Detection of Magnetically Labelled Microcarriers for Suspension Based Bioassay Technologies

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Microarrays and suspension-based assay technologies have attracted significant interest over the past decade with applications ranging from medical diagnostics to high throughput molecular biology. The throughput and sensitivity of a microarray will always be limited by the array density and slow reaction kinetics. Suspension (or bead) based technologies offer a conceptually different approach, improving detection by substituting a fixed plane of operation with millions of microcarriers. However, these technologies are currently limited by the number of unique labels that can be generated in order to identify the molecular probes on the surface. We have proposed a novel suspension-based technology that utilizes patterned magnetic films for the purpose of generating a writable label. The microcarriers consist of an SU-8 substrate that can be functionalized with various chemical or biological probes and magnetic elements, which are individually addressable by a magnetic sensor. The magnetization of each element is aligned in one of two stable directions, thereby acting as a magnetic bit. In order to detect the stray field and identify the magnetic labels, we have developed a microfluidic device with an integrated tunneling magnetoresistive (TMR) sensor, sourced from Micro Magnetics Inc. We present the TMR embedding architecture as well as detection results demonstrating the feasibility of magnetic labeling for lab-on-a-chip applications.

***Index Terms*—Biotechnology, biomedical measurements, magnetic field measurement, magnetic films, magnetic sensors, tunneling magnetoresistance.**

I. Bioassays: Introduction and Motivation

THE Biological Assay or test is an important tool in medical research, drug development and other biological diagnostics [1]. An assay typically uses specific molecular probes that will bind selectively to the molecule or "target" that is being tested for. This binding is referred to as hybridization and is the key to all assay technologies. Hybridization is usually detected using fluorescence and modern assays use multiple probes in order to simultaneously test for many different targets. This is known as multiplexing. Microarrays are the state-of-the-art in assay technology and a single microarray can be used to simultaneously search for millions of target molecules [2]. In microarrays, the molecular probes are localized at various positions on a 2-D surface and can therefore be identified by their spatial coordinates.

Although microarrays are ubiquitous in lab environments there are several reasons why they have had limited success as point-of-use/care devices. As the probes are confined to a 2-D plane, sufficient time must be allowed for the reactants to find their binding sites. This results in highly multiplexed assays taking many hours. Bulky optics are usually utilized to detect the results, which are encoded in a snapshot of fluorescent dots, whereas the microarrays themselves are relatively expensive and must be individually manufactured based on assay targets and design [3]. In order to circumvent some of the limitations of microarrays a variety of suspension based assay technologies (SATs) have been developed.

The key concept behind SATs is to move away from the 2-D solid phase kinetics of microarray binding towards the faster 3-D kinetics of binding in solution. This increase in reaction rates can lead to increased assay sensitivity or decrease assay times depending on requirements.

In SATs, small particles suspended in solution, known as microcarriers, are used to hold both the molecular probes and a label that can identify them. The label is necessary for multiplexing as simple spatial encoding, as with microarrays, is no longer possible. In most proposed technologies the labels are based on fluorescence [4] or graphical patterns [5]. The use of fluorescence labeling limits the number of distinguishable probes and potentially interferes with the detection of hybridization due to spectral overlap. In both cases, the need for optical decoding hampers device portability and increases device costs.

Our bioassay technology uses microcarriers with embedded magnetic thin films in order to generate a magnetization based label. As the labels are magnetically readable, there is no need for bulky optics. Furthermore, the ability to write a label to our microcarrier at the point-of-use means they can be mass manufactured and do not need to be uniquely fabricated for each label, drastically reducing the cost. The advantages and applications of our digital magnetic architecture have been discussed in more detail in previous papers [6]. Details on chemistry and biomolecular functionalization of the microcarriers can be found in Palfreyman *et al.* [7]. These aspects will not be discussed further here. The following section will give a brief overview of the microcarrier design and fabrication in order to provide a context for the exciting new progress towards magnetic label detection.

