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Towards MRI microarrays

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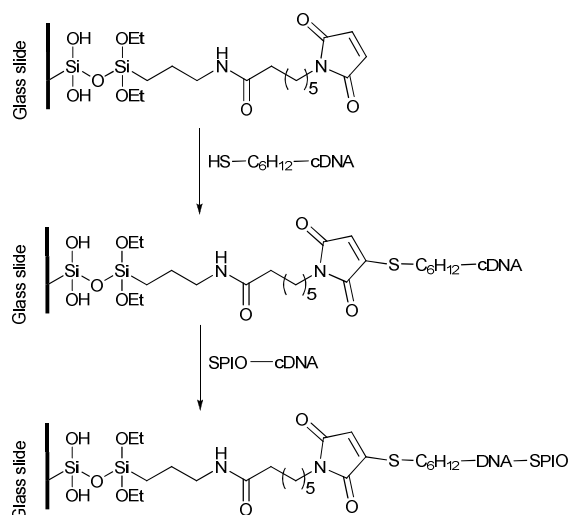
Super paramagnetic iron oxide nanometre scale particles have been utilised as contrast agents to image staked target binding oligonucleotide arrays using MRI to correlate the signal intensity and T_2^* relaxation times in different NMR fluids.

Microarrays are a high-capacity technology that facilitates the simultaneous monitoring of expression of thousands of genes or proteins,^{1–3} and often constitute a critical start-point for further analyses. Commonly, assays involve the detection of interactions between hybridised probes and a target sample *via* fluorescent labelling or radioisotope markers.⁴ Other techniques including Kelvin micro-probing⁵ and matrix-assisted laser desorption/ionisation mass spectrometry have also been demonstrated.⁶ In molecular biology, the use of multiple probes, typically consisting of messenger RNA or complementary DNA oligonucleotides (cDNA), can be used to identify useful diagnostic biomarkers for diseases including cancers.⁷

There are several methods that can be used for the hybridisation of probes to a substrate;^{8–10} however, robotic spotting and photolithography are by far the most common. In spotted microarrays, products from a polymerase chain reaction are printed using a robot, or alternatively with a method devised from those used in ink-jet printing.⁸ Photolithographic microarrays involve the stepwise addition of, for example, nucleotides by selective protection and de-protection of localised areas of substrate.^{9, 10} In addition to the detection of gene products, these technologies can be applied to protein arrays and chemical sensors.¹¹ Both of these techniques implement the use of fluorescent markers on probes to detect hybridised interactions on the array.¹²

The present fluorescent systems have several drawbacks, for example, non-specific binding and cross-reactivity can result in false-positive and false-negative data in complex biological samples, resulting in the requirement for further analyses by, for example, quantitative real-time polymerase chain reaction.^{13, 14} Furthermore, the sensitivity of fluorescent detection is reduced by processes such as quenching, resulting in the need for further resources to be used in order to optimise the experimental data.¹⁵ The potential scope for detecting specific entities of interest within complex samples is limited further by the presence of spectral overlap. This limits the number of fluorophores that can be utilised within

an experiment, which in turn dictates the ultimate maximum throughput. Additional problems associated with the use of spotted arrays include the presence of irregular shaped or sized spots, the use of porous substrates and the presence of contaminating material, all of which can interfere with fluorescence measurements. Herein, an alternative detection system using magnetic resonance imaging (MRI) is demonstrated that uses insertion of the microarray in a nuclear magnetic resonance (NMR) imaging fluid and which does not depend on fluorescent probes.



Scheme 1 Probe-target binding

MRI is conventionally used to non-invasively image major organs including the heart and lungs, providing evidence of structural and functional changes that may be associated with disease. It involves the use of magnetic field gradients to determine an NMR signal from localised volumes of a sample. The measurement of NMR relaxation times (T_1 , T_2 and T_2^*) and subsequent frequency analyses allows the reconstruction of complex three-dimensional images. Superconducting magnets are conventionally utilised in MRI scanners; however, instruments that utilise permanent magnets to unilaterally image samples placed above a homogenous magnetic field have recently been developed,¹⁶ thereby opening up the sample accessibility in this versatile imaging technology.¹⁷

By substituting the habitual fluorescent markers used in traditional microarray technologies with an MRI contrast agent, namely super-paramagnetic iron oxide (SPIO), a novel MRI readable DNA array system has been developed.¹⁸ The interaction between probe and target cDNA strands can be monitored and imaged using MRI to detect localised changes in the NMR signal coming from a fluid saturating the system. The addition of a SPIO marker to a complementary cDNA strand results in the enhancement of T_2^* relaxation times *via* the increased dephasing efficiency of the surrounding fluid's protons.

