selected dilution ratios. In certain testing applications, this may allow a relevant dynamic range of dilution between saturation and no response/background to be quickly identified so that quantitative results beyond simple positive/negative testing are achievable. This method also offers significant time savings over manual dilution techniques.

[0099] The level of dilution in each step of the method of gradient generation of the present invention may be controlled. For example, the amount of dilution may be controlled by controlling the relative amounts of the fluid and dilutant introduced into each fluid path. Where more dilution is desired, relatively greater amounts of dilutant may be added to each fluid path and, where less dilution is desired, relatively lesser amounts of dilutant may be added to each fluid path. In certain embodiments, it is desired to decrease the concentration of the fluid (or materials therein) by 50%. In such embodiments, the amount of fluid and dilutant added to each fluid path may be evenly matched. In other embodiments, 10%, 25%, 75%, or 90% reductions in concentration may be desired.

[0100] The amount of fluid and dilutant added to each fluid path may be controlled in any manner that creates the desired level of dilution as accurately and precisely as required. Various factors may influence the flow rate of fluid and dilutant, and thus, the relative amount of each that is supplied. For example, the feed pressure, the viscosity, the flow conditions, and other factors may all be related to the flow rate of the fluid and the dilutant. Where dilution is to take place in a microfluidic system, the flow conditions in such systems may be taken in to account. For example such systems typically are characterized by highly laminar flow conditions. Where desired, inlet pressure may be controlled, for example, by a pumping system or gravity feed. Flow conditions within the fluid paths may be controlled by controlling the physical configuration, such as size and shape, of the fluid paths. Where it is desired to feed even amounts of fluid and dilutant to fluid paths, the fluid paths from the inlets to the point at which the fluids meet may be as substantially evenly matched as is practical in a given embodiment.

[0101] Where it is desired to have even mixing of the fluid and dilutant, a mixing region downstream of the meeting place of the fluid may be desired. A mixing region may be particularly desirable where the fluids are under laminar flow conditions, such as in common in microfluidic systems, and would otherwise mix only by diffusion. In other embodiments, mixing by diffusion may be acceptable, and the length of the fluid path may be selected to allow more or less diffusion. Methods of mixing by diffusion are provided in International Patent Application PCT WO02/22264 and in U.S. patent application Ser. No. 09/954,710, titled "Method and Apparatus for Gradient Generation," and filed Sep. 18, 2001, both of which are hereby incorporated by reference herein in their entirety. Mixing regions will typically include a mixer able to create flow conditions, such as turbulent flow, which promote mixing. Mixers may be active or static. Static mixers are generally preferred as no means of energizing them are required and there are no moving parts to wear out or breakdown. One example of a static mixer suitable for use in a fluidic system of the present invention, and, in particular, a microfluidic system of the present invention is a chaotic advective mixer (CAM)—a microscale surface structure that mixes small volumes of liquids relatively efficiently. CAMs are described in PCT Application No. US 02/23462 filed Jul. 24, 2002 and titled "Laminar Mixing Apparatus and Methods", which is hereby incorporated by reference herein in its entirety.

[0102] One embodiment of an apparatus for gradient generation is illustrated in **FIGS. 17 and 20**. In the illustrated embodiment, a gradient generation apparatus includes (from left to right) first, second, and third fluid paths **204** each having a cross-section of less than one millimeter. Second and third fluid paths **204** each comprising a mixing region **230**. A first inlet **232** is fluidly connected to first and second fluid paths **204** and a second inlet is fluidly connected to the second and third fluid paths **204**. A first connecting path **240** is fluidly connected to second fluid path **204** downstream of its mixing region **230** and fluidly connected to third fluid path **204** upstream of its mixing region **230**. Fluid paths and inlets in this embodiment of the present invention may be constructed as described above for other embodiments of the invention. The embodiment illustrated in **FIGS. 17 and 20** has been constructed such that the fluid will be diluted 50% at each mixing stage be supplying equal amounts of fluid and dilutant to each fluid path where mixing takes place.

[0103] Fluid paths **204** may supply fluid to any location where the gradient generator is to b used. **FIG. 17** illustrates one possible use for the gradient generation apparatus of the present invention, where the gradient generation apparatus provides fluid to a microfluidic system comprising interaction material **200** patterned onto a substrate, as described previously herein. It should be appreciated that other microfluidic systems, including other embodiments of the present invention using crossing fluid paths may also be fed fluid from the gradient generation apparatus of the present invention.

[0104] Other uses and configurations of the present invention are envisioned. For example, the convection controller might contain multiple membranes separated by a series of spaces. If a flow gradient were created and if the membranes had smaller pores with each successive membrane in the direction of the gradient, materials could be separated by size. Such a system might be used to separate proteins where very small samples are involved.

[0105] As an alternate example, a system according to the present invention may allow layers of material containing fluid proteins to be removed or detached from layers of material containing one or more convection controllers.

[0106] In one aspect, the invention provides a platform for performing chemical or biological titrations, or otherwise combining different fluids together in various ratios. Chemical or biochemical reactions within fluids or between fluids may be evaluated, monitored or measured. Titrations typically involve reacting a reagent (titrant) with an analyte (sample). The titration reaches an end point when all, or substantially all, of the analyte has reacted with reagent. Traditionally, when performing a titration, the state of the reaction is monitored while reagent is added and addition of reagent is stopped when an end point is reached. Titrations may be automated. In continuous flow titrations, the amount of reagent added to a stream of analyte is increased over time so that the ratio of reagent to analyte exposed to a detector at the end of the stream increases over time. Where "reagent, ""titrant," or "sample" or "analyte" is used in describing the embodiment illustrated in **FIGS. 27 and 28**, it is to be