

droplets. The Reagent zone is where reagents (enzymes, binding ligands, labels, primers, probes, buffers, wash buffers, etc.) are stored, either as dried reagents or as confined liquids or in blister packages above the surface, which, when burst, release the reagents into these zones, or both. The processing zone (labeled herein as the amplification zone as the target analytes are nucleic acids in this particular embodiment) is where the electrowetting fluidic technology allows the microdroplets to travel over different thermal zones (in this case, provided by resistive heaters in the bottom bay where the chip contacts the bay, although in some embodiments, on-chip heaters and sensors, such as resistive copper traces or thin-film thermocouples, could be utilized) to facilitate PCR. The eSensor™ zone is where the detection occurs as described herein. In some embodiments, as described herein, a Peltier element or a resistive heater is included (again, preferably within the bay, but in some embodiments could on-chip as well).

[0087] FIG. 22 is another rendering of one of the embodiments of the present biochip invention. Several optional manual reagent introduction ports are shown, as discussed herein that may have blister packages (or other methods of storing reagents) above the zones, resident in the LRM and accessible to the chamber formed by the substrate and the top plate using holes or vias in the top plate, which optionally include one way valves to prevent sample from entering the LRM. The amplification area is divided into three zones, that can be used individually (e.g. three droplets are processed essentially simultaneously) or together (e.g. one droplet is processed on the three tracks). This can allow, for example, one 21-plex reaction to be run as a group, or as 3×7-plex reactions; in some cases, particularly when multiplex PCR reactions are done, lowering the multiplexity of the reactions (e.g. primer sets, etc.) can give better results. It will also be appreciated by those in the art that multiple droplets may be used in each PCR track, e.g., 2, 3, 4 or more droplets per track (for example which may be combined together either prior to or during dispersment on the detection zone. In addition, as noted herein, these amplification areas need not be PCR reactions, isothermal amplification techniques can also be used.

[0088] FIGS. 23A and 23B depict a general schematic of one configuration the bottom substrate of the cartridges of the invention. The substrate is divided into the sample reservoir, showing the larger pads of the electrowetting electrode grid that are used in sample preparation, based on the volume of the sample and the amount of lysis buffer, binding buffer and elution buffers needed for sample preparation. FIGS. 23A and 23B depicts a magnet area, where the capture beads are mixed with lysed sample (usually with the addition of binding buffer), washed, and eluted (using elution buffer). From there, in FIGS. 23A and 23B, the drops are loaded onto the Center Transport Lane, and moved into the reagent storage and delivery area. Moving through this area (as more fully described below), the droplet(s) move to the PCR reagent staging area, where they pick up the required reagents, primers, probes, enzymes, etc. for PCR. This figure depicts two amplification pad pathways and three heat zones for the PCR thermocycling. The drops travel back and forth through these thermal zones for an appropriate number of cycles, and then move back along the center transport lane to pick up detection reagents, signaling probes, etc., to be moved onto the detection electrode array. Also shown are a plurality of reservoirs, including the reconstitution reservoir (for use when the dried reagents are to be reconstituted by buffer and not by using the

sample droplet to resuspend the reagents), a wash reservoir, and three additional reservoirs for the storage of buffers, etc. as needed.

[0089] FIG. 24 shows the optional addition of dry reagents in the embodiment of nucleic acids and amplification on the overlay of one general embodiment of the bottom substrate configuration. These can be used alone or in combination with liquid reagents as described herein, and the placement of either type of reagent should not be considered limiting. The bottom substrate of FIGS. 23A and 23B depicts three amplification tracks, which as described herein can be used for three separate PCR reactions or for one reaction done along multiple pad pathways. The three amplification tracks are shown on the right, with three perpendicular thermal zones, depicted as 95 C, 72 C and 64 C (although these can be adjusted based on the individual primer/probe PCR reactions as is well known in the art). The interconnects (herein shown as pin connectors) are at the edges of some sides of the substrate. The electrowetting electrode grid on the right are larger pads, allowing for sample handling, including lysis, capture bead mixing (in binding buffer), etc. The electrowetting grid on the left hand side contains smaller pads for smaller droplet size.

[0090] FIGS. 25A, 25B, 25C, 25D and 25E show a number of possible configurations of the electrowetting electrode grid, the dried reagent pad locations and the reagent pathways. "XT-1" and "XT-2" refer to solutions comprising the appropriate label ligands (e.g. signal probes) for the detection of the analytes.

[0091] FIG. 26 depicts one schematic of an apparatus of the invention, including a depiction of several biochip cartridges. The apparatus shows the base station with a touch screen display with biochip icons with a one-to-one spatial correspondence to the biochip cartridge bays (shown here in two towers, one on each side of the display). As discussed herein, the apparatus can be made with one back of bays, two (as shown or both on one side), three (two on one side and one on the other, or three on one side), four (two on each side), etc. In addition to the biochip icons, there are optional function icons on the bottom of the screen as described herein, along with an optional display of the time and date. Each bay has an insertion slot, configured to only allow asymmetric insertion (both requiring "right side up" insertion as depicted here by the half-moon shape, e.g. the bottom of the biochip is rounded in this embodiment although other such shapes can be used) as well as a groove/protrusion system in both the chip and the bay that allows only one end of the cartridge to be inserted into the bay ("right end in"; depicted herein where the groove is in the cartridge and the protrusion is in the bay, although this can be reversed and/or other well-known techniques for asymmetrical insertion can be used). Above the insertion slot is a curved light display, configured to show the status of the bay using any combination of colors, flashing lights or the absence of light to depict the status of the individual bay (e.g. empty, ready to load, assay underway, assay complete, cartridge ready to remove, error, etc.). A USB port is depicted with an attached barcode reader (although as will be appreciated, more than one USB port can be included). A power button is shown. In addition, the bottom of the middle component shows a cover, which conceals a built-in barcode scanner if a hand held design is not preferred. In addition, several biochip cartridges are shown, with the housing with the blister actuator sites covered by a label (optionally including one or more trademarks, barcodes, identifying labels,