The preparation of the MRI readable DNA arrays is summarised in Scheme 1. Typically two target oligonucleotides were immobilised onto a glass surface *via* a silylated *N*-malimidoalylamide bridge into a 13×6 array. The glass microscope slides (76×26 mm) were prepared by etching 78 circular spots (diameter 2 mm) into a 13×6 arrangement with separations of 2 mm using a Hobart's laser cutter linked to coreIDRAW software. Slides were then cleaned by being immersed in a methanolic solution of sodium hydroxide overnight, rinsed in distilled water, immersed in HCl (37 %) for 2 hours, further rinsed in water and stored in diethyl ether. Freshly prepared *N*-(3-triethoxysilylpropyl)-6-(*N*-maleimido)-hexanamide, TPMH, in acetonitrile (0.01 M, 10 μ L)¹⁹ was manually spotted onto the dried template surface using an automated pipette and irradiated for 3 × 15 seconds in a domestic microwave oven (800 W).

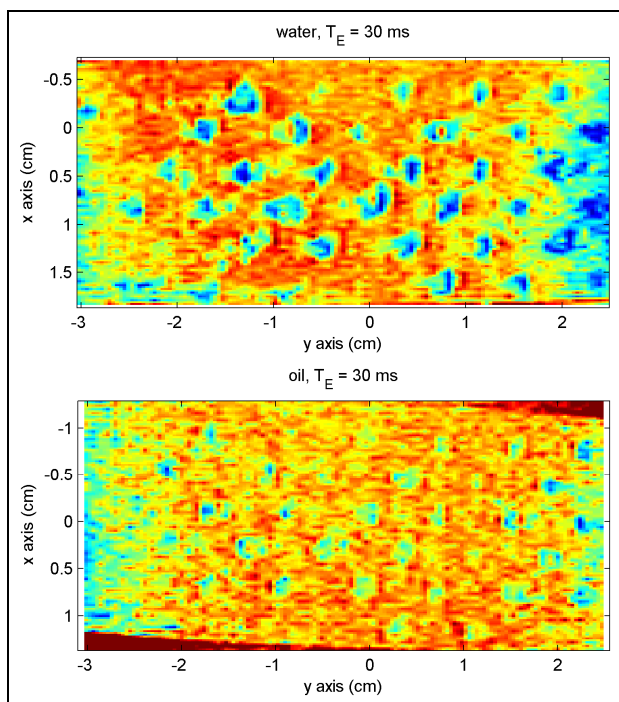


Fig. 1 Colour-coded MRI images of our system saturated with (a) water and (b) silicone oil (MRI parameters: $T_E = 30$ ms, $T_{E_{\text{eff}}} = 1.905$ s, 16 averages, slice thickness = 0.7 mm, imaging time = 61 s, RARE factor = number of lines = 128). Misregistration of the spots between the two images is due to the different sample's relative position when the fluids were changed. Although MR images demonstrate alternatively 'labelled' spots, the image with the fluid exploiting the highest self-diffusion coefficient (water) clearly exhibits superior contrast.

Complementary and non-complementary oligonucleotides (5'-GTCCAGCAGACCTTCTCTCAGGAG-3') and (5'-CTCCTGAGGAGAAGGTCTGCTGGAC-3'), respectively, were modified with the incorporation of a terminal thiol group linked to the 5' end *via* a hexane spacer (Sigma-Genosys)[‡] were spotted (1 μ L) on top of the pre-spotted TPMH in an alternating arrangement. The array was then incubated at 37 °C in a humidified chamber for 1 hour and washed with water. Unmodified 5'-CTCCTGAGGAGAAGGTCTGCTGGAC-3' oligonucleotide was labelled with dextran-coated SPIO,²⁰ being added to the freshly prepared oligonucleotide array and left to hydrolyze in a humid chamber for 24 hours. The arrays were subsequently washed with water and arranged into a fluid filled stacked microscope slide tray for analysis. Two separate NMR imaging fluids were tested, water with a self diffusion constant of $D_0 = 2 \times 10^{-9}$ m² s⁻¹ and a silicone oil with $D_0 = 1.9 \times 10^{-10}$ m² s⁻¹.

