Bi-functional Magnetic Microcarriers for Point-of-Care Diagnostics

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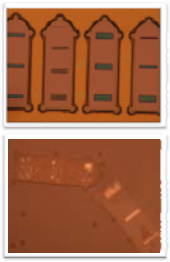
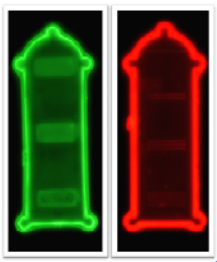
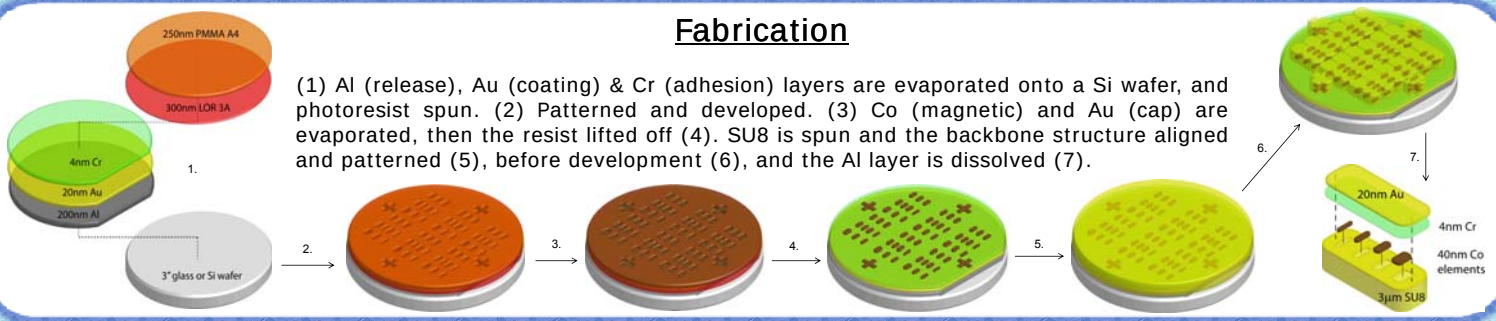
Introduction



Encoded microcarriers are finding great application in the multiplexed detection of microscopic analytes, where the identity of an attached probe molecule (ss-DNA/RNA, protein, antibody etc.) is given by the microcarriers' unique signature. This signature is usually optically determined (e.g. Luminex), but suffer inherent disadvantages, such as scalability and background noise (autofluorescence of biological fluids). We use re-writable magnetic thin films to carry a binary barcode, so the number of codes scales as 2^N by increasing the number of bits, N. The barcode can be read, post-assay, within a microfluidic channel containing a tunneling magnetoresistive (TMR) sensor [2]. The microcarriers themselves are 100 by 35µm in size and have two distinct surfaces, an epoxy polymer (SU8) [3] and a gold base. Onto either we can attach probe molecules, and use fluorescence merely to identify a positive binding event.



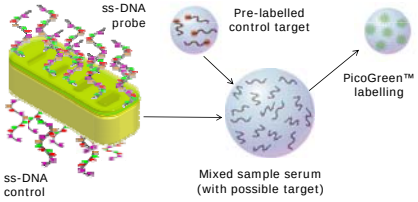
Fabrication



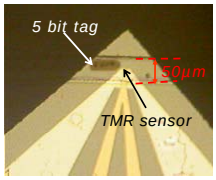
(1) Al (release), Au (coating) & Cr (adhesion) layers are evaporated onto a Si wafer, and photoresist spun. (2) Patterned and developed. (3) Co (magnetic) and Au (cap) are evaporated, then the resist lifted off (4). SU8 is spun and the backbone structure aligned and patterned (5), before development (6), and the Al layer is dissolved (7).

DNA Assay

By designing the chemical pathway we can add different probes to the SU8 and gold sides, and is demonstrated here by labelling with fluorescein (AMF) and rhodamine (TAMRA) respectively. This concept can be easily adapted for use as a positive control in a bioassay, for example:



... single stranded (ss) DNA, which is both labelled and complementary to the control ss-DNA, is added to the sample serum before the microcarriers are introduced and allowed to hybridise. PicoGreen™ is a dye used to differentiate between single and double stranded DNA, showing enhanced fluorescence with the latter [4].

