Hybridization of Electrodeposited Magnetic Multilayer Micropillars

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A novel magnetic encoding technique for performing high-throughput biological assays using molecular probes such as antibodies and DNA oligonucleotides is presented. Electrodeposited Ni/Au multilayer pillar structures with diameters from 5–20 μm and thicknesses from 1–10 μm are presented as "magnetic barcodes," where the number of unique codes possible increases exponentially with a linear increase in pillar size. We have successfully functionalized these pillars using a HS-C_{10}-NHS self-assembled monolayer (SAM) grown on the Au cap layer and attachment of an NH_2 modified primer oligo sequence and have shown hybridization and denaturation to a fluorescent template. In addition we have developed a novel release layer methodology that allows a large library of probes to be inkjet printed on a single 5 mm^2 chip prior to release.

Index Terms—**DNA, electrodeposition, fluorescence, hybridization, magnetic barcodes, microarrays, microfluidics.**

I. Introduction

THE DEVELOPMENT of microarray technology has revolutionized the biotechnology industry, allowing one to analyze the expression of thousands of genes in a single assay. Indeed, microarrays are fast becoming standard laboratory tools for many applications from drug development and proteomics to single nucleotide polymorphism (SNP) analysis and gene sequencing [1], [2]. However, there are numerous challenges facing researchers in this field as reliable data are often hard to extract and a large number of repeats (biological replicates) are recommended to reduce error rates [3]. Extraneous factors can also greatly influence microarray measurements, which are often difficult to control within large experiments [4]. Even if these complications are overcome in the future, fundamental limitations still exist: 2-D positional data used to identify a particular "spot" cannot be shrunk indefinitely without crosstalk from its neighbors. Ultimately, optical resolving power will limit the density of probes on a single chip.

Conceptually different high-throughput technologies are being developed to address the limitations of the microarray-based methods. For example, bead-based technologies, where solid beads are used as vehicles for various biomolecules offer significant advantages, mainly due to their 3-D concept. Thus beads are functionalized with probes and following hybridization or other coupling reactions take place in microfluidic channels and chips, where the beads flow freely [5]. However, the efficient use of bead-based technologies necessitates the development of encoding methods for the effective tagging of the beads. Current well established tagging schemes include the use of optical beads (Luminex), fluorescent dyes, radioactive isotopes and quantum dots (Quantum Dot Corp.), but these all have inherent disadvantages and limitations to the number of codes they can provide [6], [7]. To address these limitations, we are developing a novel encoding method utilizing advanced magnetic sensors and magnetic tags that can provide a vast number of unique codes [8] and offer unique advantages compared to the more traditional encoding techniques [9], such as the ability to write or rewrite the code at any stage of the assay. We have recently shown that single ferromagnetic beads with relatively high-magnetic moments can be successfully taken up by biological cells; hence, the cells can be manipulated, detected, and sorted by means of magnetic integrated microfluidic devices [10].

To realize this magnetic approach we must first overcome several challenges: multilayer magnetic tags must be fabricated; they need to be chemically and biologically functionalized; large libraries of different tags need to be created; the tags need to be released into solution preassay; postassay they need to be manipulated and individually addressed for successful coupling and decoding. In this paper, we report on the development and functionalization of the magnetic multilayer tags, and a strategy to efficiently generate a large library of tagged biological probes in solution.

II. Experimental and Results

The multilayer micropillars comprise nickel magnetic layers, typically 1–5 μm high, separated by gold insulating layers that allow the independent alignment of spins in each layer, creating a "magnetic barcode." The micropillars are fabricated by electrodeposition and capped with a gold layer onto which a SAM can be grown to facilitate the attachment of biological probe molecules, such as oligonucleotides. The entire pillar-tag is supported on a p-doped silicon substrate that is coated with an evaporated Cu(35)/Al(400) release layer so that the tags can be released in solution in order to be flown and interrogated in microfluidic channels. The addition of the Cu layer is useful on three counts: it increases the conductivity of the substrate, aiding the electrodeposition; it prevents the formation of a protective aluminium oxide layer that would be hard to dissolve; in aqueous environments Cu and Al form a galvanic cell, aiding dissolution of the layer.

