A composite element bit design for magnetically encoded microcarriers for future combinatorial chemistry applications

David M. Love,* Kunal N. Vyas, Amalio Fernández-Pacheco, Justin Llandro, Justin J. Palfreyman, Thanos Mitrelias and Crispin H. W. Barnes

We present a new composite element (CE) bit design for the magnetic bit encoding of suspended microcarriers, which has significant implications for library generation applications based on microfluidic combinatorial chemistry. The CE bit design consists of high aspect ratio strips with appropriate dipolar interactions that enable a large coercivity range and the formation of up to 14 individually addressable bits (16,384 codes) with high encoding reliability. We investigate Ni$_{80}$Fe$_{20}$ and Co CEs, which produce coercivity ranges of 8–290 Oe and 75–172 Oe, respectively, showing significant improvements to previously proposed bit designs. By maintaining the total magnetic volume for each CE bit, the barcode design enables a consistent stray field for in-flow magnetic read-out. The CE bit design is characterised using magneto-optic Kerr effect (MOKE) measurements and the reliability of the design is demonstrated in a multi-bit encoding process capable of identifying each bit transition for every applied magnetic field pulse. By constraining each magnetic bit to have a unique switching field using the CE design, we enable sequential encoding of the barcode using external magnetic field pulses. We therefore discuss how the new CE barcode design makes magnetically encoded microcarriers more relevant for rapid and non-invasive detection, identification and sorting of compounds in biomolecular libraries, where each microcarrier is for example capable of recording its reaction history in daisy-chained microfluidic split-and-mix processes.

Previously, magnetic encoding of microcarriers was proposed for multiplexed point-of-care diagnostic applications, where nominally identical tags are encoded using coercivity tuned magnetic bits by externally applied magnetic field pulses,\textsuperscript{5,10,11} offering unique advantages in mass fabrication and scaling. The idea formed an unconventional approach\textsuperscript{12} to other suspension assay technologies, where carrier detection was typically based on nano-particle binding,\textsuperscript{13} optical barcodes\textsuperscript{14–18} or fluorescence.\textsuperscript{17,18} Coercivity tuning on the other hand, is achieved by patterning magnetic strips with different aspect ratios (Fig. 1a), giving each bit a unique field amplitude at which its magnetisation reversal occurs (i.e. switching between a 0 and 1 state) and allowing identical microcarriers to be uniquely encoded with a multi-bit code. It has been shown that batches of such encoded microcarriers can be functionalised with two different probe molecules on two distinct surfaces,\textsuperscript{19,20} using the incorporated thiol and epoxy surface groups. By also including control molecules on each microcarrier, a bioassay is capable of identifying true positives/negatives by probing the assay conditions using control targets purposely included in the analyte. When fluorescence of the target molecules indicates positive binding, the magnetically encoded microcarriers can be identified by the magnetic stray fields of their unique barcode in-flow, as has previously been demonstrated* using an

1 Introduction

Combinatorial chemistry provides significant scaling advantages and has formed a cornerstone of medicinal chemistry over two decades ago. While modern drug discovery relies heavily on rational design and computer modelling, the use of combinatorial chemistry techniques is re-emerging\textsuperscript{1,2} in areas such as the formation of peptide libraries and formulation chemistry of drugs. Early efficiency estimates\textsuperscript{3} of combinatorial chemistry already showed that 1000 times more compounds can be generated for at least 600 times less the cost per compound compared to traditional methods, prompting pharmaceutical companies to adopt the technique in their drug development and study of related compounds. With modern economies of scale favouring automation and especially with the rise of microfluidic technologies this efficiency trend has since progressed exponentially.\textsuperscript{4,5} Here, we present advances in magnetic barcode design that make the use of suspended and magnetically encoded microcarriers (or tags) highly relevant for the development of future library generation and screening applications using lab-on-a-chip based combinatorial chemistry.

Cavendish Laboratory, Department of Physics, University of Cambridge, Cambridge CB3 0HE, UK. E-mail: david.michael.love@gmail.com
integrated tunnelling magnetoresistance (TMR) sensor (illustrated in Fig. 1b).

