current or charge and convert it to a signal. Alternatively an electrode can be defined as a composition which can apply a potential to and/or pass electrons to or from species in the solution. Preferred electrodes are known in the art and include, but are not limited to, certain metals and their oxides, including gold; platinum; palladium; silicon; aluminum; metal oxide electrodes including platinum oxide, titanium oxide, tin oxide, indium tin oxide, palladium oxide, silicon oxide, aluminum oxide, molybdenum oxide (Mo_2O_6), tungsten oxide (WO_3) and ruthenium oxides; and carbon (including glassy carbon electrodes, graphite and carbon paste). Preferred electrodes include gold, silicon, carbon and metal oxide electrodes, with gold being particularly preferred. In a particularly useful embodiment, both the electrowetting electrode grid and the detection electrodes are gold, and are fabricated simultaneously on the PCB.

[0161] The present system finds particular utility in array formats, i.e. wherein there is a matrix of addressable detection electrodes (herein generally referred to "pads", "addresses" or "micro-locations"). By "array" herein is meant a plurality of capture ligands on electrodes in an array format; the size of the array will depend on the composition and end use of the array. Arrays containing from about 2 different capture ligands to about 50 to 100 can be made. In some preferred embodiments, 80 or 100 working detection electrodes are split into four or five distinct zones of 20, with each zone having up to 60 capture probes (three different capture probes per electrode).

[0162] The detection zone of the substrate comprises one or more arrays of electrodes that is in fluid communication with the droplet pathway. That is, the droplets containing the amplicons will pick up necessary detection reagents such as label probes and then be dispersed on the detection zone. In general, each detection zone receives one or more sample droplets which are generally dispersed on the array of electrodes, which is considered one larger "pad".

[0163] Each detection electrode comprises an independent lead (interconnect) to transmit input and electronic response signals for each electrode of the array. In contrast to previous systems which require the ability to independently alter only input signals to each electrode but not electronic response signals, it is important in the present invention that both input and electronic response signals be independently monitorable for each electrode; that is, each electrode is independently addressable.

Additional Components

[0164] In addition to the components of the bottom substrate described above, the bottom substrate can also optionally comprise an EPROM, EEPROM or RFID to identify the cartridge, for example containing information about the batch, treatment or contents of the biochip. This can include information about the identification of the assay, for example.

Top Plate

[0165] The bottom substrate, described above, together with a top plate form a chamber or chambers for the reactions and processing described herein. In most embodiments, the top plate is substantially parallel to the bottom plate, to form a reaction chamber of uniform depth. In some embodiments the top plate may be optionally slanted, for example to drive air bubbles to the highest point of the chamber to avoid interference with the reactions on the surface or for access to an air vent as discussed herein. As outlined herein, and as will be appreciated by those in the art, the top plate can take on a number of configurations and can be made of a variety of materials. Suitable materials include, but are not limited to, fiberglass, Teflon®, ceramics, glass, silicon, mica, plastic (including acrylics, polystyrene and copolymers of styrene and other materials, polypropylene, polyethylene, polybutylene, polycarbonate, polyurethanes, Teflon®, and derivatives thereof, etc.), etc. A particularly preferred top plate material is polycarbonate.

[0166] In one embodiment, the top plate and bottom substrate mated together form a single chamber that is filled with the immiscible fluid and through which the droplets are moved, merged, split, etc. Generally, this is accomplished by three dimensional ridges formed on the top plate to form the sides of the chamber(s). In alternate embodiments, the top plate and the bottom substrate can be separated into more than one chamber as needed. For example, the top plate can define two chambers, one for general sample handling and purification, and a second chamber, connected through a fluid passageway in the top plate, for the reagent loading, amplification reactions and detection. This approach could be valuable for keeping different parts of the reaction separated. In addition, top plate design can include varied gap heights to allow for expected fluid volumes within different areas of the cartridge, i.e. higher gap heights where a larger volume of fluid is present (e.g. over the sample handling zone) and lower gap heights where smaller volumes of fluid are present (e.g. over the amplification and/or detection zones).

[0167] The top plate generally includes a seal to confine liquids (and particularly any biological samples) to the chamber(s). This can also be used to mate the top and bottom plates together, and can include gaskets (e.g. silicone, rubber, etc.), adhesives and glues, etc. The seal comprises an epoxy polymer that is curable by ultraviolet (UV) light. The present inventors discovered that plasma treatment of the PCB helps to improve the sealing and reduce leakage of the immiscible liquid from the chamber.

[0168] The top plate is designed with a plurality of entry ports to the bottom substrate that define delivery locations for sample, reagents, and the immiscible fluids (e.g. oil(s)). These entry ports, also referred to herein as "fluid passages", "fluid passageways" or "fluid ports" are in fluid connection with the pads which they serve. That is, whether abutting or remote, two elements will have a fluid passageway between them; in some cases this is the droplet pathway of the electrowetting grid, while in others it is a fluidic channel between two components of the system. In many embodiments, these entry ports are perpendicular to the bottom substrate, allowing the fluids to flow downward onto the pads (via either gravity or the pressure used for the blister pack delivery discussed below). This can be referred to as a type of "one-to-one spatial correspondence". Alternatively, some ports may be channels within the top plate such that delivery of the fluid can occur remotely, e.g. at a location distant from the actual reagent storage blister, such that the blister exit port is connected to a fluid channel whose exit vents at the desired corresponding pad location for delivery of the fluid (that is, the blister volume (once ruptured) and the pad are in "fluid communication").

[0169] In optional embodiments, passive one way valves can be used to prevent backflow.

[0170] In addition, the top plate may optionally include one or more vents to reduce air bubble formation and/or remove