II. The Planar Tag: Microcarrier Design and Fabrication

Our microcarrier, referred to as a planar tag, consist of a series of thin film magnetic elements that are encased in an SU-8 backbone and is shown in Fig. 1. The SU-8 protects the magnetic material from oxidization and provides a substrate on which the molecular probes can be attached. Each magnetic element is elongated and therefore, due to shape anisotropy prefers to be magnetized "up" or "down" relative to its long axis, thereby

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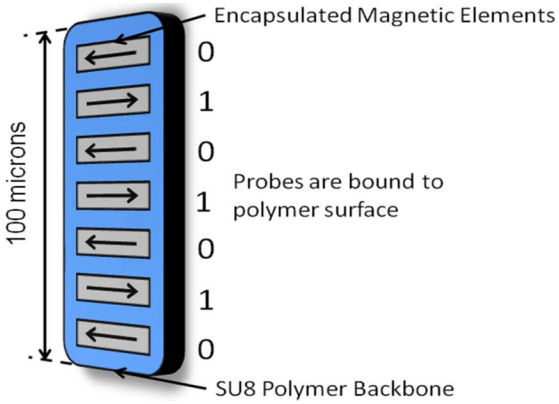

Fig. 1. The planar tag: A digital magnetic carrier. The magnetic elements are fully encapsulated in the SU8 and their magnetization defines a digital label.

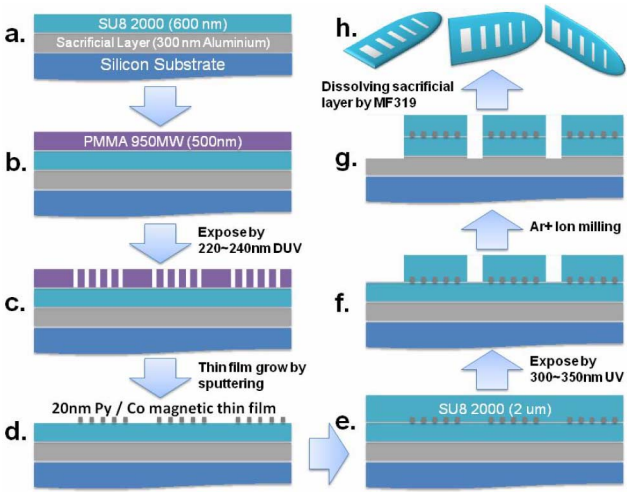

Fig. 2. Schematics of the fabrication procedure of SU8 encapsulated digital magnetic carriers. Figure reproduced from [7].

encoding a bit. Multiple magnetic elements thus provide a bit string that defines the magnetic label.

By changing parameters such as the aspect ratio and magnetic material we can increase or decrease the coercivity of each successive element and thereby make each bit switch at a different characteristic field strength. This allows us to use a global external magnetic field to write our unique label. We first address the bit that is hardest to switch. Applying a field strong enough to do this will switch all the bits and, for example, write $\{11111\}$. We then address the bit with the second highest switching field. By applying exactly this field, we can, for example, switch all the remaining bits to $\{0\}$ without affecting the state of the first. We now have $\{10000\}$. By continuing in this way we can apply a quickly varying global field that can write an arbitrary code onto a single tag or a whole group of tags simultaneously.

Fig. 2 gives an overview of how our magnetic microcarriers are fabricated. First an aluminium sacrificial layer is deposited onto a silicon wafer; this will later be dissolved in order to release our microcarriers into solution. On this, a 600 nm layer of SU8 is spun and then cross linked by flood exposure and baking [Fig. 2(a)]. A thin layer of LOR (for undercutting) and a 500 nm layer of PMMA are now spun on the wafer [Fig. 2(b)].

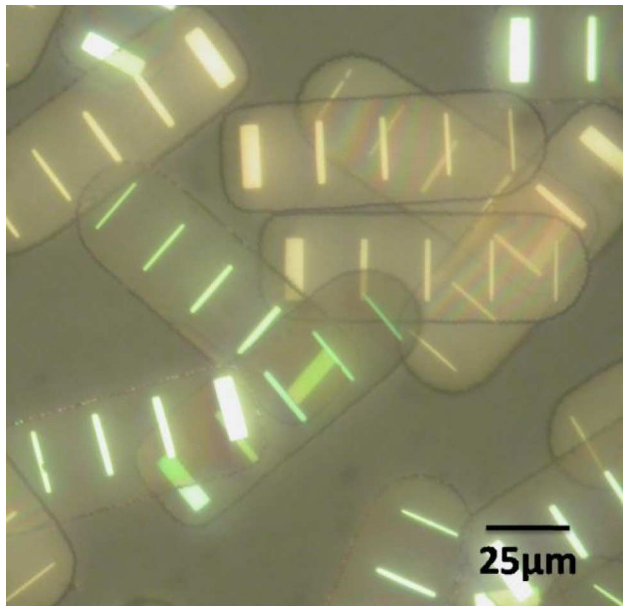

Fig. 3. Microscope image of released 5 bit planar tags in solution. The magnetic elements can be easily distinguished due to their high reflectance.

