DNA MELTING CURVE ANALYSIS ON SEMI-TRANSPARENT THIN FILM MICROHEATER ON FLEXIBLE LAB-ON-FOIL SUBSTRATE

A.Ohlander[1], T. Hammerle[1], G. Klink[1], C. Zilio[4], F. Damin[4], M. Chiari[4], A. Russom[2], K. Bock[1,3]

[1]Fraunhofer EMFT, Munich, Germany
[2]Div. of Nanobiotechnology, KTH Royal Institute of Technology, Sweden.
[3]TU Berlin, Center for Technologies of Microperipherics, Berlin, Germany
[4]ICRM-CNR, Milan, Italy

ABSTRACT
This paper presents genotyping on a novel microheater concept using semi-transparent copper microheaters manufactured by roll-to-roll and lift-off on polyethylene napthalate (PEN) foil. Using a mesh structure, heater surfaces have been realized in one single metallization step with a manufacturing robustness higher than conventional meander structures. The thermal distribution of the meshes, evaluated using thermochromic-liquid-crystals (TLC), produced more homogenous heating characteristics compared to meanders. Parylene coated heaters were functionalized using co-polymer poly(DMA-NAS-MAPS) to enable covalent DNA immobilization and successful melting curve analysis was performed differentiating between match and mismatch oligonucleotides.

INTRODUCTION
Melting curve analysis is a powerful diagnostic tool for point mutation detection. We have previously demonstrated bead-based genotyping on chip, proving the benefits of downscaling the assay to microscale [1]. However, relying on conventional MEMS manufacturing technologies on glass substrate rendered a chip unsuitable for low-cost diagnostics. Lab-on-chips on foil substrates (lab-on-foil) has gained interest with the aim to reduce manufacturing costs for disposable tests. Polymer-foils promote this having low material cost and compatibility with high-throughput roll-to-roll manufacturing. Optical transparency of the substrate facilitates online monitoring and observation of an assay. Transparent heaters for lab-on-chip applications have been produced by structuring ITO (Indium-tin-oxide) on glass [2], [3]. We propose a system merging the need of heating, optical transparency and low-cost manufacturing technologies using foil substrates and thin-film metallization. We demonstrate the feasibility of the heater concept by performing melting curve analysis to detect single base mutations in oligonucleotides.

FABRICATION AND FUNCTIONALIZATION
15 nm Ti and 100 nm Cu was evaporated onto a 125 µm thick PEN foil (Teonex, DuPont Teijin) using a BAK 760 evaporator (Balzers, Germany). Mesh and meander heater structures with an area of 3x1.5mm^2 and 15 µm lines and 150 µm spaces were structured in roll-to-roll manner using photolithography and wet etching. Mesh heaters of the same area but with 5 µm lines and 50 µm spaces were structured sheet wise, on the same type of PEN foil in a double resist lift-off process using LOR 7B (Micro Chem) and AZ1514H (Clariant). For electrical insulation, a 1 µm thick layer of Parylene C was deposited by CVD process (PDS 2010 Labcoter 2, SCS Specialty coating system 2010). Contacts were covered with tape for accessibility. To evaluate the thermal distribution in the different heater designs, a 15 um thick layer of encapsulated thermocromic liquid crystals (LCR Hallcrest) was spincoated on the heaters. Heaters were heated and kept at 62 °C while observing the color changes under microscope.

For covalent attachment of oligonucleotide probes onto the heater surface, the parylene surface was functionalized with poly(DMA-NAS-MAPS) in a physi-/chemi-sorption process. The parylene surfaces were oxidized by plasma treatment at 1.2 bar and 29.6 W for 10 min in a Plasma Cleaner, Harrick Plasma (Ithaca, NY, USA). Immediately after oxygen plasma treatment, the ox-heaters were immersed in poly-(DMA-co-NAS-co-MAPS) solution of 2 % w/v copolymer dissolved in 1:1solution of DI water and 40% $(NH_4)_2SO_4$. After rinsing in Di water and drying in nitrogen, the heaters were cured in vacuum at 80°C for 15 minutes. Amino-modified oligonucleotides were dissolved in sodium phosphate buffer (pH 8.5) to a final concentration of 10µM. Match and

