

localized field are substantially equal in terms of geometry and composition. Thus the trapping energy E may be calculated from the following equations:

$$F=M(H_x/z)=(\chi_m)(V)(H_x)(H/z)=(\chi_m)(V)/z(H_x^2/2)$$

$$E=Fdz=(\chi_m)(V)(H_x^2/2) \quad (\text{Eq. 2})$$

[0126] where H_x =magnetic field strength in the x-dimension, V =magnetic particle volume, χ_m =magnetic particle volumetric susceptibility, F is the force on the bead, and E is the trapping energy.

[0127] As an example, in certain embodiments of the invention M-280 Dynabead streptavidin beads (DynaL Biotech, Inc., 5 Delaware Drive, Lake Success, N.Y., 11042) are used. These beads have a 2.8 μm diameter and a volume V of $1.15 \times 10^{-11} \text{ cm}^3$. The manufacturer lists the volumetric susceptibility χ_m as 0.012 (cgs units). At a field strength of $H_x=1000$ Gauss (e.g., for rectangular cobalt islands with a width of approximately 3 μm separated by a gap of approximately 3 μm in length) the trapping energy is approximately $7 \times 10^{-8} \text{ ergs}=40,000 \text{ eV}$.

[0128] The trapping energy may be compared with the thermal energy of the beads. Thermal energy E_{th} is given by the following equation:

$$E_{\text{th}}=kT \quad (\text{Eq. 3})$$

[0129] where k is the Boltzmann constant and T is the temperature in degrees Kelvin. Taking $k=1.38 \times 10^{-23} \text{ Joules/}^\circ\text{K}$ and $T=300^\circ \text{ K}$ (approximately room temperature), the thermal energy is calculated as approximately $4.1 \times 10^{-21} \text{ Joules}=0.025 \text{ eV}$. Thus it is evident that the trapping energy produced by the localized magnetic fields on the magnetic biochip of the invention is several orders of magnitude greater than the thermal energy. According to certain embodiments of the invention described herein, the trapping energy is approximately a million times greater than the thermal energy. The particles are thus firmly trapped relative to thermal fluctuations. In addition, reactions such as hybridization, PCR amplification, or other reactions that may be performed at temperatures above room temperature will still result in a thermal energy several orders of magnitude lower than the trapping energy. Of course the initial process of the particle finding its way to the area of the localized magnetic field between magnetic regions is influenced by both sample flow and diffusion into that region.

[0130] The trapping energy may also be used to estimate the likelihood that a magnetic particle will escape once trapped. The probability of escape is proportional to $e^{(-E/E_{\text{th}})}$ where E =trapping energy and E_{th} =thermal energy. Thus when the trapping energy is 5 times the thermal energy, the likelihood of escape is approximately 1%; when the trapping energy is 4 times the thermal energy, the likelihood of escape is approximately 2%; when the trapping energy is 3 times the thermal energy, the likelihood of escape is approximately 5%. The trapping energy decreases linearly with volume of the magnetic particle. Thus for nanoparticle (e.g., a spherical particle of approximately 30 nm diameter), the trapping energy is still greater than the thermal energy.

[0131] The preceding calculations suggest that even magnetic trapping fields far weaker than those generated by the magnetic islands described above would still be sufficient to strongly trap and retain magnetic particles. Similar calculations can be performed using different magnetic particle parameters and chip dimensions and designs. These calcu-

lations indicate that the concept of using localized magnetic fields to strongly yet reversibly trap magnetic particles is highly generalizable and may be implemented using a wide variety of designs and materials.

[0132] The trapping energy is also relevant in terms of procedures for removing the magnetic particles from the substrate. The kinetic energy E_k of the bead may be computed as follows:

$$E_k=\frac{1}{2}(m)v^2 \quad (\text{Eq. 4})$$

[0133] where m =mass of magnetic particle and v =velocity of magnetic particle. Thus for a particle of $m=1.5 \times 10^{-11} \text{ g}$ (the approximate mass of an M-280 Dynabead) the trapping energy of the particle is equal to its kinetic energy when moving at approximately 1 m/sec. Thus if a particle were in a fluid flow at approximately 1 m/sec or greater, it would overcome the trapping energy. A corollary to this is that once trapped, a sufficiently fast fluid flow is enough to decouple the particles from their attachment sites and hence prepare the chip for reuse. For example, a fast fluid flow perpendicular to the length of the gap (i.e., a fluid flow in the y-direction on FIG. 3) and in the plane of the substrate may be used to effectively remove magnetic beads from the chip. Much slower fluid flows (e.g., on the order of less than one to several cm/sec) are used to initially assemble the array of beads on the chip and to remove excess unbound beads when the arraying is complete.

[0134] E. Extensions

[0135] (1) Microfluidics

[0136] In certain embodiments of the invention a microfluidic assembly is integrated with the magnetic chip for ease of sample introduction and removal. The microfluidic assembly may be made of glass, quartz, polymers such as plastics, or any other suitable material. The microfluidic assembly includes a plurality of channels. The channels may be of any appropriate width, e.g., between 0.1 μm and 500 μm , though for some applications somewhat greater widths (e.g., in the mm range) are desirable. For many applications channel widths of between 5 and 50 μm are useful. Channel depth may fall within similar ranges. Selection of appropriate dimensions for channels may depend on the dimensions of the chip and beads to be used. The microfluidic assembly may also contain features such as wells (e.g., for holding samples, solutions, reagents), sample inlet and outlet ports, fluid valves, mixing chambers, etc.

[0137] According to certain embodiments of the invention, each array on the chip is addressed by two crossed channels. A solution containing magnetic particles is introduced with a gentle flow, e.g., via the channel that is oriented along the long axis of the magnetic islands although the other channel may also be used. The sample may be moved back and forth over the array to enhance trapping of the magnetic particles. After several seconds to minutes, a buffer flow is introduced to clear out untrapped, excess bead particles. If hybridization is to be performed on-chip, then beads with attached probes are introduced first in the above manner and then, after clearing out excess beads, the sample containing probe is introduced similarly and given time to hybridize. The hybridization process may take several hours or longer and may be performed at elevated temperature (e.g., 45 C. or higher to enhance hybridization specificity). The channels may also be used to introduce and remove