A NEW BIO-MOLECULES DECRYPTION PROTOCOL USING SHAPE ENCODED PARTICLES (SEP)

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ABSTRACT

We had introduced the possibility of using shape-encoded particles (SEP) as DNA carrier (versus physical 2-dimensional array) to physically track the footpath of bio-molecules [1-2]. This proof-of-concept experiment demonstrated SEPs as effective tracers for application such as DNA microarray. Starting with the central concept, however, significant advances had to be made to build the entire new system to demonstrate a user-friendly protocol. In this paper, we report the completion of a new biological analysis *system* using SEPs as the platform. Furthermore, we increased the power of encoding from 2^5 to 2^{20}, increased the particle array density by factor of 4, and developed software for image capture and data interpretation. Completed with substrates design, probes preparation, microassembly, detection, all the way to data analysis, this system creates a large number of distinguishable particles, allows flexibility in experimental design, and enables an elegantly simple tracking mechanism when massive multiplexed assays are desired.

INTRODUCTION

Traditionally, DNA hybridization assays are performed on one-dimensional membranes or microtiter plates to analyze fluorescence or radioactivity. Recently, the advancement of the analysis method has enabled scientists to perform DNA analysis on spatially differentiated on-chip arrays via mechanical printing [3], piezoelectric ink-jetting [4], or by direct synthesis of DNA on the chip [5]. All of the above methods rely on the location of the probe (x-y position) for identification. In most cases, thousands of probes are examined and analyzed simultaneously. Considering the vast amount of data, direct synthesis of DNAs on the chip (such as Affymetrix) can be fabrication intensive as the length of oligos increases. Mechanical contact printing is limited by its serial process (print one or several spots per contact), printing variations from spot to spot and batch to batch, inconsistent spot morphology, misprinting, and slide surface variations, all of which are undesirable for DNA microarray analysis.

An alternate method different from the 2-dimensional array is developed by encoding a specific signature with biological molecules. This approach is pursued both academically [6-10] and commercially [11-14]. Such signature can act both as an identifier and a carrier. It is physically coupled to DNA probes so that multiple samples can be assayed in one run. The results can be later deciphered by decoding the information on the signature. .

We had previously demonstrated the use of shape encoded particles (SEP) as DNA carrier [1-2]. Building on the results reported, we proposed a novel platform for encoding silicon particles based on the shape of the particle with broad applications in biological analysis. This platform is capable of performing multi-parallel-multiplexed assays such as gene expression analysis and genotyping. It utilizes shape-encoded silicon particles as substrates for oligonucleotide attachment as well as a data-tracking system for identification of a corresponding probe. Our motivation is to design a system that can provide a much better solution for current DNA microarray analysis compared to the traditional printing technique. Furthermore, compared to other barcode-type approaches currently reported or available on the market, it provides a much simpler and efficient decoding step since the shape of the particle and the information it carries are scanned and analyzed simultaneously. It can further provide low cost production for large amounts of shape-distinguishable microparticles that can be read and analyzed in multi-parallel, high throughput fashion.

METHODOLOGY

Design

The basic shape of SEP is described in figure 1. The center is the active region where biological events take place, while the barcode region is used for recognition. Utilizing the N notches (drawn as 6 notches in figure 1) to define shape as binary codes, SEPs have 2^N possible shape arrangements. The batch processing capability of

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MEMS further enables us to create a vast amount of particles with unique shapes, thus increasing data redundancy for accuracy. Furthermore, SEPs are mobile particles, providing the power of multiplex probes, multianalyte and simultaneous analysis of an immense amount of genetic information.

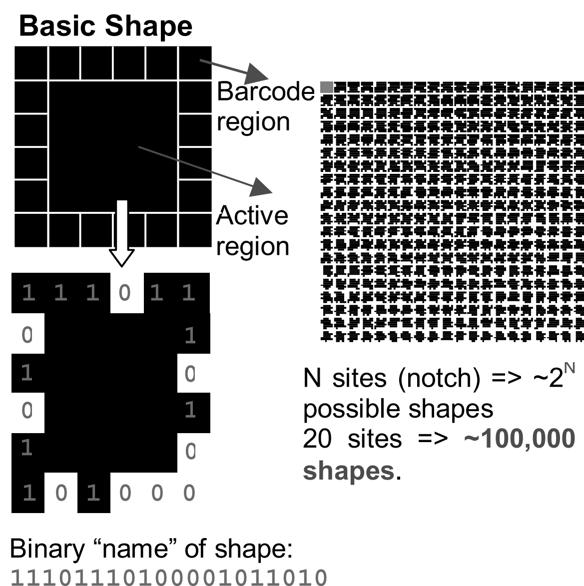


Fig. 1. SEP layout.

