

trode. The method may include the steps of washing the electrode and then treating the electrode with solution containing the assay reagents. The washing step preferably employs a washing solution comprising a surfactant; e.g., a non-ionic surfactant selected from the surfactants known by the trade names of Brij, Triton, Tween, Thesit, Lubrol, Genapol, Pluronic (e.g., F108), Tetricon, Tergitol, and Span, most preferably Triton X100. Additionally, after the washing step and prior to the treating step, the electrode may be rinsed with a surfactant free solution. Preferably, the electrode is soaked in the surfactant free solution for about one hour.

[0019] In accordance with a still further aspect of the invention, a method of forming an assay domain comprising an assay reagent is disclosed. Preferably, in accordance with such method, a predefined region of a surface is treated with an avidin solution so as to form an adsorbed avidin layer within the predefined region of the surface. Next, the adsorbed avidin layer is preferably treated with a solution comprising the assay reagent, the assay reagent being linked to biotin. More preferably, the avidin solution is dried on the surface prior to treatment with the assay reagent solution. The method may also employ the step of washing the adsorbed avidin layer prior to treatment with the assay reagent solution. The surface may be a carbon ink electrode. The predefined region is preferably defined by a boundary adapted to confine the avidin and/or assay reagent solutions to the predefined region (most preferably both solutions are confined to the pre-defined region). The boundary can be defined by a dielectric layer.

[0020] According to another aspect of the invention, a method of forming a plurality of assay domains is disclosed wherein one of a plurality of predefined regions of a surface are treated with an avidin solution so as to form an adsorbed avidin layer within the predefined region of the surface. The adsorbed avidin layer is then preferably treated with a solution comprising an assay reagent linked to biotin. These steps may then be repeated for each of the plurality of assay domains. More preferably, the avidin solution is dried on the surface prior to treatment with the assay reagent solution. The method may also employ the step of washing the adsorbed avidin layer prior to treatment with the assay reagent solution. The surface may be a carbon ink electrode. The predefined region is preferably defined by a boundary adapted to confine the avidin and/or assay reagent solutions to the predefined region (most preferably both solutions are confined to the pre-defined region). The boundary can be defined by a dielectric layer.

[0021] The assay reagent in each domain may be the same or may be different. Assay reagents that may be used include, but are not limited to, antibodies, fragments of antibodies, proteins, enzymes, enzyme substrates, inhibitors, cofactors, antigens, haptens, lipoproteins, liposaccharides, cells, sub-cellular components, cell receptors, membrane fragments, viruses, nucleic acids, antigens, lipids, glycoproteins, carbohydrates, peptides, amino acids, hormones, protein-binding ligands, pharmacological agents, membrane vesicles, liposomes, organelles, bacteria or combinations thereof. Preferably, the assay reagents are binding reagents capable of specifically binding to an analyte of interest or, alternatively, of competing with an analyte of interest for

binding to a binding partner of the analyte of interest. Especially preferred assay reagents are antibodies and nucleic acids.

[0022] According to one embodiment, the avidin solution for forming one, or a plurality, of assay domains may comprise a polymeric form of avidin. The polymeric form of avidin may be formed by forming a solution of avidin and a cross-linking molecule, the cross-linking molecule preferably having a plurality of biotin groups. The ratio of the cross-linking molecule to avidin is preferably between 0.01 and 0.25. The method of forming an assay domain can preferably include the step of washing the assay domain or plurality of assay domains. More preferably, the wash solution comprises blocking agent, wherein the blocking agent can be a protein or biotin.

[0023] The invention also relates to assay cartridges employing the electrode arrays and/or binding domains employing these electrode described above (and adapted for carrying out the methods described above for using these arrays and domains) and assay cartridge readers for operating and analyzing these cartridges. The invention also relates to assay systems comprising these cartridges and cartridge readers. The cartridges and readers, preferably, comprise the necessary fluidics and control systems for moving sample and reagent fluids, collecting waste fluids, removing and/or introducing bubbles from liquid reagents and/or samples, conducting physical measurements on the samples and/or extracting samples.

[0024] The invention also relates to assays cartridges comprising a sample chamber preferably having a sealable closure, an optional waste chamber and a detection chamber (preferably, a detection chamber having one or more binding domains having immobilized binding reagents, more preferably, one or more binding domains on one or more electrodes, most preferably an electrode array of the invention as described above). The detection chamber is connected to the sample chamber via a sample conduit and, if present, to the waste chamber via a waste conduit. The assay cartridge may also include a sample chamber vent port connected the sample chamber and/or a waste chamber vent port connected to the waste chamber. The sample can include a capillary break, preferably a z-transition. The z-transition preferably includes a fluid conduit segment that connects two planar fluidic networks of the cartridge. The capillary break may alternatively comprise a double z-transition.

[0025] In another embodiment of an assay cartridge that includes: a vented sample chamber with an introduction port and a sealable closure; a vented waste chamber; and a detection chamber (preferably, a detection chamber having one or more binding domains having immobilized binding reagents, more preferably, one or more binding domains on one or more electrodes, most preferably an electrode array of the invention as described above) connected to the sample and waste chambers via sample and waste conduits, respectively, one or more fluidic networks may be defined within the cartridge's body by one or more cover layers mated to a side of the cartridge body. A second cover layer, or set of cover layers, may be mated to a second side of the cartridge body to form one or more additional second side fluidic networks therebetween, the first and second side fluidic networks being in fluidic communication by at least one