Deep UV lithography is used to define the shape of the magnetic elements [Fig. 2(c)]. Sputter deposition is used to deposit the metallic layers. These typically consist of a chromium adhesion layer, a magnetic layer and a capping layer of gold. After deposition, the LOR and PMMA are dissolved to lift off the unwanted material leaving us with the magnetic elements on the surface of our sample [Fig. 2(d)]. A second, 2 μm thick layer of SU8 is then spun on [Fig. 2(e)] and the shape of our planar tags is defined using UV lithography [Fig. 2(f)]. After baking, ion milling is used to reduce the thickness of the second SU8 layer whilst imprinting the planar tag shape into the layer below. This eventually leaves us with free standing microcarriers [Fig. 2(g)]. Finally, the aluminium layer can be dissolved using sodium hydroxide to give us free floating microcarriers [Figs. 2(h) and 3]. More details of the design and fabrication procedures and parameters can be found in [8].

III. Magnetic Biochip: Microfluidics and Detection

For biological applications, we must be able to bring the molecular probes and therefore our microcarriers in contact with the testing solution and check for hybridization after the testing solution has been washed away. Finally, we must be able to read the label in order to decipher which target molecules have been found. This whole process can be miniaturized using microfluidics into a lab-on-a-chip or biochip.

For a fully integrated biochip, we must show the "on chip" ability to check for hybridization and detect the label. For the former, we intend to use a traditional fluorescence based approach and, as shown in Fig. 4, it is feasible to detect fluorescence whilst the microcarrier is in our fluidic channel. The latter however is a far greater challenge. The distance between our thin film elements and the sensor, on the order of a few microns, causes the detectable stray field to not only be very weak but also for there to be a high spatial overlap between the signals from successive elements [9]. This means we need a highly

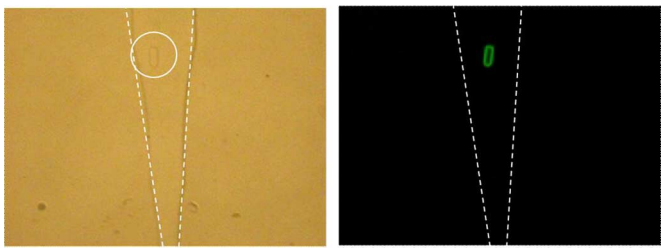

Fig. 4. The image on the right shows the fluorescence emission detected from an FITC functionalized planar tag in a microfluidic channel. The image on the left is a corresponding white light image.

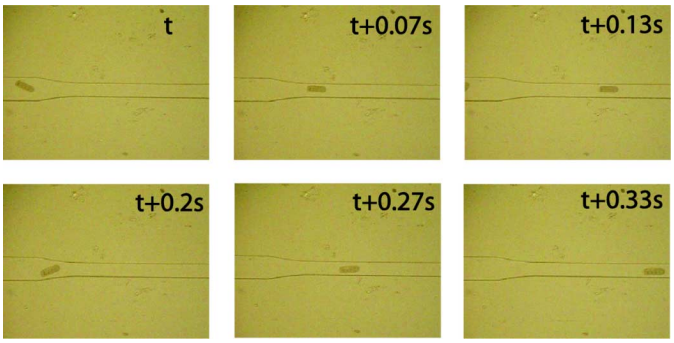

Fig. 5. Microscope images showing two released 5 bit planar tags flowing through a PDMS microfluidic channel in quick succession. The elapsed times can be seen on the top right of each image. The tags are flowing at approximately 3.6 mm per second. It can be seen that the constriction in the channel helps to align the microcarrier.

sensitive sensor with as small an active area as possible. With this in mind, we have chosen to use a commercial tunnel-magnetoresistive (TMR) sensor supplied by Micromagnetics [10]. We connected the sensor in an AC Wheatstone bridge configuration and used a lock-in amplifier to extract a dc signal. Using this setup, we have found we can achieve a sensitivity of 0.02 mT at time constants as low as 10 ms.

For the channel construction, we chose to work with PDMS as an elastomer was ideal for providing a reliable seal around our sensor chip, which was not large enough to be used as the complete base for our microfluidic system. The fluidic channels were constructed by first fabricating an SU8 mould using photolithography. PDMS and curer were then mixed, degassed and poured over the mould. The PDMS was cured in an oven at 75°C for 4 h. After this, the PDMS can be peeled off the mould to reveal a surface with channels that can subsequently be bonded to either glass or another PDMS piece once treated with oxygen plasma for 20 s.

