An integrated hybrid system for genetic analysis combining EWOD sample preparation and magnetic detection.

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Abstract. Over the last decade microelectronic technologies have delivered significant advances in devices for point of care diagnostics. Complex microfluidic systems integrate components such as valves, pumps etc. to manipulate liquids. In recent years, the drive is to combine biochemical protocols in a single system, delivering “sample in answer out”. An Electrowetting on Dielectric (EWOD) device offers the possibility to move and manipulate 64nl volumes implementing biochemical processes, while the magnetic sensor facilitates hybridisation detection. We outline an injection molding approach where EWOD and magnetic devices are integrated into a hybrid microfluidic system with the potential to implement “sample in answer out” biological protocols.

1. Introduction
In a recent review Haeberle et al. [1] described how microfluidic platforms should implement defined unit operations (e.g. valving, pumping, mixing etc.). The system should exploit low cost well defined fabrication techniques and be capable of implementing bioassays with ease and flexibility. They reviewed a diverse range of fluidic handling approaches including wicking, electrowetting, centrifugal technologies and microdroplet systems. Simple lateral flow systems are limited in the assays they can be applied to, however emerging platforms integrating a number of technologies such as the Cepheid GeneXpert [2] implement a series of complex assays such as cell lysis and PCR amplification. Many publications have demonstrated individual biochemical protocols in lab on chip systems, but relatively few have combined sample preparation and detection in a single system. Whooley et al. [3] implemented a monolithic system with capability to amplify and detect specific DNA using capillary electrophoresis. Anderson et al. [4] described an integrated monolithic device capable of nucleic acid extraction and PCR amplification. Quake et al.[5] demonstrated a fully integrated microfluidic system implementing DNA sequencing by synthesis, discriminating up to four consecutive base pairs,
suggested this approach can be integrated with upstream protocols e.g. DNA extraction and PCR amplification. Capillary electrophoresis (CE) detection has been used extensively with integrated all silicon or glass systems, however CE systems don’t lend themselves to multiplex detection a requirement for high throughput biological screening [6].

Three strategies exist for integration and packaging of hybrid microfluidic systems: (i) vertical stacking where chips are bonded to substrates containing fluidic and electrical interconnects [7], (ii) monolithic horizontal integration where all components are fabricated on a single substrate with compatible fabrication materials and processes. The third approach involves using hybrid integration as illustrated in the commercially available i-STAT blood analyser, where a polymer microfluidic cartridge is combined with an electrode substrate for electrochemical detection.

![Integrated System](image)

**Figure 1.** The functionality of the EWOD component in sample preparation and the magnetic sensor in hybridisation detection is outlined within the integrated platform.

The implementation of the biochemical protocols within the system are outlined in figure 1, with the EWOD device potentially implementing cell lysis, PCR amplification and sample preparation for hybridisation detection. While the magnetic sensor array detect’s mutations associated with specific diseases. The EWOD device has been used to successfully implement PCR amplification on a mutation associated with Cystic Fibrosis (CFTR ΔF508) with 200nM yield for amplicons 79bp in length, while the magnetic sensor has demonstrated hybridisation detection sensitivities in the fM target DNA range.

2. **Device Integration**

This work outlines a hybrid integration approach to devices fabricated in silicon and glass using injection molded polymer microfluidics. The system is designed to implement a sequence of biochemical protocols on a biological sample i.e. cell lysis, PCR amplification and hybridisation. Our approach integrates a number of devices, (i) electrowetting on dielectric (EWOD) silicon device [8] to move and mix liquid droplets, (ii) copper micro-coils on silicon [9] to manipulate magnetic beads for biomolecule capture, (iii) a magnetic sensor device [10] for mutation detection. Each device type is fabricated at wafer level, diced, packaged, interconnected and mounted on a modified printed circuit board (PCB) specific to each component. The devices are then “sandwiched” (figure 2) in a polymer (Zeonor) structure providing leak proof packaging, with horizontal microfluidic interconnects between modules. The microfluidic design contains reservoirs for reagents and reactions, with microchannels for sample input and waste. Each device is positioned and fixed within a plastic carrier substrate containing pockets, the microfluidic lid and device carrier substrate are then aligned and the system sealed.
Our integration strategy addresses key challenges including: (i) maintaining the functionality and performance of each device during the integration process and (ii) maintaining the functionality of biomolecules immobilised on the magnetic and optical devices during integration.