However, the original bit design\(^6,7\) was prone to forming multi-domainal states, which heavily limited encoding reliability and therefore also the total number of distinct bits. Here, we expand on the concept of coercivity tuning and present a new composite element (CE) bit design (Fig. 1c) that is capable of encoding up to 14 bits (16,384 codes) with unique coercivity values to a high degree of accuracy. The CE bits consist of multiple strips with high aspect ratios and are engineered to enable a significantly wider coercivity distribution while maintaining sharp magnetisation reversal behaviour for reliable encoding. This makes the CE bit design highly relevant for the development of future microfluidics-based combinatorial chemistry applications, where each bit within a microcarrier can be individually encoded (when written from highest to lowest coercivity value) after each split-and-mix process. Compounds of interest could then be identified using multiplexed detection of the microcarriers’ magnetic stray fields, all within a lab-on-a-chip platform. Here, we present the CE bit design and the intricacies that govern its encoding properties in order to demonstrate the reliability of the new barcode design.

Fig. 1 (a) Illustration of the coercivity (H\(_c\)) tuning technique and the original barcode design\(^6,7\) where the coercivity distribution of ‘bits’ is determined by varying the aspect ratios of the ferromagnetic elements. (b) Illustration of the use of magnetically encoded microcarriers, with fluorescence detection and in-flow magnetic identification as previously demonstrated\(^8\) using polydimethylsiloxane (PDMS) moulded microfluidics with an integrated tunnelling magnetoresistance (TMR) sensor. (c) SEM image of the new composite element (CE) bit design, where each bit consists of high aspect ratio strips and maintains a consistent magnetic area.

2 Composite element bit design

As the magnetic barcodes are intended to be read out using a TMR sensor in-flow, we constructed a TMR-scanning microscope for detection optimisation. This instrument consists of an in-plane sensitive TMR head (Micro Magnetics Inc.) on a three axis nano-positioner (Thor Labs), which detects the in-plane stray magnetic field components of patterned nanostructures.\(^9\) In single axis scans, inconsistencies in signal amplitude of identically encoded bit codes of the original design\(^6,7\) were observed. The magnetic field and spatial resolution of the TMR sensor allows us to perform detailed 2D raster scans at set heights to map out the variation of magnetic stray field produced within the area of each element of the original design. Fig. 2 shows the real component (X) of the sensor’s impedance obtained from scanning a 3 bit magnetic tag containing Co elements with low aspect ratios. The 2D scan reveals information of both the amplitude and orientation of the magnetic fields produced by the elements along the sensor’s sensitive direction, which is along the length of the elements. Domain formation within the low aspect ratio Co bits becomes apparent, particularly in the amplitude (R) and phase (θ) data, since zero amplitude in R indicates a domain wall while
between a single element and a CE bit in Fig. 3. As coercivity

opposite saturation in $\theta$ (and $X$) indicates the north and south

It is evident that the domain breakdown observed for the

high aspect ratio (high $H_c$) bits do not suffer from

which limits the distance at which they can be detected in-

further increasing the risk of unintentional switching of

neighbouring bits when writing a desired magnetic bit code

using magnetic field pulses.6

The new CE bit design is able to overcome all these limita-

tions by creating elements consisting of strips with significantly

larger aspect ratios coupled by dipolar interactions. Here shape

anisotropy is able to dominate the magnetic behaviour within

each bit, as illustrated by the difference in domain formation

between a single element and a CE bit in Fig. 3. As coercivity

scales inversely with strip width,23,24 such a design is also able to

significantly widen the possible coercivity distribution, while

the appropriate dipolar interactions and use of magnetically

soft materials ensure sharp magnetisation reversal behaviour23,24

for reliable encoding. In the CE bit design the magnetic volume

is maintained between bits by increasing the number of strips

for CEIs consisting of narrower strip widths, allowing for

consistent magnetic read-out of each bit’s stray field in-flow.

The in-plane easy axis coercivity ($H_c$) value of a patterned

strips at set spacings is determined by23-24

$$H_c = \alpha \left( \frac{s}{w} \right) \times \left( \frac{s}{w} \right) + H_0$$ (1)

where $t$ is the film thickness, $w$ the strip width, $s$ the strip

spacing, $H_0$ a constant and $\alpha(s/w)$ a complex function for the
dipolar interactions between strips, presented in detail by
Adeeye $et$ $al.$24 For a large coercivity range one would therefore
select a relatively thick film (still within the single domain
regime) with narrow strip widths compared to the strip
lengths.23,24 In using large aspect ratios the coercivity values

remain dominated by the CE width and thickness values.25 The

spacings between strips within each CE can further be used to
fine tune the element's coercivity and switching behaviour, by
utilising the dipolar interactions between neighbouring strips.
It is possible to adjust the onset and mode of the magnetisation
reversal process26-29 at separations typically equal to or below
the strip width.

