

statistics, which can be used to predict the likelihood that a domain will be occupied by 0, 1, or more beads. One of ordinary skill in the art will be able to select an appropriate number of beads to dispense. As discussed above, the trapping of beads on the chip can be optimized by appropriate selection of chip geometry and size of the magnetic domains. For example, too small a gap between magnetic domains will prevent bead trapping in the center of a gap while too large a gap instead allows trapping of multiple beads on the edges of the magnetic domains. This effect is demonstrated in Example 2.

[0158] Once the beads have been dispensed on the chip, they can be trapped by the localized magnetic fields created by the magnetic domains. This process may take from seconds to minutes. Trapping may be aided by gently moving or agitating the chip to allow an even dispersal of beads across the chip. A low surface tension liquid medium may be used to facilitate dispersal of the beads. A surfactant (e.g., a detergent such as SDS or Tween®) may be included in the bead solution to help in spreading the beads over the chip by reducing the hydrophobic interactions of the beads with the chip surface and the surface tension interactions with the drop surface. For example, diluting beads in 1×TE (Tris-EDTA) with 0.1% SDS maybe appropriate. Concentrations of SDS tenfold higher or lower may also be used. However, when reactions (e.g., hybridization or enzymatic reactions) are performed prior to introducing the beads to the chip surface, care must be taken to ensure that the detergent concentrations do not interfere with such reactions. When reactions are to be performed on-chip (i.e., after bead trapping), the chip can be washed sufficiently to remove detergents prior to introduction of sample, reagents, etc.

[0159] As described in Examples 2 and 3000000, the arraying behavior of the beads may be examined experimentally, e.g., by using fluorescently labeled beads and obtaining a laser fluorescence scan of the chip surface after allowing the beads to attach. Alternatively, an optical microscope can be used (e.g., with unlabeled beads) to observe their arraying behavior. Laser scanning may be preferable, however, because it readily allows quantification of signal to noise ratio.

[0160] Once the beads have been captured the remaining solution (containing uncaptured or weakly attracted beads that may cluster at the edges of an occupied gap region) can be removed, e.g., using a gentle fluid flow. The beads and/or associated probes or targets can then be detected as described below. Alternately, samples or other reagents may be introduced to the chip and reactions or assays performed prior to detection.

[0161] B. Disassembling an Array of Magnetic Particles

[0162] After detection is complete, magnetic particles can be removed from the surface of the chip, e.g., by applying a rapid fluid flow over the chip sufficient to overcome the trapping energy. (Trapping energy for magnetic beads is discussed above.) For example, a fluid flow of approximately 1 m/sec is sufficient to overcome the trapping energy of 2.8  $\mu\text{m}$  M-280 Dynabeads. The fluid flow for removing the beads can be applied according to any of the procedures used for introducing the beads to the chip surface.

[0163] Another approach is to remove beads by applying an alternating magnetic field (e.g., with a small electromag-

net and AC current) while flowing a solution such as wash buffer over the chip. The average magnetization would then be zero, and particles could therefore be removed by a gentle fluid flow (e.g., in the cm/sec range). The magnetic regions would then be remagnetized, e.g., using DC current. However, since this approach would require chip magnetization/demagnetization between runs it may be less convenient than simply using fluid to remove the beads.

[0164] V. Encoding and Decoding

[0165] For arrays in which probe is bound to substrate (e.g., conventional oligonucleotide arrays), the identity of each probe is positionally encoded, i.e., the identity of a probe may be ascertained based on the position of the probe on the substrate. This is not the case, however, for random order arrays such as those of the invention. Therefore, in many situations (e.g., most situations involving multiple different probes) a method for determining the identity of the probe and/or target is needed. In some instances determining the identity of a probe or target can be performed directly (e.g., by sequencing a nucleic acid probe or target). However, typically the identity of the bead and/or probe is encoded prior to performing an assay in order to facilitate subsequent determination of the identity of the probe (decoding).

[0166] Any of a variety of methods well known in the art may be used for encoding and decoding beads, probes, and/or targets. These methods may also, in general, be used in combination, e.g., to increase the number of possible encodings. Encoding typically involves imparting some sort of detectable property to the bead, probe, and/or target to be encoded, wherein the nature or value of the detectable property differs between different populations of beads, probes, and/or targets. The nature or value of the detectable property corresponds to the identity of the bead, probe, and/or target, so that determining or measuring the detectable property provides information as to the identity of the bead, probe, and/or target. The descriptions of encoding and decoding techniques provided herein are intended to be exemplary and are not to be considered as limiting the invention in any way. These methods and others are all well known in the art, and methods not described herein can also be used with the invention. Various encoding and decoding strategies are described in, for example, WO9967641, WO0048000, WO0071995, and WO0075373.

[0167] Typically the purpose of encoding beads or probes is to allow the mixing of populations of beads (where each population of beads bears a different attached probe or probes) prior to performing an assay in which the mixed population of beads is exposed to target. After performing the assay the identity of probes (either all probes or only those that interacted with target) can be determined by decoding. In general, if the probes themselves are encoded the beads need not be encoded (although they may be). When the probes themselves are not encoded (or when it is desired to use a different encoding/decoding scheme from that employed in encoding the probes), the beads may be encoded. The encoding of a bead then serves to identify the attached probe.

[0168] In general, a bead encoding strategy may be implemented in any of at least four different ways (and combinations thereof may also be used). Magnetic beads can be "color-coded" by providing them with one or more optically