

etc.) for example or in one case with the housing on one side removed (as described herein, in some cases the housing does not completely cover the components, for example being only on the sides and the top (this finds particular utility for the heating elements of the bottom bay, to reduce the amount of layers between the heater and the PCB board).

[0092] FIGS. 27A, 27B and 27C depict an additional schematic of the systems of the invention, with an integral barcode scanner and two biochips. FIG. 27A shows the two instrument bays and the base station with user interface.

[0093] FIG. 28 depicts another view of one embodiment of some of the thermal and electrical connections between the bay and the chip. The cartridge has electrode pads that connect with pogo pins in the bay (see FIG. 29). The three thermal zones are shown as well as the thermal zone for uniform control of the detection zone, depicted herein as the Peltier zone; the resistive heating elements and the actual Peltier are contained in the bottom bay as shown in FIG. 29. One embodiment for a lock in mechanism for the insertion of the cartridge is also depicted.

[0094] FIG. 29 shows an embodiment of the electrical and thermal connections of the bottom bay. There are pogo pin connectors for the PCR amplification zone heaters, pogo pin connectors for the optional heating of the sample zone, pogo pin connectors for the Peltier heater of the detection zone (again, detection is optionally and preferably done at a uniform temperature), and the edge connector pogo pins to connect the electrowetting grid electrodes and the detection electrodes.

[0095] FIG. 30 depicts a side view of one embodiment of a biochip of the invention; in this case the LRM does not completely cover the top plate.

[0096] FIG. 31 depicts another schematic of an embodiment of the apparatus of the invention.

[0097] FIGS. 32A, 32B, 32C, and 32D depict several suitable latching mechanisms for locking the chip into the bay. FIGS. 32A, 32B, 32C, and 32D show two views of an embodiment comprising alignment pin holes in a biochip cartridge to “lock in” the electrode/pogo connectors.

[0098] FIG. 33 depicts an overview of the operation steps for an exemplary assay run on the system of the invention.

[0099] FIG. 34 depicts a schematic a three pathway amplification zone for use in “tandem amplification” as described below.

[0100] FIG. 35 shows a schematic of one embodiment of the PCB, the top plate, and the two mated together. FIG. 35 shows that the top plate has ridges such that the chamber height is different at different locations on the substrate.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

[0101] One major challenge in the area of clinical and molecular diagnostics is the ability to have a “sample to answer” system that allows minimal sample handling and preparation as well as no requirement for trained clinical lab personnel. While many systems have been proposed, to date there are virtually no such commercial systems. The present invention provides such an integrated, multiplex system. One of the significant benefits of the present system is that in many embodiments, the chip itself needs no moving parts, such as valves or pumps, due to the unique transport properties of the electrowetting system described below.

[0102] The present invention provides molecular diagnostic methods and compositions based on the detection of target analytes, including nucleic acids. The systems described herein are complete integrated “sample to answer” systems, in contrast with current commercial systems that require some off chip handling of the sample, generally including sample extraction (cell lysis, for example), and sample preparation prior to detection. Thus, in the current system, a patient sample is loaded onto the cartridges of the invention and the target analyte sample is extracted, amplified as necessary (for example, when the target analyte is a nucleic acid using polymerase chain reaction (PCR) techniques, although isothermal amplification methods can be utilized as well), and then detected using electrochemical detection, all on a microfluidic platform, generally referred to herein as an “integrated biochip cartridge”, “biochip” or “cartridge”.

[0103] In general, the system relies on two components: the cartridge, into which the sample is loaded and processed, and the apparatus into which the cartridge is inserted to result in the sample processing and final detection of the target analytes and the generation of a report to such.

[0104] The basic microfluidic platform used herein is based on systems developed by Advanced Liquid Logic (ALL, currently a subsidiary of Illumina, Inc.), as more fully described below. In general, these technologies rely on the formation of microdroplets and the ability to independently transport, merge, mix and/or process the droplets, using electrical control of surface tension (i.e., electrowetting). In general, liquid samples are contained within a microfluidic device between two parallel plates. One plate contains etched drive electrodes on its surface while the other plate contains either etched electrodes or a single, continuous plane electrode that is grounded or set to a reference potential (“bipolar electrowetting”). Hydrophobic insulation covers the electrodes and an electric field is generated between electrodes on opposing plates. This electric field creates a surface-tension gradient that causes a droplet overlapping the energized electrode to move towards that electrode. In some embodiments, the active electrowetting electrodes may be adjacent and on the same plane as the neighboring ground reference electrode, which is referred to as “coplanar electrowetting”). Through proper arrangement and control of the electrodes, a droplet can be transported by successively transferring it between adjacent electrodes. The patterned electrodes can be arranged in a two dimensional array so as to allow transport of a droplet to any location covered by that array. The space surrounding the droplets may be filled with a gas such as air or an immiscible fluid such as oil, with immiscible oils being preferred in many embodiments of the present invention.

[0105] As the droplets containing the target analytes move across the surface, they can pick up reagents and buffers. For example, when dried reagents are placed on the bottom substrate (generally described herein as printed circuit board, although as will be appreciated by those in the art, additional substrates can be used), a droplet moving through that zone will pick up and dissolve the reagent for use in a biological process such as PCR amplification. In addition, as more fully described below, addition from the liquid reagent module (“LRM”), positioned above the substrate, allows for specific addition of buffers and other reagents such as wash buffers, etc. to drops captured at specific locations.

[0106] One of the significant benefits of the present system is that in many embodiments, the chip itself needs no moving