We found that we could easily align the planar tags as they flowed through the fluidic system by introducing a constriction as seen in Fig. 5. In order to integrate the sensor chip into our fluidic device, we constructed a PDMS base with a cavity molded using a dummy chip. We then replaced the dummy chip with a working sensor chip. This had to be contacted before we united it with the top channel piece. This scheme can be seen in Fig. 6. The constriction in our channel was precisely aligned with the sensor and brought into contact using a mask aligner. Fig. 7 shows a 5 bit planar tag with cobalt magnetic elements flowing through the resulting device.

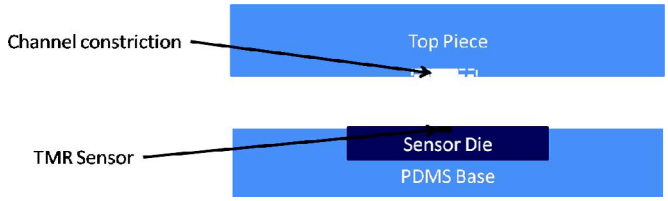

Fig. 6. The figure shows how the chip is embedded into a base piece in order to bond a microfluidic channel over the TMR sensor. A photo-lithographically defined SU8 mould is used to make the channels and a dummy chip serves the same purpose for the base.

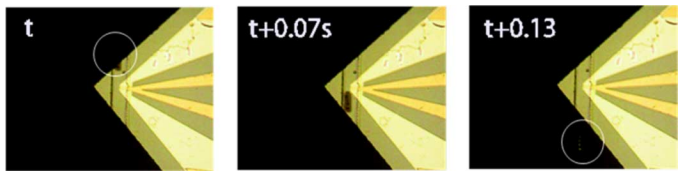

Fig. 7. A 5 bit planar tag is flown through a constriction and over a TMR sensor. The elapsed times can be seen on the top right of each image.

Since we have not yet integrated a write head into our device we have so far been working with the "saturated" $\{11111\}$ configuration. This is the configuration that displays the largest overlap effects and as such we expect to see a combined signal from all our elements in the TMR sensor output. Although it is desirable to be able to detect each bit in isolation, it is not strictly necessary as long as each configuration produces an unambiguously different shape. The wedge shaped signal observed for the $\{11111\}$ in Fig. 8(a) is a representative example and is in agreement with the simulations presented in [9]. It is consistent with the shape of the signal we would expect to see should the tags flow over the sensor at a height of 5 μm. It is important to note that PDMS cannot sustain large aspect ratios, therefore our channels, which span 100 μm at their widest, had to be made 40 μm deep in order to avoid collapse. Unfortunately, this means that though we are able to control the lateral position of the tag as it passes the sensor it is not possible to control the fly height.

The signal observed in Fig. 8(b) is unique and shows a much larger distinction between the signals for the different elements than predicted by Hayward *et al.* [9]. It cannot solely be explained by a lower fly height and we suspect it is due to a more complex domain configuration in our magnetic elements. As yet, we are not sure whether this can be utilized in any useful way, however, we are pleased to report unambiguous magnetic detection of our planar tags in microfluidic flow using a TMR sensor.

IV. Conclusion

Our goal is to replace optical label recognition methods in suspension bioassays with solid state magnetic sensors. Although fluorescence may still be used to detect hybridization, single frequency and single pixel detection can be miniaturized and does not need a complicated optical set up. This will allow devices to be portable, quick and inexpensive, providing greater potential for point-of-care devices.

We have demonstrated a further step towards this goal by detecting magnetically labeled microcariers in flow using a PDMS

