

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.

[0153] 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 BioDot.

[0154] 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 by adding surfactants to the liquid, however, in many circumstances it is undesirable to change the physical properties of the surface or liquid. Alternatively, we have found that excellent and well controlled spreading of liquids can be achieved on surfaces, such as carbon ink electrodes, having high contact angle hysteresis (i.e., large differences in the advancing and retreating contact angle of the liquid on the surface, preferably differences greater than 10 degrees, more preferably greater than 30 degrees, more preferably greater than 50 degrees, most preferably greater than 70 degrees) by using impact-driven fluid spreading. Such results can be achieved without surface modification or the use of surfactants. Fluid is deposited (preferably, using a fluid micro-dispenser such as a micro-pipette, micro-syringe, solenoid valve controlled micro-dispenser, piezo-driven dispenser, ink-jet printer, bubble jet printer, etc.) on the surface at high velocity (preferably greater than 200 cm/s, more preferably greater than 500 cm/s, most preferably greater than 800 cm/s) so as to drive spreading of the liquid over the surface, despite the high advancing contact angle, to a size dictated by the volume and velocity of the

dispensed fluid. The low retreating contact angle prevents significant retraction of the fluid once it has spread. Using the impact-driven spreading technique, it is possible to coat, with a predetermined volume of liquid, regions of a surface that are considerably larger (preferably, by at least a factor of 1.2, more preferably by at least a factor of two, even more preferably by at least a factor of 5) than the steady state spreading area of the predetermined volume of liquid on the surface (i.e., the area over which a drop having that volume spreads when touched to the surface at a velocity approaching zero).

[0155] Preferably, the region to be coated is defined by a physical boundary that acts as a barrier to confine the deposited fluid to the pre-defined region (e.g., a surrounding ledge or depression, a boundary formed of patterned materials deposited or printed on the surface, and/or a boundary formed via an interface with a surrounding region that varies in a physical property such as wettability). More preferably, the liquid has a higher receding contact angle on the surrounding region than on the pre-defined region (preferably, the difference is greater than 10 degree, more preferably greater than 30 degrees, most preferably greater than 50 degrees). Even more preferably, the surrounding region also exhibits a low contact angle hysteresis for the liquid (preferably, less than 20 degrees, most preferably, less than 10 degrees). By using a surrounding region having high receding contact angle and/or low hysteresis, the tolerance for imprecision in deposition velocity or spreading rate becomes much improved. In a preferred deposition method, a small volume of reagent is dispensed onto the pre-defined region with sufficient velocity to spread across the pre-defined region and slightly onto the surrounding region, the liquid then retracts off the surrounding region (due to its high receding contact angle) but does not retract smaller than the size of the pre-defined area (due to its low receding contact angle). In especially preferred embodiments of the invention the pre-defined area is an exposed area of an electrode (preferably, a carbon ink electrode) and the surrounding region is provided by a dielectric ink patterned on the electrode.

[0156] FIG. 8 illustrates typical observed contact angles of 250 nL drops of water deposited using a solenoid valve-controlled micro-dispenser (Bio-Dot Microdispenser, Bio-Dot Inc.) on a preferred dielectric ink and a preferred carbon ink. The figure plots the contact angle as a function of the velocity of fluid as it leaves the tip of the dispenser. At low velocity, the observed contact angle is close to the advancing contact angle of water on the surface. As the velocity increases, impact-driven spreading causes the liquid to spread over a greater area and the observed contact angle decreases. At the high velocities, the observed contact angle becomes relatively independent of velocity as it approaches the receding contact angle of the liquid on the surface, the receding contact angle being the lowest contact angle the liquid can have on the surface (a lower contact angle would cause the drop to recede till it achieves the receding contact angle).

[0157] As described above, assay reagents such as antibodies or other specific binding reagents may be patterned by depositing (e.g., via impact driven spreading) solutions comprising the reagents on pre-defined locations on a surface (e.g., an electrode surface, preferably a carbon ink electrode surface) and allowing the reagents to become