Templates for the pillars were fabricated using standard lithography techniques. AZ6290 was used to create a 20-μm-thick layer of resist on top of the substrate, and a quartz

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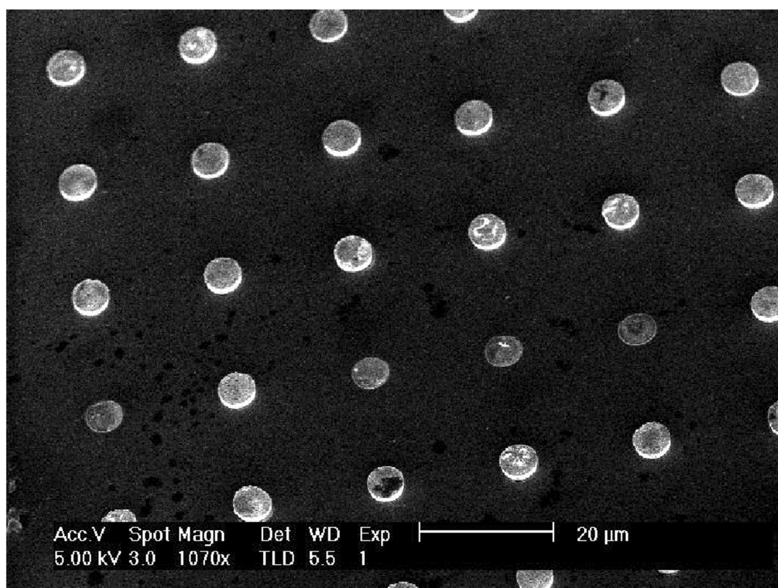

Fig. 1. SEM image of electrodeposited multilayer Au/Ni pillars.

mask with dots (5, 10, 15, and 20 μm diameter) was used to create the pattern. The sides and back of the template were protected by Kapton tape and an electrical contact was made by removing part of the resist. The sample was dipped in a home-made Ni electrolyte (0.1 M $NiSO_4$ + 0.1 M boric acid as a buffer +10 mM saccharine) and Ni was deposited using a potential of -1.5 V (using a Princeton Verstastat Potentiostat with PowerPulse software). For the gold layer, a commercially available gold solution was used (Engelhart). All deposition was done at room temperature with occasional agitation by means of a magnetic stirring bar. A platinum mesh was used as a counter electrode and a saturated calomel electrode (SCE) as the reference electrode. After deposition the sample was soaked in acetone to remove the resist and facilitate removing the tape. Fig. 1 shows an SEM image of the silicon chip following deposition, demonstrating the successful deposition of the pillars.

After imaging with the SEM the sample was measured in a superconducting quantum interference device (SQUID) to determine the magnetic moment. Measurements were done at 110 K and the hysteresis curves are shown in Fig. 2. The sample's total magnetic moment was 1.5×10^{-3} emu, which sets a minimum moment per pillar of 2.4×10^{-8} emu, since there is a maximum or 62 500 pillars per sample. This is an order of magnitude larger than the saturation magnetization of 4.5 μm diameter ferromagnetic beads (Spherotech, 10^{-9} emu per bead.) and over three orders of magnitude larger than the saturation value of 10 μm diameter superparamagnetic beads (Bangs Lab, 10^{-11} emu per bead). The theoretical magnetic saturation value of nickel pillars of 15 μm diameter and 8 μm height is 3.7×10^{-7} emu. The difference with the observed value is attributed to inaccuracies in the amount of pillars present (Fig. 3), an uneven distribution of pillar heights over the sample, surface oxidation, calibration errors, and porosity in the nickel which makes the saturation value lower than the theoretically expected value.

To functionalize the metallic tags we make use of the known strong affinity of thiols for gold [11] to grow a SAM on the gold

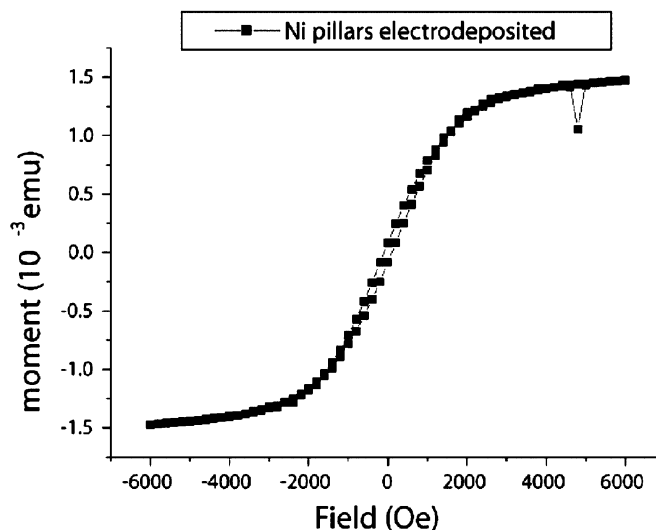

Fig. 2. SQUID measurement of an assembly of Nickel structures.