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mismatch oligonucleotides were spotted using a non-contact microarray spotter SCENION sci-FLEXARRAYER S5 assembled with a 80 µm nozzle. The heaters were blocked in 0.1 M TRIS/HCl buffer (pH 9) and 50 mM ethanolamine at 50°C for 15 minutes. Finally, they were incubated with a complementary oligonucleotide target at a concentration of 1 µM, labelled with Cyanine 3 for fluorescence detection.The heaters were assembled under microscope and connected electrically. A drop of 5x SCC buffer was applied on the heater surface and covered with a glass slide. Heaters were ramped from 25 to 120°C at a speed of 0.2 or 0.6 °C / sec while following the intensity decay taking microscopic images. Pictures were evaluated using Image J to extract intensity profiles of each DNA spot.

RESULTS AND DISCUSSIONS

To evaluate the heat distribution of the various designs, a 15µm thick encapsulated-TLC layer (bandwidth 60-65°C) was spin coated on the heater. Figure 1 and 2 reflect the thermal distribution at 62°C, in a conventional meander heater structure with 15µm lines and 150µm space and a mesh heater with 5 µm lines and 50 µm space. The transparent foils are put on a dark background for clarity.

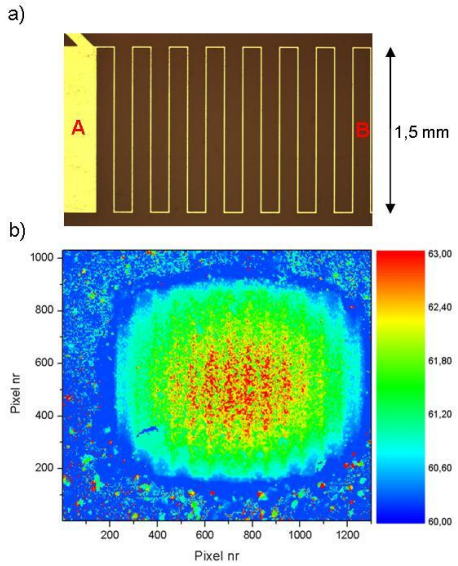

Fig. 1 a) *Roll-to-roll manufactured Cu meander with 15 µm lines and 150 µm spaces on 125 µm PEN foil.* ***b)*** *The corresponding thermal profile at the surface in 1a) at 62 °C using a 15 µm thick TLC layer.*

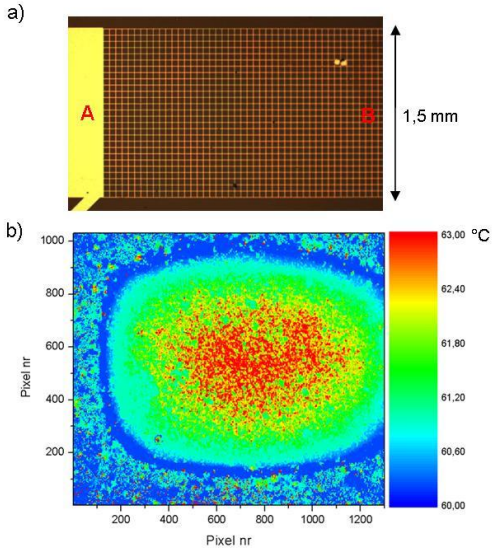

Fig. 2 a) *Lift-off processed mesh heater with 5 µm line and 50 µm space on PEN foil.* ***b)*** *The TLC response in the mesh heater in 2a) at 62 °C.*

It can be seen that the meander, although having the same size as the mesh, is heating a smaller area in the given temperature range. Further, the current carrying conductors in the mesh structure are less pronounced in the TLC layer than in the corresponding image of the meander, indicating a more homogenous heat spreading over the 1.5 x 3 mm heater area when using the mesh structure. Hence, by using a mesh structure, heat can be distributed over a larger area and by making the mesh with finer metal lines, thermal homogeneity on the micro scale in the heater can be improved. Furthermore, the mesh heater constitutes a more robust design from a manufacturing point of view. In the case of an interruption on the metal conductor in a meander, the heater would be unusable whereas a mesh heater still would possess its functionality.