For a maximised coercivity range we therefore select a rela-
tively thick film of 15 nm for both the Co and Ni$_{80}$Fe$_{20}$ CEs to
illustrate the difference between materials with and without

crystalline anisotropy, respectively. The pitch between elements

should be sufficiently large (e.g. $>5$ $\mu$m) to prevent bits from

interacting and to allow for in-flow stray field detection for set

TMR sensors properties and rapid flow rates.6 By varying strip

width, length and spacing we investigate their effects on the

coercivity distribution and switching behaviour of the barcodes
in order to optimise the CE bit design for reliable use in combinatorial chemistry based applications.

3 Methods

Samples were fabricated using e-beam patterning of a polymethyl-methacrylate (PMMA) resist bi-layer on a 3° Si wafer, comprised of a 100 K (1 : 2 PMMA : anisole diluted) bottom layer and 950 K (3 : 5 PMMA : anisole diluted) top layer. The lower molecular weight resist was spun at 2000 rpm and soft baked at 180 °C for 90 seconds to give a 48 nm thickness measured on an ellipsometer. The higher molecular weight resist was subsequently dispensed and spun with maximum acceleration at 2700 rpm and soft baked at 180 °C for 90 seconds to give a combined thickness of 77 nm. To prevent significant inter-diffusion between resist layers, the second coating was quickly dispensed using a 10 ml syringe and immediately spun, as otherwise the solvent from the 950 K resist would penetrate the first layer within the order of one second.

Patterns for the CEs were designed using CleWin 4 (WieWeb software v.o.f.) and exposed using a Leica VB6 UHR e-beam writer at a 100 kV beam voltage, and developed in a 5 : 15 : 1 ratio of methyl–isobutyl–ketone : isopropanol : methyl–ethyl–ketone (MIBK : IPA : MEK) for 5 seconds followed by a thorough IPA rinse and N$_2$ gas dry. The Co (99.95%) and Ni$_80$Fe$_{20}$ (99.97%) sources were purchased from Goodfellow Ltd. and were both thermally deposited, at a rate of 0.025 nm s$^{-1}$, using an Edwards Auto306 evaporator with a base pressure of 2 × 10$^{-7}$ mBar to a thickness of 15 nm, each with a 5 nm Au (99.99%) capping layer to prevent oxidation. All growth heights were confirmed using atomic force microscopy (AFM). The hysteresis loops were measured by longitudinal magneto-optic Kerr effect$^{29}$ (MOKE) on elements with appropriate pitch using a NanoMOKE magnetometer, produced by Durham Magneto Optics Ltd., capable of focusing the laser down to a 5 μm spot diameter. Each presented MOKE coercivity value was obtained by averaging 200 hysteresis loops measured on each of at least 16 identical barcodes.

4 Coercivity tuning

The MOKE characterisation of the Ni$_{80}$Fe$_{20}$ and Co CE bits show a wide coercivity range, effectively increasing the operation margin of the bit design by utilising narrower strip dimensions. Fig. 4 [top] shows the average coercivity values plotted against strip widths, for bit designs where strip spacings are equal to strip widths, revealing a coercivity range of 8–290 Oe for Ni$_{80}$Fe$_{20}$ and 75–172 Oe for Co. These ranges are significantly wider than the 27–60 Oe for Ni$_{80}$Fe$_{20}$ and 32–120 Oe for Co elements reported for the original bit design.$^7$ The magnetically soft Ni$_{80}$Fe$_{20}$ elements are clearly preferable and were also measured at two lengths, 25 μm and 10 μm, and plotted on top of each other showing no significant variation. This behaviour was expected due to the extreme aspect ratios within the CE bit design, causing the length demagnetisation factor to be suppressed, and leaving the elements’ demagnetisation value determined predominantly by the width and thickness values.$^{25}$

The spacing between strips can itself further effect a CE’s coercivity value and switching behaviour. Fig. 4 [bottom] shows Ni$_{80}$Fe$_{20}$ elements with strip widths between 100–800 nm, each varying by spacings of 50–400 nm. It becomes evident that decreasing the separation between strips decreases the overall element’s coercivity ($H_C$) while maintaining consistently sharp switching behaviour, as seen in the inset hysteresis loops of elements with 400 nm Ni$_{80}$Fe$_{20}$ element at 50–400 nm spacings. Every averaged data point represents the mean of 16 replica measurements of the same element geometry, with the error bars showing the ±1σ variation in coercivity. Point labels represent values along the x-axis.
cascade-like switching of strongly interacting strips, resulting in a slanted hysteresis loop.

