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).

[0152] 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.

[0153] FIG. **8** illustrates typical observed contacts angles of 250 nL drops of water deposited using a solenoid valve-controlled micro-dispenser (Bio-Dot Microdispensor, 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).

[0154] 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 immobilized on the surface (e.g., via covalent bonds, non-specific interactions and/or specific binding interactions). 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) so as to form a fluid containment region.

[0155] In certain preferred embodiments, antibodies or other binding reagents (preferably proteinaceous binding reagents) are immobilized on carbon ink electrodes by non-specific adsorption. It may be advantageous to allow the assay reagent solution to dry on the electrode during the immobilization procedure. Preferably, the immobilization procedure further comprises blocking un-coated sites on the surface with a blocking agent such as a protein solution (e.g., solutions of BSA or casein), washing the surface with a wash solution (preferably a buffered solution comprising surfactants, blocking agents, and/or protein stabilizers such as sugars) and/or drying the surface.

[0156] In a preferred immobilization procedure of the invention, imprecision due to variations in the ability of different assay reagents to adsorb on a surface such as a carbon ink electrode are reduced by immobilizing via a specific binding interaction involving a a first and second binding partner. Such an immobilization technique is less likely to be affected by small variations in the properties of the surface. By way of example, antibodies may be patterned by patterned deposition of antibody solutions (the first binding partner) on a surface coated with an antibody binding reagent (the second binding partner, e.g., an anti-species antibody, protein A, protein G, protein L, etc.). Alternatively, assay reagents labeled with the first binding partner (preferably, biotin) may be patterned by patterned deposition of the assay reagents on a surface coated with the second binding partner (preferably, anti-biotin, streptavidin, or, more preferably, avidin). Most preferably, the second binding partner is deposited in the same pattern as the assay reagents. By analogy, the method can be adapted to use any of a variety of known first binding partner-second binding partner pairs including, but not limited to, hapten-antibody, nucleic acid-complementary nucleic acid, receptor-ligand, metal-metal ligand, sugar-lectin, boronic acid-diol, etc.

[0157] Accordingly, one embodiment of an immobilization method of the invention comprises forming an assay domain comprising an assay reagent by: i) treating a predefined