For melting curve analysis, the probe needs to be immobilized to a solid support. The parylene coated Cu heaters were functionalization using co-polymer poly(DMA-NAS-MAPS). This copolymer obtained by radical polymerization of dimethylacrylamide (DMA), N-acryloyl-oxy-succinimide (NAS) and 3-(trimethoxysilil)propyl-methacrylate (MAPS) forms a thin-film on the surface of parylene by physi-/chemi-sorption [4]. The film bears active esters that allow covalent binding of amino modified oligonucleotides. Consequently amino coupled probes were spotted on

mesh heaters (15µm line and 150 µm space) and Cy3 labeled complementary strands with or without point mutation were hybridized onto the immobilized probes. In a first experiment, a heater with spotted match and mismatch probes was ramped from 25-120°C at a speed of 0.2 °C /sec and microscope images was taken each 10th second. In figure 3 the normalized intensity versus temperature of 3 matching and 3 mismatching DNA spots are shown. In a second experiment, the same type of heater was ramped at a speed of 0.6 °C / sec taking pictures every 5th second (figure 4).

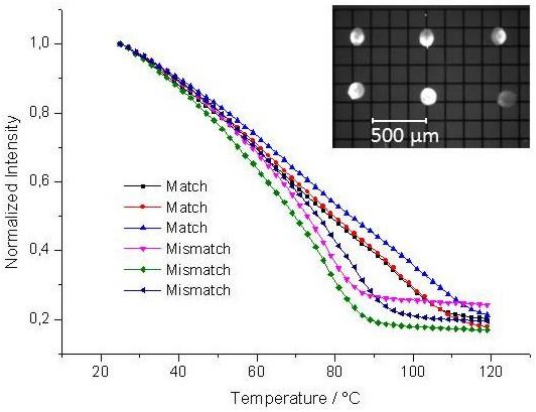

Fig. 3 *Melting curves of match and mismatch oligos spotted on a Cu mesh heater (upper right). Heating rate was 0.2 °C/s.*

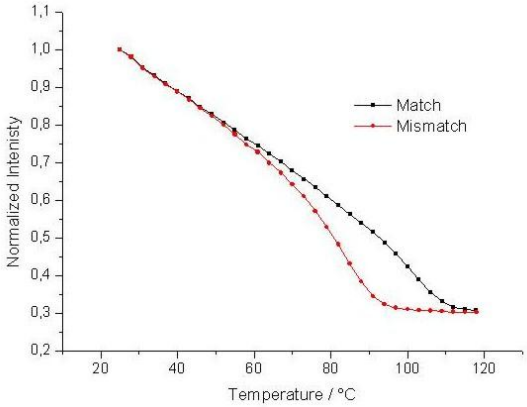

Fig. 4 *Melting curves of a match and mismatch spot on a Cu mesh heater, ramped with a rate of 0.6 °C/s.*

A clear distinction between matching and mismatching DNA can be observed in both ramping speeds. The prescence of a single base mismatch causes the dissociation at a lower temperature of a mismatching duplex than a matching one, hence the intensity drop at lower temperature. The increased temperature ramping speedby a factor of 3 does not affect discrimination power of the assay and resulted in a total time of 2.5 minutes.

CONCLUSION

We demonstrate single base mutations by melting curve analysis using a novel foil-based semi-transparent heater-concept enabling low-cost multiplexing of diagnostic assays. Using a metal mesh, a heater which produces more homogenous thermal distribution than a conventional meander structure was manufactured using only one metallization step. Through co-polymer functionalization of the heater surfaces, oligonucleotides were covalently bound to the surfaces and melting curve analysis was performed being able to distinguish between match and mismatch samples. Future work will include integration of upstream processes in lab-on-foil system for applications in low cost molecular diagnostics.

REFERENCES

1. "Genotyping by dynamic heating on monolayered beads on microheated surface"; A. Russom et al; *Electrophoresis* 2004, *25*, 3712–3719
2. "A heater-integrated transparent microchannel chip for continuous-flow PCR"; K. Sun et al; Sensors and Actuators B 84 (2002) 283–289
3. "A transparent cell-culture microchamber with a variably controlled concentration gradient generator and flow field rectifier"; J-Y. Cheng et al; *Biomicrofluidics. 2008 June; 2(2): 024105*
4. "Characterization of a new polymeric adsorbed-coating for DNA microarray glass slides Pirri G., Damin F., Chiari M., Bontempi E., Depero L. E., *Anal. Chem*. 2004, 76, 1352