Optical and impedance detection of DNA hybridisation using barcoded micro-particles in a microfluidic device

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ABSTRACT

Micron-sized polymer particles are commonly used in chemistry as substrates for attaching proteins and DNA. The analysis of such particles is important for a wide variety of biochemical assays [1]. One area of particular interest is the development of encoded or barcoded particles. The use of such particles allows for the realization of a number of novel combinatorial chemistry processes and multi-analyte assays. In this paper we describe a microfluidic device for the analysis of microfabricated barcoded particles. This allows specific particles to be identified by their optical diffraction pattern as they pass through a flow channel in a microfluidic device. The device also has optical (fluorescence and diffraction) and broadband impedance measurement capabilities allowing the full characterization of the encoded particles as they pass through the device. Barcodes are fabricated from the SU8 photo-epoxy using standard photolithographic techniques. DNA chemistry is performed on these particles. In this paper we present data showing the ability of the system to detect DNA hybridization on the surface of polymer beads.

Keywords: microFACS, flowcytometry, encoded particles, diffraction, impedance based detection

1 INTRODUCTION

A common problem with high throughput combinatorial chemistry is the inability to easily analyse and track the multitude of molecules produced. This bottleneck is a major problem for the big pharma and other researchers working in the fields of drug screening, DNA analysis, etc. To this end we have developed a microfluidic device capable of rapidly analysing the optical and impedance properties of individual polymer beads. Such beads are commonly used as solid supports for combi-chem and DNA synthesis. We have further developed the system by the use of barcoded microparticles [2]. Each bead is uniquely identifiable by its optical diffraction pattern and can be tracked within the device allowing correlation between this unique code (which can be associated with the beads chemical history) and the optical and impedance measurements performed on the individual beads. The device is capable of processing particles at high-speed and we are developing on-chip sorting techniques to allow particle sorting based on the analysis of the bead [3].

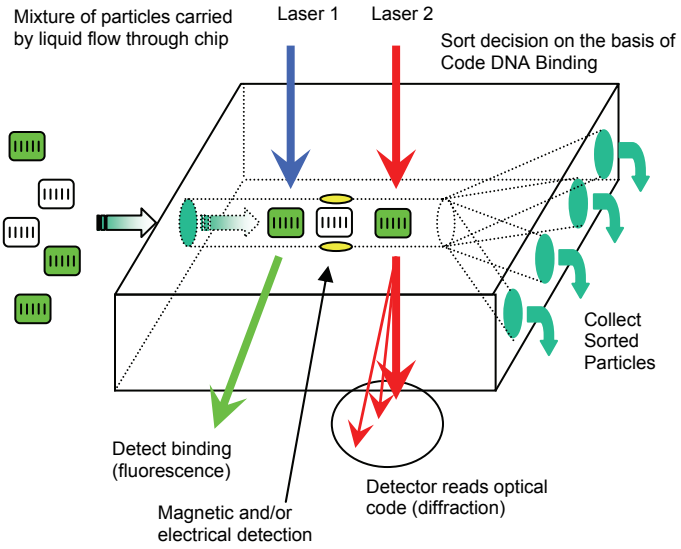

Figure 1: Schematic illustrating the operation of the microfluidic device.

Figure 1 shows a schematic diagram of the system. Barcoded particles enter from the left and flow through the devices. Electric fields generated by electrodes fabricated on the inner walls of the flow channel are used to position the particles as they flow through the channel. The manipulation of the particles is critical to allow accurate impedance and optical measurements to be performed. Alignment of the barcoded beads along the flow axis of the microfluidic channels is also critical to ensure the diffraction pattern is readable by the detection system. We use a combination of hydrodynamic focusing and dielectrophoresis [3,4] to allow the precise positioning and orientation of the particles, ensuring all the particles entering the device flow through the detection zones in single file with constant velocity and orientation. As the particles pass through the detection zone information about their fluorescence, impedance and diffraction pattern is

collected. This data is processed and stored on a pc. Particle sorting is achieved by the use of electric fields (again dielectrophoretic forces) to deflect the particle into one of the outlet channels (for discussion of the sorting mechanisim see [3]). The sort decision is user definable and is based on the information gathered as the particle passes through the detection zone.