![Image](image_url)

**Figure 2.** The concept of hybrid device integration with specific functions within a POC system is illustrated (left). The “sandwich” structure is outlined with carrier substrate and microfluidic lid contains reservoirs and interconnects between devices (right).

### 2.1. Micro-fluidic Lid design and fabrication

The microfluidic lid is injection molded using a Babyplast 6/10P, a low cost hightthroughput fabrication technique [11], using Zeonor® (Zeon Europe GmbH), a popular thermoplastic with low auto-fluorescence used extensively to fabricate optical devices. Negative relief SU8 masters (figure 3) are defined on silicon substrates using photolithography, the master is placed in the injection mold cavity for fabrication (figure 3). This offers a low cost approach where multiple microfluidic design iterations are evaluated. The microfluidic channel depth was typically 100µm with feature width of 1mm, suitable for acetate mask photolithography. Design layout was carried out on Mentor graphics CAD package and the masks printed by JD Phototools (2000 dpi). A 100µm thick SU8 (Microchem Ltd) layer was spin coated onto a silicon wafer (Single side, 4 inch, 750µm thick). To achieve the fluidic channel a three spin process of 300rpm (60 seconds) 600rpm (30 seconds) and 1500rpm (4 seconds) is used to deposit the SU8 on the wafer surface. This is followed by a 30 minute bake at 90°C and 105°C respectively and a hard bake at 95°C. The substrate is exposed using a mask aligner (Zeiss) with the acetate mask defining the microfluidic design, following exposure the substrate is hard baked and developed using EC solvent. The substrate is finally diced into individual masters. For injection molding, Zeonor® pellets are dried in an oven for 40minutes at 50°C removing any moisture before being loaded onto the injection molder for fabrication. The system temperatures were set to, (i) Injection nozzle 195°C, (ii) injection chamber 190°C, (iii) plastic melt chamber 220°C, with injection parameters of 60bar pressure for a 3 second injection. Thermal shock and sonication in DI water was used to remove the SU8 master from the molded plastic part. After fabrication the lid was coated firstly with 180nm of Indium Tin Oxide (ITO) followed by 1000nm of SiOC. The ITO layer forms a planar ground electrode while the SiOC forms a hydrophobic layer for EWOD operation. To prevent plastic melting we repeated three ITO depositions of 60nm allowing the substrate to cool between evaporation cycles.
To fabricate the polymer carrier substrate, a brass mold was CNC machined. The injection cavity was 40mm x 45mm containing negative relief structures forming pockets in the polymer substrate within which the devices were inserted. The dimensions of the EWOD device pocket was 11mm x 12mm x 750µm, the dimension of the magnetic sensor pocket was 5mm x 6mm x 750µm, for the magnetic micro-coil pocket dimensions varied but the thickness was also 750µm. To compensate for polymer shrinkage with component cooling during fabrication, the microfluidic relief structures were 5% oversize.

![Image](https://example.com/image.png)

**Figure 3.** SU8 microfluidic master structures were fabricated on a 4" silicon wafer (left) which was diced and used for component fabrication within the Babyplast injection molding system (right).

2.2. Device Preparation (EWOD, Microcoil, Magnetic Sensor)

In most biosensors a probe biomolecule is immobilised on a substrate surface to capture a specific target biomolecule of interest from a biological sample. For genetic screening assays, probe DNA is immobilised on the sensor substrate upon which a hybridisation reaction takes place. Where specific binding occurs between probe and target DNA a genetic mutation of interest is identified. Thus surface chemistry and DNA immobilisation must be carried out on the magnetic/waveguide substrate before integration with microfluidic components. Following wafer level fabrication the EWOD and magnetic sensor devices are diced and mounted on PCB’s, wire bonded and encapsulated, each packaged device is then tested verifying electrical performance. In the case of the magnetic sensor chip DNA probe molecules are immobilised on the sensor surface. For the magnetic detector an array of 32 spin valve sensors screen for specific mutations of interest, a thin gold film (20nm) patterned upon each sensor acts as a base for thiol surface chemistry to which a specific combination of probe DNA is immobilised [9], DNA probes are spotted by hand using a 2µl pipette. The sensor chip is then inserted into the carrier substrate before the microfluidic cover lid is aligned and sealed, hybridisation detection takes place in a single reservoir. In a genetic assay the magnetic beads are functionalised with a surface chemistry to capture target DNA released from lysed blood cells, these then hybridise to probe DNA immobilised on magnetic sensor surfaces.

