

for the generation of an optimized magnetic field, through the careful tuning of the key parameters such as magnetic material, geometry, configuration, and initial magnetization.

[0089] The use of external magnets to hold magnetically labeled components at a designated position in a microfluidic device has been suggested previously in a number of patents, for example in U.S. Pat. Nos. 5,916,776, 5,939,291, and 6,193,892. Briefly, a magnetic field is generated by an external magnet, which will allow the immobilization of a material that is labeled with a magnetic label. In the present invention, the magnetic beads in the wall or the chamber of the microchannel will produce a local high magnetic field gradient upon the application of the external magnetic field. The magnetic gradient produced by the magnetic beads will be 1 to 4 orders of magnitude greater than would be produced by the external magnet alone.

[0090] In a preferred embodiment, the microfluidic device comprises a magnetic labeling chamber for labeling the target analyte or any other component of the sample with magnetic labels. By "magnetic label" herein is meant magnetic particles conjugated with binding ligands to which the target analyte or other components of the sample can bind. In this embodiment, the reagent for the labeling reaction may contain the necessary reagents, or they may be stored in a storage module and pumped as needed. As will be appreciated by those skilled in the art, the labeling reaction described therein can also be carried out in a separate device.

[0091] By "magnetic particles" herein is meant magnetically susceptible particles that are small enough so that they can be manipulated in a microfluidic device. In a preferred embodiment, the labels are of any suitable shape, including rods and beads, and most preferably spherical beads. The labels have a preferred diameter of from about 0.01 μm to about 25 μm , more preferably, from about 0.05 μm to about 0.8 μm , yet more preferably from about 0.05 μm to about 0.2 μm .

[0092] In a preferred embodiment, the labels are ferromagnetic, paramagnetic, superparamagnetic, or made of any other material so that they can be seized or manipulated by a magnetic field within the magnetic microchannel. The material is preferably resistant to chemicals commonly used in manipulations of biological samples.

[0093] In a preferred embodiment, the magnetic particles are paramagnetic. "Paramagnetic" materials are characterized by containing unpaired electrons which are not coupled to each other through an organized matrix. They have only a weak magnetic susceptibility and when the field is removed quickly lose their weak magnetism. A paramagnetic particle can be comprised of, for example, iron dispersed in a polymer, and can be obtained, for example, from Miltenyi Biotec (Bergisch Gladbach, Germany or Immunicon (Huntingdon Valley, Pa.).

[0094] More preferably, the magnetic particles are superparamagnetic as sold by Dynal (Oslo, Norway) and other commercial manufacturers. Superparamagnetism occurs in ferromagnetic materials when the crystal diameter is decreased to less than a critical value. Superparamagnetic materials are highly magnetically susceptible-i.e., they become strongly magnetic when placed in a magnetic field, but, like paramagnetic materials, rapidly lose their magnetism. Whereas the paramagnetic particles exhibit some reso-

nance and hysteresis, and therefore tend to clump together after exposure to a magnetic field ceases, superparamagnetic particles completely demagnetize when the field is removed, thus allowing the superparamagnetic particles to be redispersed without clumping after removal of the magnetic field.

[0095] Although the above-mentioned definitions are used for convenience, there is a continuum of properties between paramagnetic, superparamagnetic, and ferromagnetic, depending on crystal size and particle composition. Thus, these terms are used only for convenience, and "superparamagnetic" is intended to include a range of magnetic properties between the two designated extremes.

[0096] In a preferred embodiment, the magnetic particles are coated so that they can be conjugated to binding ligands that will enable them to capture the target analytes. Methods of conjugating a binding ligand to the magnetic particle are fully disclosed in U.S. Pat. Nos. 5,512,439 and 5,705,059, incorporated herein by reference. For conjugation purposes, a particularly preferred coating comprises of polymers or polysaccharide that either contain a functional group or are suitably derivatized to provide a functional group such as hydroxyl, carboxyl, sulfhydryl, aldehyde or amino groups. Such functional groups function to conjugate the coated particles to a specific binding ligand. A variety of suitable coatings are known to the art. For example, polyurethane together with a polyglycerol provides hydroxyl groups, a cellulose derivative provides a hydroxyl group, a polymer or copolymer of acrylic acid or methacrylic acid provide carboxyl groups, an aminoalkylated polymer provides amino groups. A variety of such modifications is known in the art. For example, polysaccharide can be conveniently oxidized using periodate to provide aldehyde functional groups which can then be conjugated to amino substituents on a proteinaceous binding ligand, or can be reacted with CNBr to provide this functionality.

[0097] By "binding ligands" or grammatical equivalents herein is meant a compound that can directly or indirectly bind to a component of the sample, which can either be a target analyte, or other analytes. In a preferred embodiment, the binding of the analytes to the binding ligand is specific, and the binding ligand is part of a binding pair. By "specifically bind" herein is meant that the ligand binds the component, for example the target analyte, with specificity sufficient to differentiate between the analyte and other components or contaminants of the test sample. The binding should be sufficient to remain bound under the conditions of the processing or treatment, including wash steps to remove non-specific binding. In some embodiments, the disassociation constants of the analyte to the binding ligand will be less than about 10^{-4} - 10^{-6} M^{-1} , with less than about 10^{-5} to 10^{-9} M^{-1} being preferred and less than about 10^{-7} - 10^{-9} M^{-1} being particularly preferred.

[0098] As will be appreciated by those in the art, the composition of the binding ligand will depend on the composition of the analyte to be labeled. Binding ligands to a wide variety of analytes are known or can be readily found using known techniques. As will be appreciated by those in the art, any two molecules that will associate, preferably specifically, may be used, either as the analyte or the binding ligand. Suitable analyte/binding ligand pairs include, but are not limited to, antibodies/antigens, receptors/ligand, proteins/nucleic acids; nucleic acids/nucleic acids, enzymes/