Figure 2 illustrates the general outline of genetic analysis protocol. In step 1, the particles, after

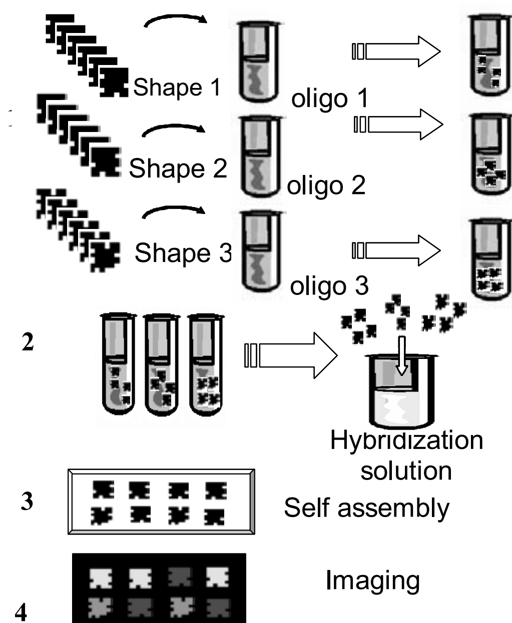


Fig. 2. Example of protocol used for SNP analysis. SEPs allow user the flexibility in experimental design.

aminosilane activation, are subjected to oligonucleotides binding. Each unique oligonucleotide binds to a shape-specific particle (for example, oligo 1 binds to shape one, etc.). Afterward, they are mixed and hybridized with fluorescently labeled targets in a microwell (step 2). Then, the mixture of different particle groups is microassembled (step 3). The microassembled chip is used for laser scanning to obtain fluorescent signal intensities and the subsequent decoding of the particles (step 4).

RESULT

Self-Assembly

The key features of SEP technology, such as its ability to perform multianalyte and simultaneous analysis of genetic/proteomic/cellular information, can not be fully exercised without microfluidic assembly. Without assembly, random spreading of particles results in large spacing between particles, hence a larger scanning area and a longer scanning time. Adversely, random spreading also results in particle overlap, hence lost of information. Therefore, the potential exists to improve packing via microassembly. The microassembly technique chosen must be capable of positioning a large number of particles simultaneously with micro-scale precision. The technique should be insensitive to its chemical environment. The fluid utilized must not interact or interfere with the detection. The working temperature should not exceed the physiological temperature of biomolecules. The technique utilized must enable the production of highest density possible chip for vast data collection and generation. Each data must not interfere with one another. The technique should promote non-overlapping of particles for arrays and be intrinsically parallel in positioning.

Our effort is concentrated on building a 2-dimensional array given a set of defined information such as geometrical constraint, chemical inertness, and the maximization of filling ratio and packing density. Therefore, we decided to utilize template-assisted assembly as the assembly method of choice for SEP.

In addition, desire for economical method calls for cheaper starting materials and easier/faster manufacturing process. To fulfill this requirement, we incorporate polymer molding as the method of choice to manufacture assembly chip. The advantage of using polymer as the assembly chip material is three fold. First, polymer can be made transparent. It allows flexibility in data scanning since the reactions can be viewed either top down or bottom up. Second, since our

desired polymer is transparent, the image analysis software has a higher confidence level to make a distinction between background and foreground. Third, polymer assembly chip is made by molding. It facilitates fabrication speed since the mold can be used repeatedly and rapidly using inexpensive polymer processing.

Figure 3 shows the microassembly result. When an "array deposition" technique, such as template-assisted assembly (figure 3(a)) is applied, the resultant arrays, shown in figure 3(b), can be easily analyzed without loss of data. This method has proven to provide geometric constraint, chemical inertness, and the maximization of packing and signal density [15]. With this approach, we obtained nearly 100% filling yield (1) and close to 80-90% packing density (2).

$$Filling\ yield = \frac{number\ of\ occupied\ sites}{total\ available\ sites} \quad \text{.........} \quad (1)$$

$$Packing\ density = \frac{assembly\ area}{total\ area} \quad \text{....................} \quad (2)$$