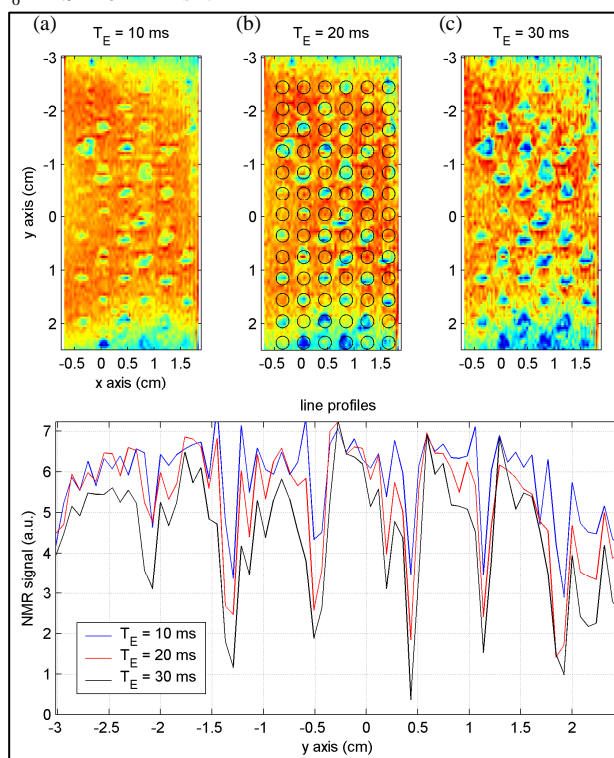


Fig. 2 Colour-coded MRI (RARE sequence) images (black circles have been added to highlight the boundaries of the alternatively SPIO-labelled spots) of the same water saturated system imaged with increasing echo time: (a) $T_E/T_{E_{\text{eff}}} = 10$ ms/635 ms, (b) $T_E/T_{E_{\text{eff}}} = 20$ ms/1.27 s, (c) $T_E/T_{E_{\text{eff}}} = 30$ ms/1.905 s). The lower plot (d) shows the line profiles for each of the three images at horizontal coordinates, $x = 0.45$ cm. All data were scaled so as to reach the same maximum pixel intensity.

A Bruker[®] 2.35 T small-bore BIOSPEC MRI scanner was used to image the fluid-saturated arrays using RARE scan protocols.²¹ This allowed a complete set of MR images of a sample volume to be obtained and 2D slices corresponding to a given array within those images to be extracted.

Results illustrated in Fig. 1 and 2 show that DNA hybridisation can be identified on spots with negative contrast. This loss of signal is caused by exploiting the T_2^* contrast, as is demonstrated by the two independent observations that using a fluid with higher diffusion

coefficient (Fig. 1) or a longer echo time (Fig. 2) produces better contrast. From Fig. 2, the ratio of NMR signals coming from two different locations, one without and one with immobilised SPIO, is seen to be as high as 14 for TE = 30 ms, whilst the signal to noise ratio is approximately 10. The definition of the spots is improved when using water (Fig 1a), which has a molecular self-diffusion coefficient ten times higher than that of the oil used (Fig 1a).

The line profiles of three experiments, wherein the TE of the sample in water is increased from 10 to 30 ms (Fig. 2), at $x = 0.45$ cm, demonstrates that there is an enhanced contrast with longer TE times. This is also clearly evident, by simply observing the relative contrast differences, by eye, in the three images of the same array, when all other MRI parameters remain constant (16 averages, slice thickness = 0.7 mm, imaging time = 61 s, RARE factor = number of lines = 128). Increasing TE improves contrast, at the cost of loss of spatial resolution and SNR. Spatial resolution is also limited by the spatial extent of the magnetic perturbation, an effect which we have not quantitatively explored.

In all measurements the selected slice was set up so as to capture the signal coming from the fluid residing just above the array. For all images the colour-coding spans all signal intensities (in arbitrary units) found in the data in order to display the genuine contrast. Signal variations on all MRI images are also seen on a large length scale due to drop in the B_1 homogeneity of the scanner's resonator and due to the selected slice being slightly off-horizontality (the horizontality of the array was set by using a spirit level).

In summary NMR microscopy has been demonstrated to be a suitable technique for microarray analysis when using magnetic particles as labelling compounds. Binding has been identified by the local signal intensity modulated by the local relaxation times. At high polarising field strength, the SPIO's effect within NMR is mostly found as a perturbation on the magnetic field surrounding the SPIO, resulting in signal loss. This effect can be modulated with outstanding flexibility by altering the nature and density of the SPIO, by changing the polarising field strength, the sequence echo time, the MRI sequence itself, and it is also dependent on the chosen NMR imaging fluid. At low field strength, SPIO can also be used for enhancing the measured NMR fluid signal by lowering the local T_1 value.²² The method we demonstrated does not require optical transparency of the sample, thereby offering potential measurements on high density porous spots. It could work with NMR gases, including hyperpolarised ones.

MRI was also found to be a suitable technique for the simultaneous analyses of multiple stacked slides (3D arrays) for high density throughput. We are working to further optimise the resolution of these array systems to sub millimetric spots which can be resolved in less than one minute.[†]

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Footnotes

[†] Electronic Supplementary Information (ESI) available: Preliminary experiments on millimetric spots have been identified within an imaging time below one minute. The binding strategies for the DNA array on a hydrophobic binding surface are also include. See DOI: 10.1039/b925020d

[‡] Complementary 5' to 3' and 3' to 5' oligonucleotide sequences (5-CTCCTGAGGAGAAGGTCTGCTGGAC-3 and 5-GTCCAGCAGACCTTCTCCTCAGGAG-3) were modified with the incorporation of thiol (-SH) groups linked to the 5' end by a 6-carbon spacer (Sigma-Genosys) and subsequently reconstituted in water to a concentration of 100 μ M. Trityl groups were removed by incubation with 0.04 M DDT in 0.17 M phosphate buffer (pH 8.0) at room temperature for 16 hours. DTT and thiol by-products were removed using NAP-10 columns, following the manufacturer's protocol (GE healthcare). Finally the oligonucleotide fractions were verified by taking readings at 260 nm, pooled and diluted to stock aliquots of 60 μ M.

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