Research Highlights

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Particles with diffractive micro bar codes for multiplexed assays

In high-throughput screening experiments, a key requirement is the fast and sensitive identification of individual molecules within a library of probes. A common approach is the use of microarray systems, in which different biomolecules can be identified by their position on a two-dimensional grid. A promising alternative is the employment of suspended arrays, in which probes are attached to the surface of microparticles that carry a unique bar code. The bar code allows precise identification of individual microparticles. For high-throughput analysis it is important that the code can be identified reliably and quickly and hence, an appropriate encoding strategy is crucial for the applicability of suspended arrays. A number of strategies have been proposed; the most common rely on spectral encoding, based on fluorescence wavelength and intensity. However, a major drawback of this method is that many biochemical assays likewise rely on fluorescence readout, which limits their use particularly for multiplexed analyses.

Researchers from the University of Southampton (UK) have recently developed a novel and robust encoding system based on microparticles that contain diffractive elements as identifiers.[1] The incorporated micrograting of the particle diffracts incident light into a unique diffraction pattern, and, hence, the spatial distribution of the diffracted light represents the "code" of each particle (Fig. 1). Image capturing by a CCD camera and automated image processing software enable the identification of the particles in less than 1 ms. The technology has the potential to provide huge numbers of encoded particles, up to 10^{18} for 50 μm-sized particles. The microparticles are fabricated by photolithography of a commercial epoxy photoresist (SU-8). On the surface, probe biomolecules can be immobilized. By using biomolecules tagged with fluorescent dyes, the reaction kinetics of molecular interactions can be studied on individually encoded microparticles by means of fluorescence spectroscopy.

The authors demonstrate the applicability of the technology for genetic assays and immunoassays by determining the ordinary functionality of biomolecules such as immunoglobulin G (IgG) and DNA that are immobilised on the particle surface. By kinetic and thermodynamic characterisation of analyte binding it is confirmed that binding assays can be carried out in short time scales, allowing small amounts of analytes (< 20 fmol in 1 μL volume) to be detected. Furthermore, the potential for multiplexed analyses is demonstrated using particles with different bar codes. Among various examples of bioassays, a multiplexed immunoassay of selective antigen–antibody binding is performed (Fig. 2). For this experiment, sets of particles are prepared and functionalised with different IgGs (human, rabbit, mouse and guinea pig IgG). Antibodies that are labelled with fluorescent dye are captured by the respective microparticle and can be identified with high sensitivity in a modified microscope setup capable of reading diffraction and fluorescence intensity. By further improvement of the system and automation of analyses the authors expect to achieve a throughput rate comparable to conventional flow cytometers, and envisage using the device for directed sorting of particles.

Remote detection of NMR with a microchip sensor

Nuclear magnetic resonance (NMR) spectroscopy and magnetic resonance imaging (MRI) are powerful techniques to obtain structural information of molecules in solution and solid-state. However, due to the low sensitivity of NMR spectroscopy, the applicability of this technique in combination with microfluidic devices was limited in the past. In a recent work, Alexander Pines and co-workers from Lawrence Berkeley National Laboratory and University of California, Berkeley, have made NMR spectroscopy compatible with microfluidics.[2] The key advance of their work is the realisation of remote detection using a compact sensor assembly (the microfabricated atomic magnetometer) that consists of a microfluidic channel, and an alkali vapour cell. The term "remote detection" refers thereby to the spatial separation of the three essential elements of a nuclear magnetic resonance (NMR) experiment, *i.e.*, nuclear spin polarisation, encoding and detection. An important advantage of remote detection is that encoding and detection occur at zero magnetic fields, which eliminates the need for a solenoid around the detection region, and hence increases the proximity of sensor and sample. This configuration allows operation of the atomic magnetometer in the so-called spin-exchange

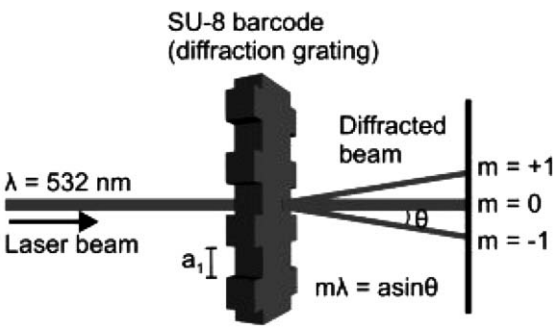

Fig. 1 Microparticles with diffractive bar codes. The sketch shows the first-order diffraction of laser light from a grating with a pitch a_1. (Reprinted from G. R. Broder *et al.*[1] Copyright 2008 American Chemical Society.)

