

only one of the molecules is freely diffusing in three dimensions, with bead-based approaches both interacting molecules can diffuse in three dimensions. This results in decreased time requirements and/or increased assay sensitivity. Multistep protocols that involve the sequential addition and/or removal of reagents may also be performed more conveniently using beads. Magnetic beads offer a particular advantage in this regard since their magnetic properties make it possible to readily isolate and/or wash the beads. In addition, the bead platform offers greatly increased potential for multiplexing and for redundancy to ensure statistical robustness even with random sampling.

[0061] While the foregoing advantages apply in general to bead-based assays, they may be of particular relevance for assays including hybridization steps, such as genotyping assays, including multiplex genotyping assays. These advantages may be summarized as follows, in the context of a comparison of bead-based and conventional DNA chip arrays (e.g., oligonucleotide arrays in which probe is bound to substrate such as those described in U.S. Ser. No. 6,040,138) for multiplex genotype assays:

[0062] (i) Reduced Hybridization Time

[0063] Tagged sample hybridization to beads in solution will reduce the hybridization time by one to several orders of magnitude. This reduction occurs because the effective concentration of an immobilized probe on the bead surface is much higher than that of a probe on a chip surface. Mobility and diffusion of both the probe and the target greatly enhance the hybridization efficiency.

[0064] (ii) Improved Sensitivity

[0065] Conversely, for the same hybridization time, the sensitivity of bead-based detection is better by orders of magnitude. Efficient hybridization using a bead-based approach has been shown over several hours using 100 fM target (or, 1 attomole of target in 10 μ l) (Ferguson, et al., *Anal. Chem.*, 72:5618-5624, 2000). Over similar periods of time, inventors have shown that conventional DNA arrays (e.g., substrate-bound oligonucleotide arrays available from Affymetrix, Inc.) typically require approximately a 12-14 hour hybridization time using 10 pM target (or, 100 attomoles in 10 μ l) for reasonable target detection. Hence, sensitivity is improved typically by two orders of magnitude. This has the advantage of reducing the level of sample amplification required, keeping it well in the linear regime. One can use less genomic template in the assay. Potentially, PCR steps could be eliminated in favor of other reduced amplification schemes.

[0066] (iii) Improved Signal to Noise Ratio

[0067] The total number of sites available for sample hybridization is similar when comparing one bead to one feature (i.e., one spot of probe) on a typical substrate-bound DNA array. However, randomly ordered bead arrays utilize built-in redundancy which allows 20-50 fold over-sampling, leading to much-improved signal to noise ratios and better accuracy in the data. A 20 μm^2 oligonucleotide (oligo) probe feature on a chip surface typically contains 200,000 full-length oligos (full-length probe densities on oligo synthesized arrays are typically \sim 500 oligos/ μm^2). Functionalized beads (e.g., approximately 3 μm in diameter) can typically bind over 100,000 oligo probes.

[0068] (iv) Flexibility

[0069] Bead arrays can easily be configured to contain from ten to several thousand different probes in real time simply by using different batches of encoded beads. This provides a great deal of flexibility from experiment to experiment. The small size of the arrays (\sim 10,000 features/ mm^2) also lends itself to integration with microfluidics as described below. Finally, the redundancy levels can be adjusted to improve sensitivity or to pack more probes per run.

[0070] (v) Improved Selectivity

[0071] Washing protocols can be more uniform and effective on a solution of beads than performing washes by flowing buffers over a surface. Flow patterns and local washing stringency are typically very hard to keep uniform in the latter.

[0072] The invention represents an efficient, cost-effective, and flexible platform for genotyping assays, among others. Accurate genotyping of 10,000 or more samples can be performed in a single run. Since the arraying and detection processes take only on the order of 10 minutes per run, very high throughput is possible. Genotyping humans for common diseases and disorders and various polymorphisms of significance may require examining thousands of individuals for approximately 500,000 markers. Each run on a chip may examine one individual, however multiplexing schemes can be used to examine multiple different individuals for all these markers at the same time. Since all samples are interrogated simultaneously the cost per sample (not including PCR costs) and the time per run may be reduced by a factor of 100 or more as compared with using singleplex detection methods.

[0073] The following sections provide details of certain embodiments of the invention, from which these advantages will become more evident. As will be clear to one of ordinary skill in the art, a large number of variations may be made.

[0074] II. Chip Design and Manufacture

[0075] A. Design Considerations

[0076] FIG. 1(D) shows a schematic cross-sectional view of one embodiment of the invention. According to this embodiment the chip comprises a substantially flat, non-magnetic substrate 25 with magnetic regions 26 projecting above the surface. In this embodiment of the invention the magnetic regions are referred to as magnetic islands, though this is not intended to limit the invention to magnetic regions that project above the substrate surface. The magnetic islands are arranged in a grid-like pattern, i.e., in mutually perpendicular rows and columns similar to those found in conventional oligonucleotide arrays. Such an arrangement may be convenient for bead detection and data processing using existing image processing tools and software. In addition, a regular arrangement allows for optimization of chip geometries in all regions. However, it will be appreciated that other configurations are possible and are within the scope of the invention.

[0077] As indicated in FIG. 1(D), the magnetic islands are separated by gap regions of sufficient length g to accommodate a bead.