

ecules within the column or channel and prevents the draining of any portion of the fluidic system.

DESCRIPTION OF THE FIGURES

[0010] FIG. 1 shows a diagrammatic representation of channel-to-droplet extraction achieved by integrating liquid-column based separation and Digital Microfluidics to enable transport of concentrated sample droplets to secondary evaluation sites.

[0011] FIG. 2 is a diagrammatic representation of A) channel pull-back which resists droplet extraction and B) use of a refill droplet which mitigates channel pull-back force and replaces fluid lost via extraction to prevent drainage of channel and subsequent mixing and dilution of concentrated solute bands.

[0012] FIG. 3 is Droplet extraction test chips (3 figs)

[0013] FIG. 4 shows a series of photomicrographs demonstrating the stages of droplet extraction using a test EWOD chip.

DETAILED DESCRIPTION OF THE INVENTION

[0014] The following description is provided to enable any person skilled in the art to make and use the invention and sets forth the best modes contemplated by the inventor of carrying out his invention. Various modifications, however, will remain readily apparent to those skilled in the art, since the general principles of the present invention have been defined herein specifically to provide a method of extracting a single droplet from a separation column or channel in a microfluidic device.

[0015] The present invention solves this problem by integrating liquid column-based transport and separation functions with Digital Microfluidic droplet handling to extract a mobile droplet that has been enriched with column-concentrated solute molecules. Extraction of a solute-rich droplet enables additional follow-on operations to be applied to the enriched sample volume, such as further in-droplet sample purifications (see e.g., "Particle Separation and Concentration Control for Digital Microfluidic Systems," Sung Kwon Cho and Chang-Jin "C J" Kim, *IEEE Conf. MEMS*, Kyoto, Japan, January 2003, pp. 686-689) and/or droplet transfer to a secondary analysis or imaging site without dilution of the concentrated solute molecules.

[0016] FIG. 1 shows the inventive process diagrammatically. FIG. 1A is a diagram of a separation column or channel shown as a tube or capillary 20 with a physical opening 22 in its side wall serving as an extraction point. In addition, fluid columns can also be bounded without physical walls, using instead surface property differences and effects to constrain and direct the column of fluid. In such a situation the "opening" would not be an opening in a physical wall and extraction could take place at any point along the column. The walled liquid column 20 in the figure is shown as containing three different populations of solute molecules or particles 24, 26 and 28. The middle population 26 is located adjacent the opening 22. In FIG. 1B this population 24 is withdrawn or extracted as a separate droplet 30. Once a droplet is extracted it can be transported along a fluidic pathway 32 to secondary evaluation sites. As shown diagrammatically in FIG. 1C, the separated droplet can also be subjected to a number of further manipulations. In the left panel of FIG. 1C the droplet 28 contains a central area of molecules 26. Surrounding these molecules are molecules originally found in population 28.

The droplet is subjected to additional forces so to cause additional within droplet separations of particles or solutes—for example according to particle charges with negative charges becoming located at one end of the droplet and positive charges at the other as shown in the right panel of FIG. 1C (the arrow represents the application of an electrical field). Once there has been an end to end separation of particles or solutes, it is then possible to split the droplet 30 (FIG. 1D) into separate droplets 30' and 30" by means of applied forces (two ended arrow) so that the separated particles or solutes can be separately analyzed.

[0017] According to the present invention droplet extraction from a liquid column for on-chip microfluidics can be achieved without mechanical structures (e.g., valves or pumps) or pneumatic effects (e.g., driving the liquid by gas pressure) by using surface-borne effects such as surface acoustic waves (SAW) and control of surface wettability via electrical, optical or chemical means. A specific example of the use of surface-borne effects is droplet extraction by means of electrowetting based Digital Microfluidics.

[0018] Surface effect-based extraction of droplets can advantageously be applied to any microfluidic function that serves to transport, focus, concentrate, or separate target molecules within a column of fluid (e.g., a continuous linear fluid volume) contained within a column. Examples of such microfluidic techniques include Capillary Electrophoresis (CE), Dielectrophoretic (DEP), Liquid Chromatography, High Performance Liquid Chromatography, and capture and release mechanisms such as immuno-magnetic-separation (IMS) using beads as well as electrophoretic capture of proteins and nucleic acids. In some of these techniques, the fluid column flows resulting in separation of solutes which move more slowly (or even become immobile) relative to the flow whereas with other techniques the fluid column is relatively stationary with the solutes moving relative to the fluid to effect separation.

[0019] Droplet extraction can be performed anywhere along a non-walled liquid column or as shown in FIG. 2A at any wall opening in between the ends of a wall bounded liquid channel. As shown in FIG. 2A, a liquid column that is bounded by physical walls 20 will typically provide greater resistance to extraction of a droplet 30 in the form of a pull-back force (indicated by the arrows in the column 20) exerted by the intermolecular attraction or cohesion within the fluidic volume at the extraction site. If this force is not mitigated, the fluid column within the channel will be disrupted and sample molecules or particles may become mixed or otherwise disturbed and/or droplet extraction may actually be prevented. Several methods can be employed to reduce this channel pull-back force, including the merging or addition of a refill droplet 34 to the extraction site 22 as shown in FIG. 2B. The refill droplet 34 mitigates the pull-back force exerted by the column by replenishing the fluid volume lost through droplet extraction. This replenishing prevents drainage of adjacent fluids within the channel, thereby preventing the mixing and dilution of any solute bands at or near the extraction point.

[0020] Other methods may be used to mitigate channel pull back force and to replace fluid volume lost through the droplet extraction. These methods can also be used on non-walled liquid columns as the pull-back force to be mitigated in such structures will be less than with channel-bounded liquid columns.

[0021] Once a droplet is extracted, the sample within the droplet can be subjected to further separation and bifurcation