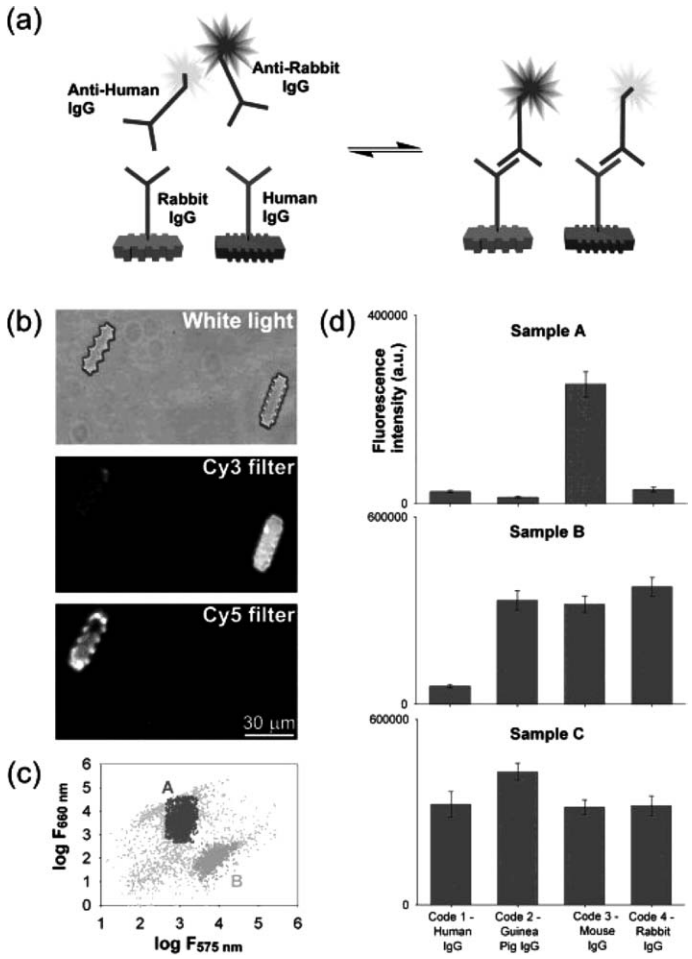

Fig. 2 Encoded microparticles are used in multiplexed immunoassays. (a) Scheme of the immunoassay, in which antibodies are immobilised on the encoded microparticles, and detection antibodies are labelled with the fluorescent dyes Cy3 and Cy5, respectively. (b) Antibodies bound to the microparticles are identified using fluorescence microscopy and (c) conventional cytometry. (d) A quadruplex immunoassay is performed, *i.e.* four types of microparticles with unique bar code are used, each of them carries a specific immunoglobulin G. (Reprinted from G. R. Broder *et al.*[1] Copyright 2008 American Chemical Society.)

relaxation-free (SERF) regime, which is currently the most sensitive technique in atomic magnetometry. The experimental setup is shown in Fig. 3. The sensor chip is fabricated in a Si substrate that is anodically bonded to glass. It consists of a vapour cell containing 5000 torr of N_2 buffer gas and Cs, which is sandwiched between two indium tin-oxide (ITO) resistive heaters. Adjacent microchannels integrated on the same chip supply the sample (water). Remote detection of pulsed and continuous-wave NMR is demonstrated. Pulsed NMR linewidths of $\sim$ 26 Hz are achieved, which is, in the opinion of the authors, limited by residence time and flow dispersion in the encoding region. The authors estimate that for an integration time of 1 s and a relatively modest prepolarising field of 10 kG, $\sim 7 \times 10^{13}$ protons can be detected in a volume of 1 mm^3 with a signal-to-noise ratio of 3, which is competitive with those demonstrated by microcoils in superconducting magnets. Hence, this novel technique offers a promising solution to NMR of mass-limited samples, and might be useful *e.g.* for drug screening.

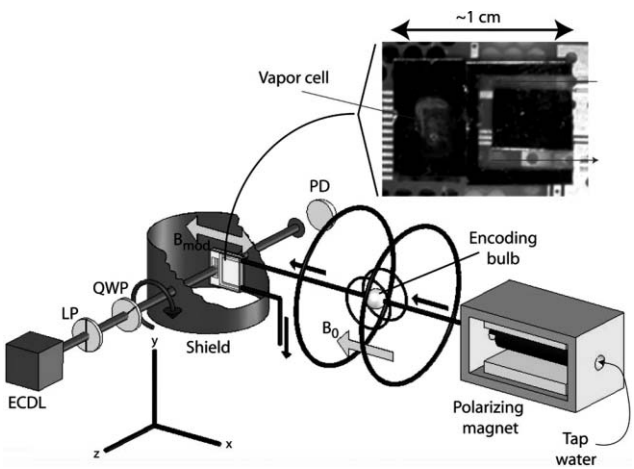

Fig. 3 Experimental setup for remote detection of nuclear magnetic resonance. The microfabricated chip consisting of an alkali vapour cell (atomic magnetometer) and a microfluidic channel is shown in the inset. (From ref. 2. Reprinted with permission. Copyright 2008 National Academy of Sciences, USA.)