2 MATERIALS AND METHODS

2.1 Microfabrication

2.1.1 Microfluidic chips

The microfluidic chips are constructed using microfabrication techniques and a full wafer thermal bonding technique [5]. The basic process was as follows; 500mm thick 4" pyrex wafers were cleaned and 100nm of Ti was evaporated onto the pyrex surface. A layer of S1813 photoresist was spin coated onto the metal layer and patterened using photolithography. The S1813 acts as a masking layer for the metal which was then patterned using ion beam milling. Onto the metal patterned substrate polyimide was spin coated and patterned photolithographically to form the flow channels. Two identically processed wafers were then thermally bonded using an EVG aligner bonder system. Individual chips were then released from the wafers via dicing with a diamond saw. Inlet and outlet holes were then drilled in the top of the individual chips to allow fluidic access to the microfluidic channels.

2.1.2 Barcoded particles

Two types of barcoded particles were fabricated; the first a single SU8 layer with the diffraction grating on the side walls of the particle, and the second a two layer process with the diffraction grating being formed on the upper surface of the SU8 particle in a second layer of SU8. In order to increase the optical contrast, SU-8 was mixed with different metallic nano-particles (e.g. Au and Ni colloids). A 50nm thick sacrificial Al layer used to allow release of the SU8 particles from the silicon substrate.

Microposit primer was spin coated on top of the Al sacrificial layer prior to SU8 coating. SU8-25 was spin coated onto the silicon substrates to give an SU8 layer thickness of 15 – 20 μm. Soft bake of the SU8 was carried out. Photolithography was then carried out followed by a post exposure bake to fully cross-link the SU8. Development was then carried out to reveal the SU8 bars. For the double layer barcodes processing was identical with a different mask being used to define the initial SU8 layer. A second layer of lower viscosity SU8, SU8-2, was then spin coated over the entire substrate to achieve a 2 μm thick layer of SU8 on top of the previous SU8 layer. This was followed by a soft bake and a second photolithography step was then carried out to define the diffraction gratings in this thin layer of SU8. A schematic of the fabrication process is shown in figure 2.

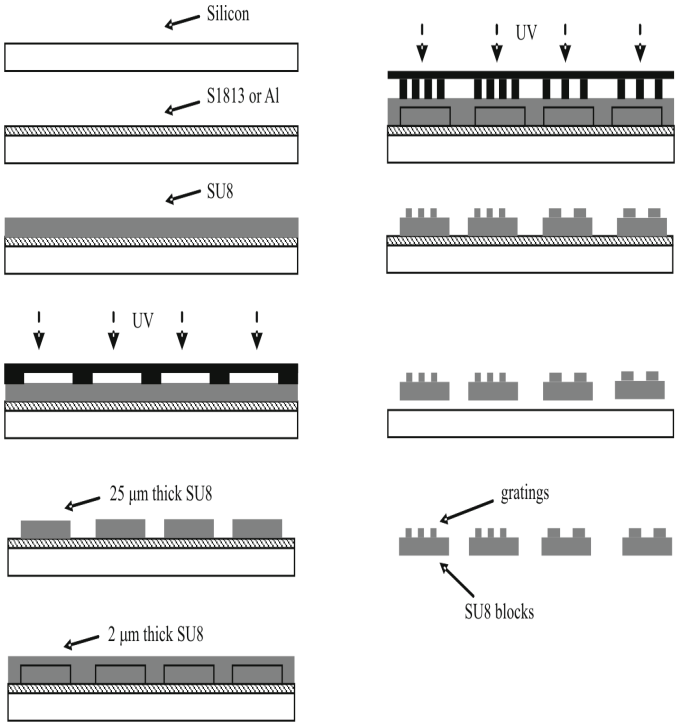

Figure 2. Schematic of the barcode fabrication process.

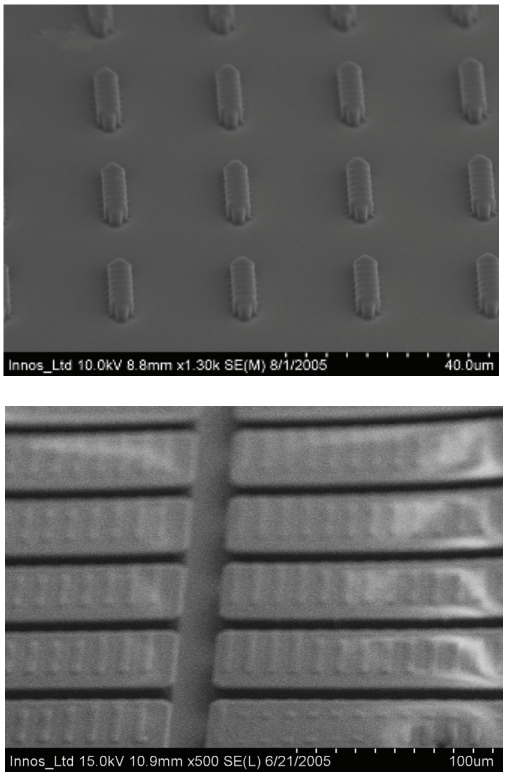

Figure 3: Microfabricated SU8 barcodes (a) single layer (b) dual layer (pictured on wafer prior to release).

