32, wherein the method of deposition is RF sputtering at a low temperature.

34. A method according to claim 32 or claim 33, wherein the deposition temperature is less than that at which damage occurs to any piezoelectric properties of the material.

35. A method according to claim34, wherein the deposition temperature is about 200ºC.

36. A method according to any one of claims 27 to 35, wherein the thin films are coated and the electrode patterning is effected by photolithographic techniques.

37. A method according to any one of claims 27 to 36, wherein the patterned electrodes are aligned using double-sided alignment on the surfaces of the substrate.

38. A method of analysing or monitoring a molecule, said method including simultaneously conducting micro-gravimetric sensing and SPR sensing, utilising bio-analysis sensing apparatus according to claim 25 or claim 26, and any one of claims 1 to 13.

39. A method of analysing or monitoring a molecule, said method including simultaneously conducting micro-gravimetric sensing and optical sensing, utilising bio-analysis sensing apparatus according to claim 25 or claim 26, and any one of claims 14 to 22.

40. A method according to claim 39, wherein the optical sensing includes measuring a selected optical signal selected from one or more of fluorescence, photoluminescence, Raman scattering, absorption, reflection and transmission.

41. A method according to claim 40, wherein the optical sensing includes use of surface enhanced Raman scattering (SERS).

42. A microchip substantially as hereinbefore described with reference to any one or more of Figures 2 to 6.

43. Bio-analysis sensing apparatus comprising one or more microchips according to claim 42.

44. A method of analysing or monitoring a molecule by using bio-analysis sensing apparatus according to claim 43.