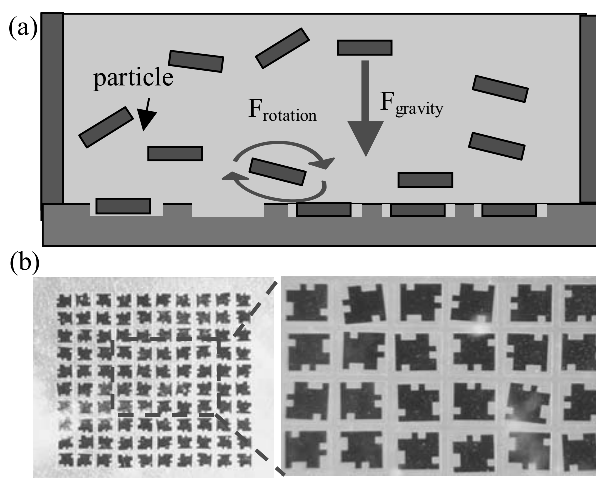
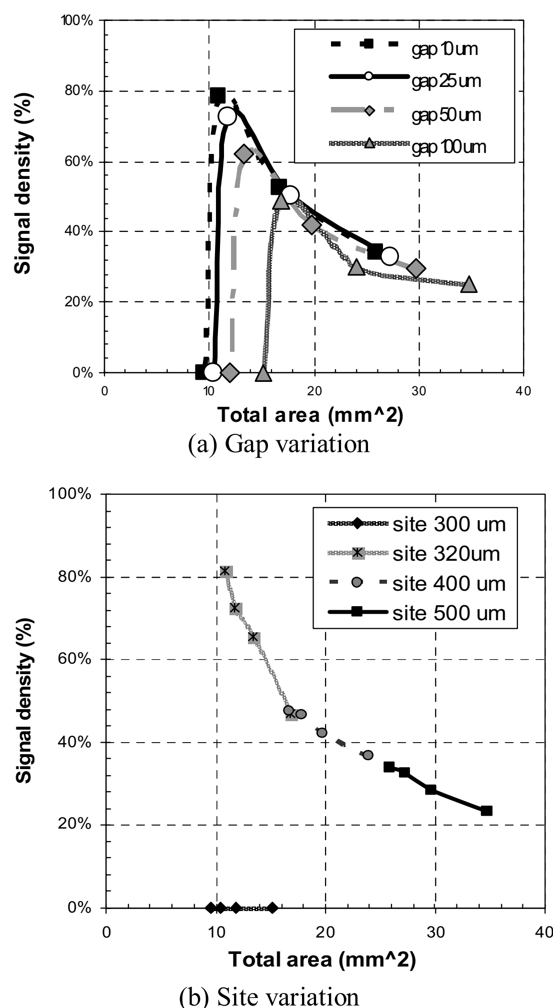


Fig. 3. Template-assisted self-assembly. (a) Particles fall into their complementary sites with agitation in aqueous fluidic environment. (b) Optical pictures of the assembly result. Transparent PDMS substrate is used for best contrast.

The assembly efficiency is analyzed by examining parameters such as site and gap size (figure 4). We found that a decrease in site and gap size increases overall signal density (observable particles per total



(a) Gap variation

(b) Site variation

Fig. 4. (a) The gaps changes (from 100 µm, 50 µm, 25 µm, to 10 µm.) shift the assembly curve up toward higher signal density. (b) whereas site size changes (from 500 µm, 400 µm, to 320 µm,) increase signal density until it reaches the threshold (300 µm) when no assembly is observed.

area) until a threshold is met. Threshold occurs when the site has the same size as the particle.

Data Analysis

The software *ShapeReader* was created in response to the increasing need for object identification in an increasingly complex monitoring of biological reaction. It has the ability to recognize particle shapes, measure fluorescent intensities and associate the signals back to the probes encoded by the shapes. Figure 5 shows an example of the scanning result.

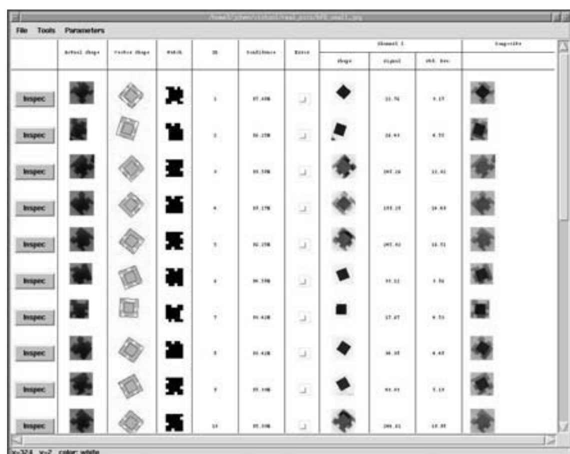


Fig. 5. Output data from *ShapeReader* software.

CONCLUSIONS

We have successfully demonstrated the use of SEP in Single Nucleotide Polymorphism (SNP) genotyping. Specifically, the microparticles with encoded shapes have been designed and fabricated. The particles have been coupled to DNA oligo probes and hybridized to fluorescently label complementary targets. The reaction products are read by fluorescent scanners, and the images are decoded and analyzed by shape analyzing software named *ShapeReader*. SEP self-assembly has been achieved by using shape-entrapping mechanism. Overall, system integration and protocol procedure have been developed.

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