However, the varied strip spacings of the CE bit designs have sufficiently low coupling to give an earlier reversal onset at reduced separations due to a lower domain formation barrier. Domains typically occur at the strip ends and rapidly propagate through all neighbouring strips without forming antiparallel intermediate states during the reversal process. The fine tuning of the dipolar interactions at the higher coercivity elements becomes increasingly difficult, as a greater number of strips (to preserve the total magnetic volume between elements) tends to result in more gradual transitions. For our final CE bit design we select spacings equal to the strip width to ensure appropriate dipolar interaction strengths.

The benefits of using magnetically soft materials for coercivity tuning are further illustrated in Fig. 5, where stacked MOKE loops of Co and Ni$_{80}$Fe$_{20}$ elements reflect the coercivity range presented in Fig. 4. Crucial for reliable magnetic bit encoding are sharp transitions where magnetisation is dominated by shape anisotropy, reflected by sharp changes in Kerr signal. The Co elements produce rounded and overlapping loops that can send neighbouring elements into minor loop positions or even full transitions and therefore heavily limit writing reliability, as was noted for the original bit design.$^{6,7}$ The Ni$_{80}$Fe$_{20}$ elements on the other hand, result in much sharper transitions that are well separated from one another. This is particularly evident in the bottom plot of Fig. 5, where the derivatives of the Ni$_{80}$Fe$_{20}$ CE Kerr signals show no overlap between bits. The broadening of the differential plots for high $H_L$ elements comprised of narrower strips, corresponding to more slanted hysteresis loop, is due to the greater difficulty of balancing dipolar interactions for growing arrays of strips, due to the tendency of forming antiparallel states during the reversal process. With growing arrays of strips there is also an increased likelihood of dispersion in CE strip features, where edge effect and lithography defects become more relevant in the reversal process. Nonetheless, it is evident that it is essential to use magnetically soft materials for the CE bit design to ensure reliable encoding of large bit codes.

5 Magnetic bit encoding

The reliable encoding process of the CE bit design can be demonstrated via MOKE by intentionally de-focusing the laser to illuminate multiple bits simultaneously to show compounded transitions of the elements characterised in Fig. 4 and 5. Fig. 6 shows the distinct transitions of three neighbouring elements in one MOKE loop, with strip widths of 200 nm, 400 nm and 800 nm resulting in transitions at 177 Oe, 81 Oe and 38 Oe, respectively. By placing the laser slightly off-centre between the three bits the measured changes in Kerr amplitude are proportional to the level of illumination, allowing for the identification of the switching bit both from its change in Kerr signal as well as its coercivity value. By then decreasing the applied field amplitude accordingly, either all three, two, one or no bits can be forced to flip.

Fig. 6 can now be used as a reference for an encoding process, as the different amplitude changes in Kerr signal of the de-focused laser position can be used to precisely identify each switching bit of the three element system. Fig. 7 demonstrates such an encoding process, where a 101 bit code is written from a 000 initial configuration. The plots show all transitions that the three bit system undergoes for every applied field pulse, thereby locking in the desired bit states from highest to lowest coercivity value with decreasing field amplitude. The orange X indicates when the field sequence has been completed and the desired 101 bit code is written. The schematic of Fig. 7 illustrates the same process, where the number of intermediate transitions decreases with decreasing applied field amplitude. The encoding process is carried out at 1 Hz due to induction limitations of our electromagnet, whereas the bit encoding itself can be performed much quicker (i.e. MHz range) and is only limited by domain formation and motion.$^{31}$ The de-focused MOKE technique can also be used to demonstrate encoding of longer bit codes, but visually distinguishing all individual Kerr amplitude changes becomes increasingly difficult.