A number of bonding approaches were evaluated, UV cured glue (Norland 68) gave a robust seal but the curing process had an adverse effect on the immobilised probe DNA. Low temperature cured epoxies also gave good bonding results, but blocking of fluidic channels was an issue. We found a thermal bond process using partially cured PDMS polymer to give the best result in terms of bond performance and maintaining the functionality of the immobilised DNA probe. A 10:1 mixture of PDMS to curing agent is formulated and dispensed on the microfluidic device, it’s partially cured in an oven at 75°C for 10 minutes forming a thin gasket layer. The microfluidic lid is aligned and sealed.
to the carrier substrate containing the EWOD and magnetic sensor device, the integrated system is placed in an oven for 30 minutes at 75°C to complete the bond process. Integration of the magnetic coil into the lid was implemented by injection molding over the device, following evaporation of ITO and SiOC the performance of the copper microcoil is maintained as illustrated by its ability to manipulate magnetic beads.

3. Integrated Component test

3.1. Integrated hardware

Following EWOD integration, tests were undertaken to verify device performance. The EWOD microfluidic reservoirs (Figure 4) were preloaded with silicone oil using a 2µl pipette, the integrated system was connected to the EWOD drive electronics. This instrumentation delivers a sinusoidal signal (120V RMS 3Khz) selectively to EWOD electrodes via a Labview™ (National Instruments) interface. As EWOD electrodes are energised the surface property at the solid/liquid interface changes from hydrophobic to hydrophilic moving droplets in a controlled fashion. Figure 4 illustrates droplets dispensed from a reservoir onto the EWOD electrode bus with test liquid TRIS buffer containing 5% TWEEN 20. The smallest size droplet dispensed and moved using the EWOD system was 64nl. Dispensing, moving and mixing reagent droplets allows biochemical protocols to be implemented within the system without the need for externally actuated pumps or valves.

Figure 4. The integrated EWOD device was tested by dispensing and moving liquid droplets (TRIS Buffer containing 5% TWEEN 20) on the EWOD device.

The integrated magnetic sensor is positioned within a hybridisation reservoir with fluidic interconnect to the EWOD device, an external magnetic field biases the sensors generating a stable baseline signal. The magnetic sensors were biased in with a 1mA DC and the magnetic polarising field was set to 30 Oe DC and 13.5 Oe rms at 211Hz. When paramagnetic nano-particles (250nm diameter) come in close proximity to the sensor surface a change in magneto-resistance is observed. To test the integrated sensor device, a reference liquid of TRIS buffer was introduced to the sample reservoir as a blank measurement giving a constant signal over time. A solution containing magnetic nano-particles was introduced causing a sharp signal “dip”, with signal change observed for all sensors, when the beads were washed away the signal returned to its original reference value in TRIS buffer (figure 5).
Figure 5. Signal from an integrated magnetic sensor, the plot outlines magneto-resistance over time, with the signal “dip” occurring where magnetic beads were introduced to the chamber. The signal returns to its baseline after the beads are washed away.

4. Conclusion

The goal of “Lab on Chip” systems is to implement biochemical protocols on whole biological samples, removing the need for time consuming sample preparation by skilled laboratory staff. The challenge is to combine liquid handling techniques on a platform to implement the required biochemistry, combining this with sample detection. We have integrated silicon and glass devices using a low cost, manufacturable hybrid approach. The EWOD device moves and manipulates liquid droplets with the capability to implement biochemical protocols and the magnetic sensor detects specific mutations of interest in biological samples. The performance of each device after integration has been demonstrated, maintaining functionality and biochemical viability. In future work, a full biochemical sample preparation and hybridisation protocol will be implemented on integrated platforms using biological samples (i.e. blood, saliva or urine specimens) as a demonstrator of this platform with point of care applications.

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