cap layer. The SAM was grown by incubating the silicon chip containing the pillar microarrays in a centrifuge tube with 1% HS-C_{10}-NHS in absolute ethanol for 2 h at room temperature. Excess was removed by repeated washing steps with absolute ethanol then drying under a stream of nitrogen gas. A 26 base pair (bp) 5' NH_2-modified oligonucleotide primer (NH_2-TTT TTT TAT GAC ACC GTC ATC AGC AG) (all oligos from Sigma Genosys) was dissolved in 90 μL phosphate buffer, pH 8.5 at a 7-μm final concentration. This solution was then micropipetted onto the surface of the chip and covered with a plastic cover-slip to prevent drying. After 2 h at room temperature the chip was extensively washed with phosphate buffer before being incubated at 55 °C for 3 h with a 2 μm template in phosphate buffer. The 25 bp target oligo containing a 19 bp complimentary overlap was 5' fluorescein-modified: (FITC—GAC

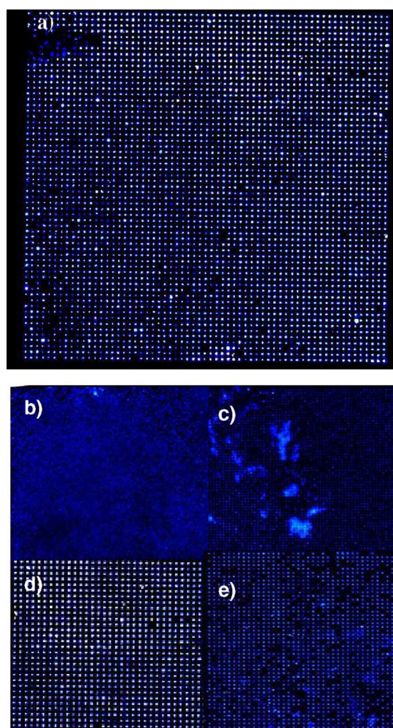

Fig. 3. CCD-scans of the fluorescently hybridized pillar arrays. (a) 61 × 63 array of 15-μm diameter pillars. (b)–(e) Sections showing 5-, 10-, 15-, and 20-μm diameter pillars respectively.

TAC TGC TGA TGA CGG TGT CAT A). The chip was then repeatedly washed with phosphate buffer and dried under N_2 gas before analysis.

CCD-scans shown in Fig. 3(a) demonstrate the successful hybridization of the fluorescent template oligo in a clear array structure, thus proving that the preceding chemistry was also successful. The missing spots in the array (other than the tweezer mark) are evidence of the ease with which it is possible to release the pillars. Further denaturing of the sample at 90 °C showed a tenfold decrease in fluorescent intensity suggesting the possibility of recycling the probes for multiple hybridization assays. Comparing the pillar diameters we found that the greatest fluorescence intensity was achieved with the 15 μm diameter pillars [Fig. 3(b)–(e)], the trend showed increasing intensity with diameter as one would expect, since more fluorophore can bind to each pillar, but the intensity reduced slightly with the 20 μm diameter pillars. This effect is believed to be due to possible quenching if the spacing between fluorescent molecules is too close—further studies comparing 15 and 20 μm diameter pillars are planned to provide more insight to this phenomenon.

III. Conclusions and Future Work

We have successfully fabricated uniform arrays of Ni-Au multilayer magnetic pillars by electrodeposition as an alternative architecture to magnetic beads; because the pillars are made of solid magnetic material it is possible to achieve magnetic moments in excess of 10^{-7} emu [12]. This is very promising since they can be easier manipulated by means of local magnetic fields applied by tapered current strip-lines on the surface of a lab-on-a-chip device, as well as easier detected by magnetic sensors [8].

These have been successfully functionalized by growth of a SAM onto the gold cap layer. We have demonstrated the coupling of a 26 bp primer oligo to the pillars by hybridization and denaturation of a fluorescent complimentary template oligo. We have fabricated new 5 mm ^{2} chips containing up to 22 500 pillars designed to be used in conjunction with a newly purchased sciFLEXARRAYER nanoprinting system to create a library of up to 2500 different probes. Our novel release layer can be dissolved under relatively mild conditions (0.1 M sodium hydroxide at room temperature for 15 min), meaning ss-DNA can be printed prior to release. A fluorescent signal will be used to distinguish successful coupling and the magnetic tags will be read and sorted in a high-throughput manner in an integrated microfluidic chip (IMC) [10], [13].

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