6 Discussion

This investigation aims to demonstrate that the CE bit design, constructed with magnetically soft materials and appropriate
Dipolar interactions, enables a wide coercivity range for reliable encoding without compromising read-out properties. This makes the CE barcode and magnetically encoded microcarriers highly applicable for future combinatorial chemistry applications. While Fig. 4 suggests that up to 20 bits (1 048 576 codes) at ±1σ separation could be achieved using Ni₈₀Fe₂₀ CEs ranging in strip widths between 50–3200 nm, this measurement is of individual bits and does not take into account the effects of field pulses on neighbouring bits. The differential plot of Fig. 5 limits the true number of uniquely addressable bits as it is important to not send neighbouring bits into partially switched states and risk detection inconsistencies, which becomes increasingly difficult at smaller widths. We therefore make a more rigorous estimate of up to 14 uniquely addressable bits (16 384 codes) based on non-overlapping regions of Fig. 5 for widths 50–3200 nm using Ni₈₀Fe₂₀ for the CE bit design. However in theory, by using more complex sensor arrays in-flow with rigorous signal processing, it is also possible to envisage longer bit codes in the future, as demonstrated by developments in the magnetic hard disk drive industry.

The scaling advantages of bit encoding for library generation using combinatorial chemistry can be modelled by a Poisson distribution using the assumptions of consistently even (50 : 50) split-and-mix processes, a 6σ accuracy in each chemistry step and a 2σ accuracy in every encoding step. This gives a joint failure probability of \( P = 1.05 \times 10^{-6} \) for which we can find the appropriate level of degeneracy (\( \lambda \)), indicating the average number of tags per end state that are required at the onset to guarantee that every split-and-mix compound and bit code is realised in the assay. Using the equation \( P = 1 - (1 - e^{-\lambda})^n \) where \( \lambda = N/2^n \), we can determine the total number of tags (\( N \)) required for a set number of combinatorial chemistry and bit encoding steps (\( n \)). Table 1 illustrates the exponential scaling advantages in library generation with the linear scaling of split-and-mix steps. Therefore, using 384 561 tags (with 14 bits) in a 14 step split-and-mix process will ensure that at least one or more tag carries every possible compound (16 384 in total) with the correct bit code. Encoding in-flow is also possible since the time scales for Néel rotation are many orders of magnitude below that of Brownian motion.

Since a 3″ Si wafer can hold several million microcarriers (100 × 20 μm footprint) the number of required microcarriers can easily be met at low cost. Mass fabrication of feature sizes down to 50 nm can already be accomplished by existing semiconductor lithography procedures, such as interference lithography. Based on material and running costs of the bi-functional microcarrier fabrication procedure we estimate a cost of $100 for 4 million tags on a 3″ wafer, which can be further reduced when using industrial wafer sizes.

As the true advantage of magnetically encoded microcarriers is scalable library generation and not rapid diagnostics, compared to for instance nanoparticle-based applications, the relatively large size of the microcarriers is not a limiting factor. Since the area of the tags is comparable to the spot sizes used in printed microarrays, optically intensive techniques such as fluorescence detection can still be used to perform quantitative analysis. Multiplexing can then rapidly identify compounds of interest based on the magnetic stray fields of the microcarriers’ barcodes in-flow. For an alternative to fluorescence detection, it has also been shown that it is possible to incorporate Au gratings on micron sized carriers to detect binding interactions using grating-coupled surface plasmon resonance (GCSPR).
7 Conclusion

We have presented in detail the characteristics which govern the magnetic behaviour and reliability of the new CE bit design in order to demonstrate that coercivity tuned multi-bit encoding is feasible for large bit codes. The appropriate design of grouped high aspect ratio strips enables a large operation margin for coercivity tuning, with high encoding reliability and without limiting read-out properties. While the successful functionalisation of the epoxy and thiol surface groups on a single microcarrier has already been demonstrated, the proposed tagging technology can be extended to incorporating other well-established surface chemistry processes, by for example adding a sputtered silicon dioxide coating. As the barcode and microcarrier designs are highly adaptable, magnetic encoding of microcarriers using the CE bit design has broad implications for the lab-on-a-chip community, with a wide range of potential applications relevant to the plethora of existing libraries of biomolecules which would benefit from a rapid method of compound detection, identification and sorting.

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Table 1

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References