The aluminium layer was etched using Rom and Hass MF 319 developer to release the bars from the substrates. After immersing the wafers into the solution and leaving for few seconds the bars were released. These were then subsequently filtered and washed with water followed by acetone. Microscope images of the single layer and double layer SU8 barcodes attached to the Si wafers are shown in figures 3(a) and 3(b) respectively.

2.2 Experimental setup

2.2.1 Optical detection

Fluorescence

The detection of fluorescent particles passing through the microchip was implemented using a bespoke inverted microscope. A region of the channel, approximately 20μm downstream of the focussing electrodes was imaged through a x20 0.75NA objective lens. Incident light from a 10mW HeNe laser (632.8nm) was beam expanded then focussed into the chamber using the objective lens to an approximate spot size of 5μm diameter. Fluorescence emission from the particles is then filtered using a Cy5 filter set, passed through a spatial filtered (pinhole 50μm) giving a small detection volume of approximately 1μm across. This light was detected using a photomultiplier (Hamamatsu).

Diffraction

In order to obtain that information encoded in the barcode the far-field optical diffraction pattern is read. The position of the first order diffracted spot in this pattern can be used to recover the period of the tag; this is used as a unique identifier. A second laser beam is used, this is beam expanded and focussed onto the back focal plane of the objective, resulting in a narrow beam of collimated light being emitted by the objective lens. This collimated laser beam produces a diffraction pattern as the particle passes through it. A CCD (or linear diode array) is used to detect the diffraction pattern from a particle. Dedicated electronics were used to read the position of the 0-order and 1st-order diffraction spots.

2.2.1 Impedance detection

The impedance measurement system can measure several discrete frequencies simultaneous, typically between 10 kHz and 10 MHz. The source signal frequencies are digitally synthesised, mixed and then applied to the electrodes through two bridge balance resistors giving typical electrode voltages of between 200 and 300 mV. Differential voltages are measured across each arm of the bridge using a high speed instrumentation amplifier and lock-in amplifiers (Stanford Research), one for each probe frequency. This circuit arrangement produces a characteristic complex voltage signal which has two peaks, each one corresponding to the movement of a particle through one half of the two sets of electrode pairs.

Figure 4 shows the experimental setup. For both optical and impedance collection, the data was digitised using a 16 bit A/D card (National Instruments), time-referenced and stored on hard disc for post-processing using N.I. LabVIEW software.

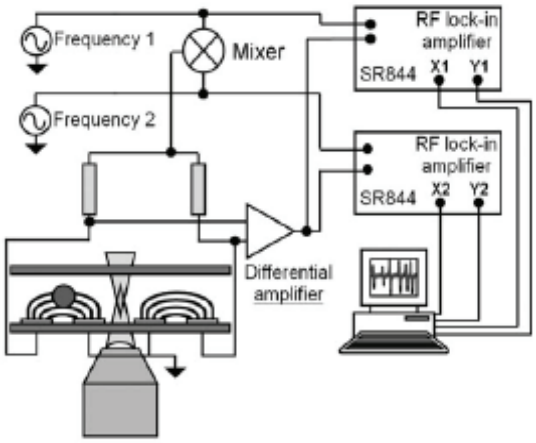

Figure 4: Schematic showing the experimental setup.

2.3 DNA chemistry

In order to attach DNA to the particles the following coupling chemistry was performed. Particles were functionalised with an Fmoc-6-aminohexanoic acid spacer using standard amide coupling conditions (HOBt, TBTU, DMF). After deprotection, the amino groups were reacted with succinic anhydride in basic media to provide carboxylic acid groups on the particle surface. The 5'-amino ssDNA probes were attached onto the carboxylic surface of the particles through amide coupling using EDC (Imidazole buffer). After coupling the particles were washed with Tween 20 (0.02%). For the hybridisation a complementary ssDNA labelled with Cy5 at the 5' end was used. A non-complementary strand was also used to study the extent of non-specific binding of ssDNA. Hybridisation was performed in SSCE buffer. Samples were heated for 1 min at 90 ºC and then shaken at 45 ºC for 2 hrs and then overnight at 35 ºC. After hybridisation the particles were again washed with Tween20.

