

nel by an elastomeric membrane that can be deflected into or retracted from the flow channel in response to an actuation force. The term "valve" refers to a configuration in which a flow channel and a control channel intersect and are separated by an elastomeric membrane that can be deflected into or retracted from the flow channel in response to an actuation force. An "isolated reaction site" or "reaction chamber" refers to a reaction site that is not in fluid communication with other reactions sites present on the device, and which is created by the actuation of control channels in the device. A "via" refers to a channel formed in an elastomeric device to provide fluid access between an external port of the device and one or more flow channels. Thus, a via can serve as a sample input or output, for example. "Guard channels" may be included in elastomeric microfluidic devices for use in heating applications to minimize evaporation of sample from the reaction sites. Guard channels are channels formed within the elastomeric device through which water can be flowed, to increase the water vapor pressure within the elastomeric material from which the device is formed, thereby reducing evaporation of sample from the reaction sites. The guard channels are similar to the control channels in that typically they are formed in a layer of elastomer that overlays the flow channels and/or reaction site. Typically, the guard channels are placed adjacent and over flow channels and reaction sites as these are the primary locations at which evaporation is the primary concern. Guard channels are typically formed in the elastomer utilizing the MSL techniques and/or sacrificial-layer encapsulation methods cited above. The solution flowed through the guard channel includes any substance that can reduce evaporation of water.

[0034] The devices incorporate flow channels, control channels and valves to isolate selectively a reaction site at which reagents are allowed to react. FIGS. 1A and 1B depict an exemplary design of a partitioning microfluidic device **01** in a valve off and valve actuated state. Referring to the figure, a sample is injected into inlet **02** which is in communication with branched partitioning channel system **03** of the device. Solution flow through flow channels of the device is controlled, at least in part, with one or more control channels that are separated from the flow channel by an elastomeric membrane or segment. This membrane or segment can be deflected into or retracted from the flow channel with which a control channel is associated by applying an actuation force to the control channels so that solution flow can be entirely blocked by valves. Actuating the control valves creates isolated reaction chambers **05** in which individual reactions can be conducted. The reaction chambers can number from 10^3 to 10^5 or more be at a density of at least 100 sites/cm² and can range up to at least 2000, 3000, 4000 or more than 4000 sites/cm². Very small wells or cavities can be formed within an elastomeric material to increase the volume of the reaction chamber. Valves can be actuated by injecting gases (e.g., air, nitrogen, and argon), liquids (e.g., water, silicon oils and other oils), solutions containing salts and/or polymers (including but not limited to polyethylene glycol, glycerol and carbohydrates), and the like into the port.

[0035] Although FIG. 1 illustrates a MPD with branched flow channels, any channel configuration (flow channel path) that can be partitioned by control channels can be used in accordance with the invention, including, for example, square, spiral or serpentine configurations.

[0036] The dimensions of flow channels in a MPD can vary widely. Typically channels are from about 0.1 μm to about

1000 μm in any dimension, sometimes from about 0.1 to about 100 μm , and sometimes from about 0.1 to about 10 μm . In one embodiment the channels have a high aspect ratio (e.g., a height to width ratio of from about 2:1 to about 10:1) to increase channel density and/or to increase signal collection from channels containing a detectably labeled moiety. For example, in some embodiments the channel has a columnar shape in which the dimensions of floor and ceiling are smaller than the dimensions of the walls, and a signal (e.g., fluorescence, infra red or visible radiation) is detected through the ceiling or floor. Appropriate channel dimensions will depend in part on the nature of the entities being partitioned. For partition of eukaryotic cells, for example, a dimension should be at least sufficient for passage of the cell (e.g., 2-5 times the dimension of the cell). However, for the purpose of restricting movement the dimensions can be on the order of 0.75 times the smallest dimension of the particle. Microfluidic manipulation and analysis of particles is also described in U.S. Patent Pub. 20040229349 entitled "Microfluidic particle-analysis systems" and incorporated herein by reference.

[0037] Reactions (e.g., nucleic acid amplification, protein binding, etc.) are allowed to occur in each chamber. For example, PCR reactions can be initiated by heating the chambers (e.g., placing the device on a suitably programmed flat plate thermocycler).

[0038] The results or products of the reaction can be detected using any of a number of different detection strategies. Because the MPDs are usually made of elastomeric materials that are relatively optically transparent, reactions can be readily monitored using a variety of different detection systems at essentially any location on the microfluidic device. Most typically, however, detection occurs at the reaction site itself.

[0039] The nature of the signal to be detected will, of course, determine, to a large extent, the type of detector to be used. The detectors can be designed to detect a number of different signal types including, but not limited to, signals from radioisotopes, fluorophores, chromophores, electron dense particles, magnetic particles, spin labels, molecules that emit chemiluminescence, electrochemically active molecules, enzymes, cofactors, enzymes linked to nucleic acid probes and enzyme substrates. Illustrative detection methodologies suitable for use with the present microfluidic devices include, but are not limited to, light scattering, multichannel fluorescence detection, infra-red, UV and visible wavelength absorption, luminescence, differential reflectivity, and confocal laser scanning. Additional detection methods that can be used in certain application include scintillation proximity assay techniques, radiochemical detection, fluorescence polarization, fluorescence correlation spectroscopy (FCS), time-resolved energy transfer (TRET), fluorescence resonance energy transfer (FRET) and variations such as bioluminescence resonance energy transfer (BRET). Additional detection options include electrical resistance, resistivity, impedance, and voltage sensing.

[0040] A detector can include a light source for stimulating a reporter that generates a detectable signal. The type of light source utilized depends in part on the nature of the reporter being activated. Suitable light sources include, but are not limited to, lasers, laser diodes and high intensity lamps. If a laser is utilized, the laser can be utilized to scan across a set of detection sections or a single detection section. Laser diodes can be microfabricated into the microfluidic device itself. Alternatively, laser diodes can be fabricated into another