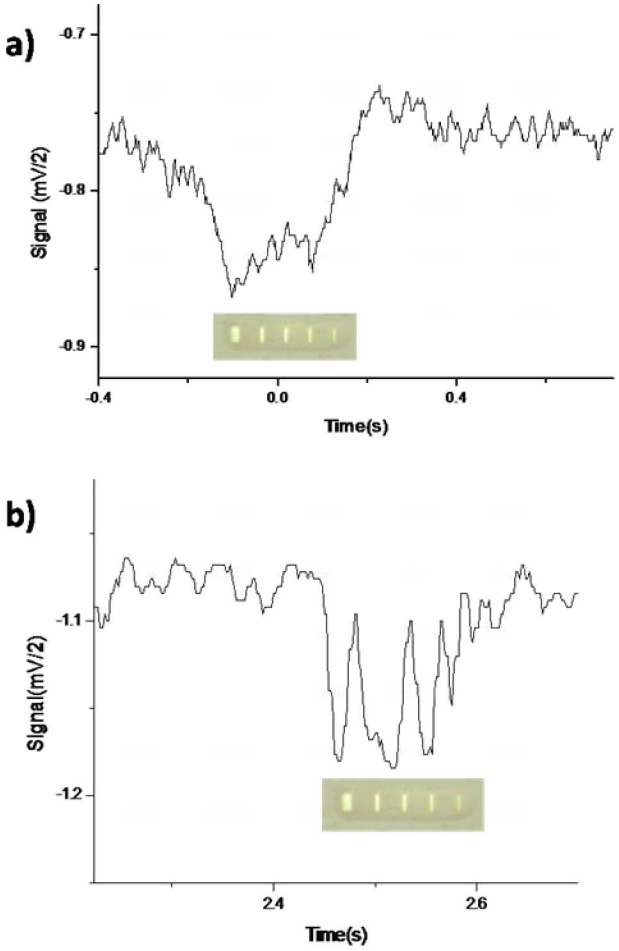

Fig. 8. The TMR sensor signal detected due to a 5-bit tag in the {11111} configuration flowing through a microfluidic channel. (a) A typical wedge shaped trace due to a tag passing about 5 μm away from the TMR sensor. (b) A trace showing distinct signals due to each bit.

microfluidic cell and a commercial TMR sensor. The next step is to integrate writing capability into our device and demonstrate full coding, decoding capability. One of the parameters we will have to control moving forward is the fly height of our microcarriers within the fluidic channel. It may be necessary to move to other materials and fabrication methods for our fluidic device in order to achieve this.

Our coding density is limited to 5 bits on a 100 μm support, corresponding to 32 codes. This is due to the capability to successfully detect and distinguish each code. A technique by which you can correlate a model signal with the sensor output will allow us to extract a code from noisy data with a much higher degree of confidence. This will enable a further increase in the coding density; allowing for a smaller microcarrier. More work on the characterization of the stray field will need to be done before correlation techniques can be employed.

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REFERENCES

[1] M. J. Heller, "DNA microarray technology: Devices, systems and applications," *Annu. Rev. Biomed. Eng.*, vol. 4, no. 129, Mar. 2002.
[2] M. Jayapal and A. J. Melendez, "DNA microarray technology for target identification and validation," *Clin Exp Pharmacol Physiol*, vol. 33, no. 5-6, pp. 496–503, May 2006.
[3] C. J. D. Gershon, "Microarray technologyan array of opportunities," *Nature*, vol. 416, no. 6883, pp. 885–891, Apr. 25, 2002.
[4] M. Han, X. Gao, J. Z. Su, and S. Nie, "Quantum-dot-tagged microbeads for multiplexed optical coding of biomolecules," *Nat. Biotechnol.*, vol. 19, no. 7, p. 631, Jul. 2001.
[5] D. C. Pregibon, M. Toner, and P. S. Doyle, "Multifunctional encoded particles for high-throughput biomolecule analysis," *Science*, vol. 315, no. 5817, pp. 1393–1396, Mar. 9, 2007.
[6] Mitrelias, T. Trypiniotis, F. van Belle, K. P. Kopper, S. J. Steinmuller, J. A. C. Bland, and P. A. Robertson, J. A. C. Bland and A. Ionescu, Eds., "Magnetic microtags and magnetic encoding for applications in biotechnology," in *Biomagnetism Magnetic Biosystems Based Molecular Recognition Processes, AIP Conf. Proc.*, Jun. 2008, vol. 1025, p. 60, 71 and.
[7] J. J. Palfreyman, P. Beldon, B. Hong, K. N. Vyas, J. F. K. Cooper, T. Mitrelias, and C. H. W. Barnes, in *Fabrication Magnetic Barcoded Microcarriers For Biomolecular Labeling: SU-8 Encapsulated Magnetic Tags AIP Conf. Proc.*, (to be published).
[8] B. Hong, T. J. Hayward, J.-R. Jeong, J. F. K. Cooper, J. J. Palfreyman, T. Mitrelias, A. Ionescu, J. A. C. Bland, and C. H. W. Barnes, "Design and fabrication of SU8 encapsulated digital magnetic carriers for high throughput biological assays," *J. Appl. Phys.*, vol. 105, p. 034701, Feb. 2009.
[9] T. J. Hayward, B. Hong, K. N. Vyas, J. J. Palfreyman, J. F. K. Cooper, Z. Jiang, J. R. Jeong, J. Llandro, T. Mitrelias, J. A. C. Bland, and C. H. W. Barnes, "Magnetic micro-barcodes for molecular tagging applications," *J. Phys. D: Appl. Phys.*, vol. 43, p. 17500, May 2010.
[10] Micromagnetics Inc. [Online]. Available: http://www.micromagnetics.com/