

entire disclosure of which is incorporated herein by reference. The fluid may be selected to prevent any significant evaporation of the droplets.

Sample Manipulation Zone

[0151] As will be understood by those in the art, the movement of droplets from pad to pad, with the addition of reagents as needed, can be used for any number of sample manipulations. In the case of the nucleic acid manipulations for nucleic acid detection, these manipulations generally include the addition of reagents such as PCR enzymes, PCR buffer, primers, exonuclease, reverse transcriptase (RT) enzymes, RT-PCR buffers, signal buffers, signal probes, etc.

[0152] In one embodiment, on-chip thermal components, e.g. resistive heaters for PCR thermocycling, are used. In this embodiment, resistive heater(s) can be placed underneath the electrode grid pathway of pads to result in thermal zones for amplification, exonuclease digestion, reverse transcription, target elution, and the electrochemical detection. As will be appreciated by those in the art, some manipulations such as PCR amplification requires from 2 to 3 different temperatures (primer binding, extension and denaturation), while others require a uniform temperature for best results, e.g. enzymatic processes such as the use of exonuclease and reverse transcriptase, specific temperature(s) for improved elution and/or reagent resuspension, or binding/assay temperatures in the case of the electrochemical detection. Isothermal amplification techniques and other PCR alternatives typically require precise temperature control.

[0153] Alternatively, these thermal components such as heaters are found off-chip in the bays of the instrument into which the cartridge is placed.

[0154] In one embodiment, the sample manipulation zones on the substrate can optionally include sensors, for example to monitor and control thermal zone temperatures, particularly in the case where specific temperatures are desirable. These sensors can include, but are not limited to, thermocouples and resistance temperature detectors (RTDs). Again, for many embodiments, as for the thermal elements, these can also be "off chip" in the bays.

Amplification Zone

[0155] As shown in the figures, in the embodiments for detecting nucleic acid targets, the substrate comprises one or more amplification pathways. As shown in a number of the figures, a bottom substrate can contain 1, 2, 3 or more amplification pathways of pads. These can be used for individual PCR reactions (e.g. one droplet is moved up one path and down another, etc.) or for multiplexing (e.g. for three pathways, three different droplets can be moved up and down a single pathway).

[0156] As will be appreciated by those in the art, each PCR reaction can additionally be multiplexed. That is, for target specific amplification, the use of multiple primer sets in a single PCR reaction can be unwieldy, and thus the present invention allows multiple reactions to achieve higher levels of multiplexing. For example, for the evaluation of 21 different target sequences (for example, in screening of respiratory viruses), it may be desirable to run 3 different reactions of seven primer sets; e.g. a first PCR sample droplet (e.g. the bottom pathway) picks up a first set of 7 primer pairs (e.g. "Primer Mix A"), a second droplet picks up a second set of 7 primer pairs ("Primer Mix B"), and a third droplet picks up a

third set ("Primer Mix C"). In some embodiments, the primers will be completely different in each set; in others, redundancy and/or internal controls are built into the system by adding the same primer sets to different tracks. The multiplexing flexibility represents one of the key advantageous and distinguishing features of the present invention. The number of multiplexes can vary easily through software without the need to modify any physical components of the system. Traditional channel based microfluidic devices lack such flexibility.

[0157] In general, the amplification reactions (as more fully described below) for use in the present systems use sets of primers wherein one primer of each set has a blocked end that is impervious to standard exonucleases. That is, it is desirable to remove one strand of the double stranded amplicons that are generated in the PCR reaction, so as to simplify the detection reactions and remove background signal. Thus, by running a first PCR reaction and then adding exonuclease, one strand of the double stranded amplicon is digested, leaving only the detection strand.

[0158] The use of heating zones perpendicular to the amplification pathway, as generally depicted in FIGS. 23 A and 23B, allows the droplets to travel through the appropriate thermal zones. As shown in FIGS. 23 A and 23B, three amplification pathways are shown with three perpendicular thermal zones (in this case, the thermal elements are off chip Peltier heaters and show desired temperatures of 95 C, 72 C and 64 C for use in PCR thermocycling). In some embodiments, two different temperature zones (e.g., about 95 C for denaturation and about 60 C for annealing and extension) can be used for a two-step PCR reaction. In other embodiments, a three-zone, two-temperature configuration may be employed, wherein a middle heater controls the denaturation temperature (e.g., about 95 C), and additional heaters on each side of the denaturation heater provide substantially the same annealing and extension temperature (e.g., about 60 C) as shown in FIG. 34. In this configuration, two-step amplification cycles can be performed with more than one droplet in each PCR track, sometimes referred to herein as "tandem amplification" or "typewriter amplification". For example, two droplets may be positioned in each PCR track and spaced in such a way that when one droplet is in the denaturation zone, the other is in one of combined annealing and extension zones, and vice versa. By shuttling the droplets in tandem back and forth between the denaturation and annealing/extension zones, one can amplify both of them in the same amount of time it would normally take to amplify a single droplet. In a three-track PCR configuration, this means that six droplet can be amplified simultaneously instead of three.

Detection Zones

[0159] The biochips of the present invention rely on the use of electrodes and electrochemical labels for the detection of target analytes. Generally, the electrode surface (optionally coated with a self-assembled monolayer (SAM), as outlined below) has capture ligands which bind the target. A second label ligand, which also binds to the target, is included, such that in the presence of the target, the label ligand is bound near the surface of the electrode, and can be detected electronically.

[0160] Thus, the detection zone of the bottom substrate comprises one or more separate arrays of detection electrodes. By "electrode" herein is meant a composition, which, when connected to an electronic device, is able to sense a