# Fabrication of Paper Based Microfluidic Devices

K. Govindasamy, S. Potgieter, K. Land and E. Muzenda, Member, IAENG

Abstract-This paper describes an inexpensive method of fabricating paper based microfluidic devices, a new point of care technology. The method uses a solid ink printer, chromatography paper and a heating source. The printer deposits wax onto the surface of the paper which is then melted to allow the wax to penetrate the depth of the paper. This results in hydrophobic barriers capable of guiding fluid movement through the paper. The paper provides a detailed study of process parameters critical to this fabrication process. It discusses the selection of the optimum line width, melting temperature and melting time required to generate impermeable hydrophobic barriers. It was found that line widths play a predominant role in the development of effective wax barriers, more so than other fabrication parameters. A comparison between the melting effectiveness of a hot plate and an oven is also given. To test barrier effectiveness, square chambers were printed and flooded with coloured dye. It was found that barriers narrower then 300µm do not form impermeable hydrophobic barriers.

Index Terms—Microfluidics, paper based microfluidics, fabrication, wax

#### I. INTRODUCTION

PAPER based microfluidic devices have recently found a new application in the area of point of care/in the field testing. This is due to their ease of operation which requires no supporting equipment and external power and ability to provide results that are easily interpreted [1]. Home pregnancy tests (lateral flow tests) which operate on a similar principal, are an example of point of care diagnostics. Paper based microfluidics aims to address the inherent inadequacies of standard lateral flow tests. This includes improving the sensitivity and specificity of the tests, incorporating multiple assays onto a single device, and providing semi to fully quantitative results, all while

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- K. Govindasamy is with Micro Manufacturing Department, Material Science & Manufacturing Unit, CSIR, Pretoria 0001 SA (phone: 0027-12-8412556; fax: 002-12-8414209; e-mail: <a href="mailto:KGovindasamy@csir.co.za">KGovindasamy@csir.co.za</a>).
- S. Potgieter is with Micro Manufacturing Department, Material Science & Manufacturing Unit, CSIR, Pretoria 0001 SA (e-mail: SPotgieter@csir.co.za).
- K. Land is with Micro Manufacturing Department, Material Science & Manufacturing Unit, CSIR, Pretoria 0001 SA (e-mail: KLand@csir.co.za).
- E. Muzenda is with the Department of Chemical Engineering, Faculty of Engineering and the Built Environment, University of Johannesburg, Doornfontein, Johannesburg 2028 SA (e-mail: <a href="mailto:emuzenda@uj.ac.za">emuzenda@uj.ac.za</a>).

maintaining low cost and simplicity. Development of low cost diagnostics is vital for developing countries like South Africa, where rural communities lack access to basic health care and clean drinking water. These tests provide a rapid alternative to conventional medical testing which requires patients to visit sophisticated laboratories located long distances from their homes. In the case of environmental monitoring, such tests will enable in the field, real time monitoring of air and water quality. Such information can be used to warn communities of possible health threats and be used to alert the responsible authorities. Other advantages of paper based microfluidic tests include low reagent, sample and energy consumption, ease of transportation to remote locations, and disposability. Various paper based microfluidics fabrication techniques exit. These include photolithography, polydimethylsiloxane (PDMS) plotting, wax printing, laser cutting, and plasma and inkjet etching [2]. The photolithography technique, while expensive, creates devices of high resolution. This method requires the use of clean room facilities which include UV light sources, expensive photoresists, and oxygen plasma [3]. The PDMS manufacturing technique makes use of a standard desktop plotter to deposit PDMS droplets onto paper in a desired pattern. It is less expensive than photolithography, but has a lower resolution [4]. It produces devices that are flexible, an important requirement since these devices are often exposed to mechanical stresses such as bending and folding [4]. Wax printing uses a solid ink printer which deposits wax onto the surface of paper. The wax is then melted into the depth of the paper using a hot plate or an oven. Total fabrication time is less than 5 minutes and does not require the use of organic solvents or any pretreatment of the paper. A solid wax printer, while expensive, is capable of manufacturing devices at approximately \$0.001per device [6], which is less expensive than all other techniques mentioned. This is mostly due to the fact that wax is cheaper than both PDMS and UV curable photoresists [5]. This printing technique is also more easily upscaled for mass production compared to the other processes. Large scale production is envisioned to be similar to newspaper printing techniques [6]. Wax is also more environmentally friendly, readily available, and stable at high temperatures (60°C), temperatures which are common in developing countries. The resolution of wax printed devices is typically lower than other manufacturing methods [2], and this is due to the melting step required in the process. Other groups have developed screen printing methods [2] to remove the need for an expensive solid ink printer. However, up scaling such a production technique