3 RESULTS AND DISCUSSION

Figure 5 shows a typical diffraction pattern obtained from a single layer barcoded particle within the microfluidic chip. The 0-order is clearly visible along with the 1st-order diffraction. The distance between the 1st-order and the 0-order is calculated to allow identification of the particle. Due to restrictions in photolithographic resolution, the period of barcode patterns currently cannot be smaller than 3 - 4 μm, corresponding to a small diffracted angle of 10 -

12°. Nano-imprinting of the barcodes will allow us to achieve higher quality diffracting particles.

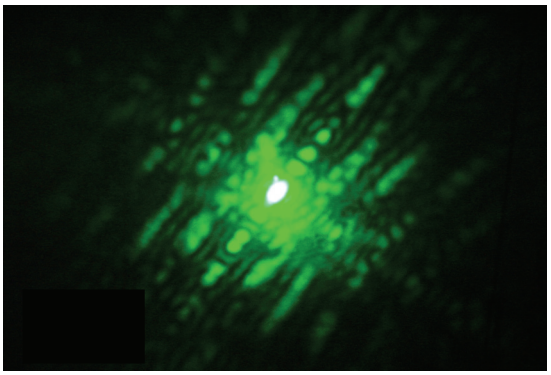

Figure 5: Diffraction pattern from an individual barcoded particle.

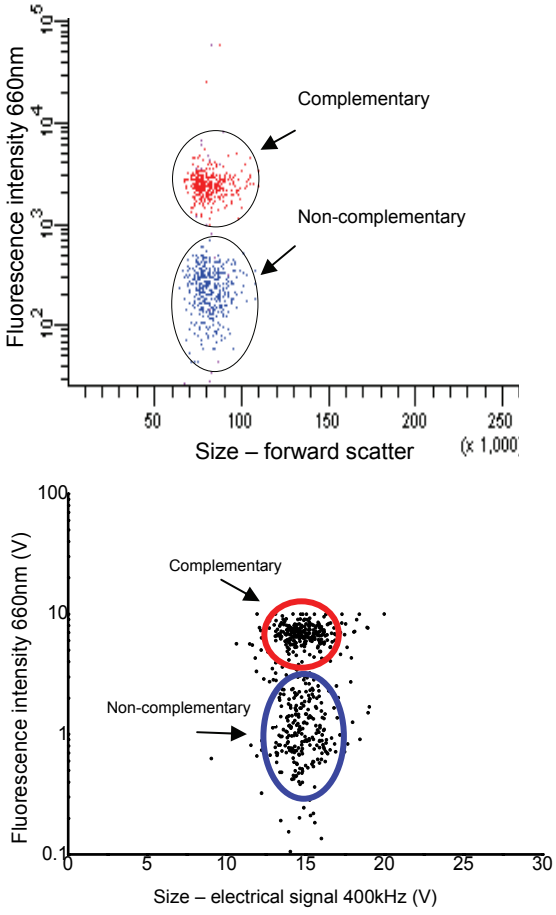

Figure 6: Comparison of (a) FACS and (b) microfluidic chip data for a mixture of two populations of beads, one complementary and the other non-complementary DNA.

Figure 6 shows data obtained from a mixture of particles. The data is compared with that from the same sample run on a commercial flowcytometer (BD FACSAria). Either of two different ssDNA sequences is attached the particles surface. When incubated with the fluorescently labelled ssDNA, the particles with complementary ssDNA on their surface are seen to fluoresce while those with the mismatch sequences which do not hybridise have a lower fluorescence intensity. The impedance signal in this case is used to trigger the signal acquisition. It may be possible to detect hybridization at the particle surface using impedance measurement, we are currently investigating this.

4 CONCLUSION

Diffractive barcodes were fabricated from SU8 using photolithography. DNA chemistry was then performed on these particles. A microfluidic device was developed and has been shown to be capable of the analysis of the barcoded particles. The optical (diffraction pattern and fluorescence) and electrical properties of the particles are analysed as the particles flow through the flow system. We show the utility of the system to recognize DNA hybridization events on the surface of the beads. We are currently investigating the use of the system for DNA sequencing and other bio-chemical assays.

Theoretical predictions suggest that several million distinct patterns can be encoded using such a technology on particles as small as 50 μm in length [2]. Patterning of the barcodes using nano-embossing techniques will allow the production of large numbers of such particles cheaply and with nanometer resolution.

5 AKNOWLEDGEMENTS

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