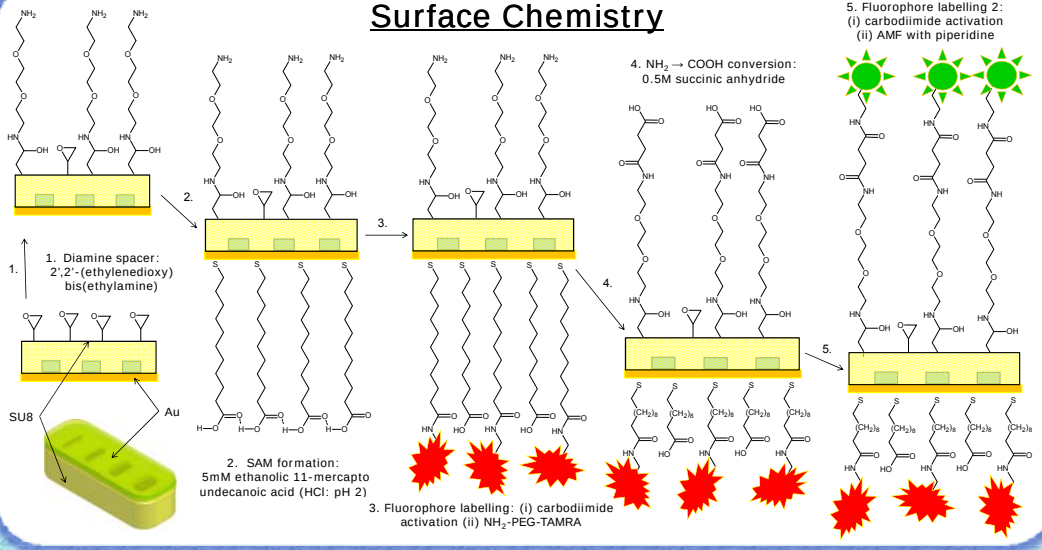


The microcarriers are washed free of non-bound reactants, and then flown through a microfluidic reader, which extracts all the information:

- Red fluorescence → confirms hybridisation,
- Green fluorescence → shows presence of target,
- Magnetic signature → identifies the probe.

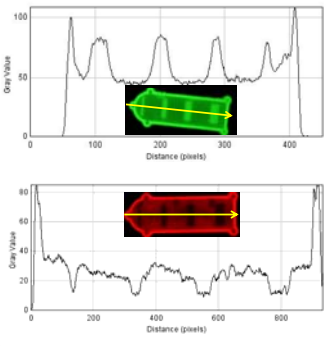
Alternatively, the first check can be made before adding PicoGreen™.

Surface Chemistry



Results

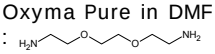
Following the above fabrication and surface chemistry procedures, the microcarriers were imaged with a fluorescence microscope.



Line profiles clearly distinguish the microcarriers' orientation, i.e. if the fluorescence is on the top side there are reflection peaks from the metal elements, whereas the inverse is true for the underside.

The fluorescence intensity from the AMF was so high at neutral pH that it masked the TAMRA. On addition of HCl the AMF signal decreased, which allowed us to verify that the TAMRA target had bound.

Conclusions

- We are pioneering a novel microcarrier for multiplexed diagnostics, based on a magnetic encoding technology.
- We have demonstrated an increase in the flexibility of possible assays by introducing a second chemical functionality to our microcarriers, for instance, as a control against false negatives.
- Using different fluorophores (e.g. cascade blue with a small Stokes shift) would prevent potential problems due to spectral overlap.
- Preliminary surface chemistry studies compared homobifunctional spacers of varying chain length [3]. We also optimised the activation step, and found that DiC and Oxyma Pure in DMF gave the highest overall yields with the spacer: 

References and Acknowledgements

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