

terned dielectric layer printed over the carbon ink. The impedance measurements were taken at 25 degrees C. using an excitation voltage equal to 0.010 V rms at the frequencies indicated in the tables. For capacitance measurements, since it is desirable to use a frequency where all (or nearly all) of the voltage drop occurs across the capacitive element, a frequency of 200 Hz was utilized as this was found to result in greater than 95% of the voltage drop to occur across the double layer capacitance; i.e., the solution losses were almost negligible. Resistance and capacitance were calculated using a series RC model.

[0146] As can be seen in Tables 3 and 4, the capacitance varied little between the different sample matrices, however, the resistances showed much greater variation among

TABLE 3

Sample Discrimination Using Capacitance Measurements (phase angles 76 to 82 degrees).	
Matrix	Capacitance, uF at 200 Hz
Assay buffer	0.023
Saline	0.021
Serum	0.019
Plasma	0.018
Blood	0.020

TABLE 4

Sample Discrimination Using Resistance Measurements (includes 700 ohms of lead resistance; phase angles 12 to 16 degrees)	
Matrix	Resistance, ohms at 20,000 Hz
Assay buffer	2516
Saline	3722
Serum	3996
Plasma	4158
Blood	7039

[0147] In certain preferred embodiments the electrochemical current measured during the induction of ECL, may be used to detect the presence of trapped air over an electrode since trapped air may cause a significant decrease in the electrochemical current (e.g., current from TPA oxidation during ECL). FIG. 5 depicts an image of ECL emitted from an electrode array. One of the electrodes has a small dark spot **500** due the presence of a small air bubble on the electrode surface. Even such a small bubble gave a detectable change in the electrochemical current measured at that electrode during the ECL experiment; the current in the presence of the air bubble (178 uA) was significantly different (by 5%) than the average of the current at the other electrodes (187 uA). Other factors besides trapped air, e.g., errors in the printing of the electrodes, may change the effective area of an electrode and thus the measured current. The measurement of current during ECL can be used to check for these factors as well as for bubbles and can be used to trigger error flags if the current values fall out of an acceptable range or, alternatively, to allow for normalization of the reported ECL signal to compensate for the actual electrode area.

[0148] The bubble detection methods described above can also be employed to detect the presence of fluids, the presence of bubbles in fluids and/or identify classes of samples in compartments in an assay cartridge outside the detection flow cells. For example, certain preferred embodiments of assay

cartridges comprise fluid inlet and/or outlet lines for introducing and removing fluids from the cartridge flow cells, wherein these inlet and/or outlet lines comprise fluid detection electrodes for detecting the presence of fluid, the presence of air bubbles in fluids and/or for identifying samples. These fluid detection electrodes may have independent electrode leads and contacts. So as to reduce the number of electrical contacts to the cartridge, these fluid detection electrodes, preferably, comprise exposed surfaces of the leads to assay electrodes (e.g., assay electrodes in the assay cartridge flow cells). In this arrangement, it is further preferred that the exposed leads in a given fluid volume (e.g., an inlet line or outlet line) do not comprise leads from two electrodes that will be fired together in an assay measurement (e.g., used as a working electrode counter electrode pair in an ECL measurement). In this fashion it is ensured that the assay measurements are not affected by low resistance current paths between exposed leads.

[0149] With reference to the simplified embodiment depicted in FIG. 4, use of the impedance sensors **425** for detection of fluid presence and/or discrimination within the fluid input line **450** will now be discussed. Impedance sensors **425** are regions of electrically conductive surfaces on the electrode leads between electrodes **401-407** and electrode contacts **420**. The electrically conductive surfaces are, preferably, exposed via apertures in a patterned dielectric layer that is patterned over the electrode leads. As fluid is directed into and through the fluid input line **450** (e.g., by use of pumps, valves, capillary flow, and the like), the impedance sensors **425** may be activated by a controller (not shown) that applies interrogation potentials between sensor pairs to detect and/or discriminate the fluid (the interrogation potentials being preferably lower than those required to induce ECL at the assay electrodes). The position of bubbles or fluids in the input line can be determined by sequentially measuring the impedance between different sensor pairs and comparing the values. The sensors are on alternating electrode leads so that when adjacent electrodes are fired during, e.g., an ECL measurement, the potential across the assay electrodes is not short circuited by current between sensors.

[0150] According to another aspect of the present invention, the electrode surfaces are coated with assay reagents such as antibodies or other specific binding reagents by dispensing solutions comprising the reagents to one or more appropriate locations on the electrode array, i.e., the capture surfaces. Preferably, the assay reagents collect on the surface (e.g., via the formation of covalent bonds, non-specific adsorption or specific binding interactions) to form an immobilized layer on the electrode. In a preferred embodiment, accurate volume delivery to a specified location results in complete coverage of only the desired electrode surface and/or a desired portion thereof. Accurate volume delivery to a specified location can be readily accomplished with commercially available dispensing equipment; e.g., commercially available equipment from Bio Dot.

[0151] Attaining complete coverage of a pre-defined region on a surface (e.g., an assay electrode) via localized deposition of a liquid (e.g., an assay reagent or a liquid comprising an assay reagent) can be difficult to achieve if the advancing contact angle of the liquid on the surface is high, thereby inhibiting spreading of the liquid on the surface (as has been observed for surfactant-free aqueous solutions on untreated carbon ink electrodes). Spreading can be accelerated by chemically modifying the surface to make it more wettable or