Visualisation of flow in a microreactor by NMR imaging

In various studies, it has been proven that highly efficient reactions can be performed in flow-mode microreactors that contain catalysts immobilised on solid support. To fully optimise microsized catalyst bed reactors, computational models as well as experimental validations are required. With the goal to characterise packed-bed microreactors Alexander Pines and coworkers present in a further work a spectroscopic method based on magnetic resonance imaging (MRI) that uses hyperpolarised spins derived from *para*-hydrogen (p-H_2).[3] The use of MRI to characterise microreactors offers several benefits compared to alternative techniques, because the technique is non-invasive, can probe optically opaque media, and various parameters can be mapped with considerable chemical and spatial selectivity. The researchers employed two model catalytic reactors to visualise the gas-phase flow and the density of active catalyst. Both microreactors are filled with propylene and p-H_2, and the reaction of the gases, catalysed by Wilkinson's catalysts, yields

propane. While the first type of microreactor contains a tightly packed bed of the silica-immobilised catalyst for the reaction, the second microreactor comprises powdered catalyst and represents a more heterogeneous packing. The method enables correlation of the spatial distribution of the reactive conversion inside a reactor with the morphology and packing of the catalyst. In future, it might be applied to a wider range of hydrogenation reactions and support the development of improved reactor and catalyst design.

RNA sequencing by tip-enhanced Raman spectroscopy

Established methods to sequence DNA or RNA require substantial amounts of molecules and are not suitable to directly read the base composition of a single strand. Many attempts to sequence DNA or RNA on a single molecule level were based on fluorescence spectroscopy and failed due to the lack of appropriate labelling strategies to differentiate between the four individual bases. Label-free techniques that utilise the inherent information of the distinct base are thus extremely valuable in the context of single-molecule sequencing. In a current study by Elena Bailo and Volker Deckert from ISAS–Institute for Analytical Sciences, Dortmund (Germany), tip-enhanced Raman scattering (TERS) is employed to directly read the sequence along a single isolated strand of RNA.[4] While standard Raman spectroscopy facilitates the straightforward identification of individual bases, a high lateral resolution is achieved by tip-enhanced Raman scattering. Using a silver-coated AFM tip diameter of < 20 nm, high signal sensitivity in short acquisition times is observed with a resolution down to a few tens of nucleobases. The authors measured the topography as well as the Raman spectra at several positions of a poly(cytosine) RNA strand. The spectra show all the main features of cytosine and single-base sensitivity has been realised. It is important to note that a lateral resolution of single bases is not required. Although the evaluation of data is easier when only a few bases contribute to the spectrum, it is sufficient if single base sensitivity is achieved while the tip and the sample are shifted in intervals of at least base-to-base distance. Spectral changes from one position to the next can then be attributed to variations in the sequence. The authors expect to employ this technique in future to obtain sequence information of other biological macromolecules such as DNA and peptides.

Shrinky-Dink microfluidics

A cheap, non-photolithographic alternative to fabricate microchips is presented by researchers from the University of California, Merced and Berkeley. The technique uses "Shrinky Dinks", a children's toy, onto which one can draw a picture and subsequently shrink it to a small fraction of its original size. This toy was first exploited to fabricate moulds for PDMS chip fabrication.[5] In these experiments, the microfluidic channel pattern is printed onto a Shrinky-Dink transparency (a polystyrene thermoplastic sheet). The height of the pattern is determined by repetitive printing on the sheet. After being baked, the pattern shrinks by 62.5%, and further post-bake improves the smoothness of the ink features. In the current *Lab on a Chip* issue, more complex three-dimensional, stacked microchips are fabricated directly in the Shrinky Dink polystyrene sheets.[6] Different layers are prepared by scribing the pattern into the sheet using a syringe tip. The scribing process can be likewise adapted to milling machines or computer-controlled plotters. After punching holes and aligning the layers, the assembly is placed in an oven for simultaneous shrinking and bonding to form the three-dimensional microfluidic chip.

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