ISBN: 978-988-19252-2-0 ISSN: 2078-0958 (Print); ISSN: 2078-0966 (Online) has proved difficult. In this paper we explore the wax printing manufacturing technique in detail, providing information on the selection of optimal fabrication conditions such as melting times and temperatures and selecting the type of heating source. Finally, the influence these optimal parameters have in creating effective wax barriers is discussed.

# II. MATERIALS AND METHODS

#### A. Materials

All devices were designed using Design CAD and printed onto 20 cm × 20 cm sheets of Whatman chromatography paper (no. 1). Chromatography paper was selected as it is readily available, relatively inexpensive and reproducible. A Xerox Color-Qube 8870 wax printer was used. The printer heats up the resin based solid wax cartridges and deposits the molten wax droplets onto the paper surface. Once the wax comes into contact with the paper, it cools quickly, preventing any further spreading. The printed device is then placed on a heating source (either an oven or hot plate) to allow the wax to melt into the depth of the paper. An Ecotherm oven (Model 22, Labotec) was used. cooling for >10s, the device is ready for use. Standard blue food colouring, diluted in DI water in a ratio of 1:6 was used to test for barrier effectiveness. For each experiment, unless otherwise stated, a series of 5mm long lines ranging from 30 µm to 1000 µm in width, were printed on a single sheet of paper and then melted on a hot plate (StableTemp, DLM 51806-15, Cole Parmer) for 1 min at 200°C. For validation of results, 5 lines of each size were printed and anlaysed after melting. The extent of melting through the depth of the paper was examined both visually and using image analysis software, Image J. Image J is able to perform a grey scale analysis of an image by measuring its black, white and grey colour intensities. A dark (or black) image will have low grey scale values, while lighter images will have high grey scale values. All photo's used for image analysis were captured using a Canon Powershot G-11 digital camera.

# B. Determination of optimum printed line width

Lines of different width were melted on a hot plate at the same temperature and for the same time period. The image formed on the reverse side of the printed surface (the melted surface) was examined (Fig. 1). The optimal line width is that which forms the darkest and clearest image on the melted surface. This indicates that wax from the printed surface has successfully penetrated through the paper. To further analyse the wax penetration a cross sectional analysis was performed. The line width that resulted in complete vertical penetration of the wax through the cross section of the paper was considered the optimal line width.

# C. Comparison of the melting effectiveness between an oven and a hot plate

Printed lines were melted using both an oven and hot plate at temperatures ranging from 50°C to 250°C, in

increments of 50°C. The images formed on the melted surface at each temperature were compared for each process. The optimal heating source was selected as that which forms the darkest image on the melted surface.

# D. Determination of the optimum melting temperature

Lines of different width were melted at temperatures ranging from 50°C to 250°C, in increments of 50°C. Although the optimal line width was already identified, using a range of line widths helps to better understand the influence of temperature on the formation of barriers. The optimal melting temperature was selected after analyzing the melted surface.

#### E. Determination of the optimum melting time

Wax lines were melted at a constant temperature for time intervals ranging from 1 min to 7 min. The optimal melting time period was selected by analyzing the image formed on the melted surface.

## F. Determining the effectiveness of barriers

5mm squares with line widths ranging from 50  $\mu m$  to 1000  $\mu m$  (1 mm) were printed. Each square was melted at 200°C for 1 min on a hot plate to form hydrophobic barriers. 10 $\mu$ l of food dye was pipetted into printed squares and monitored for any leakage.

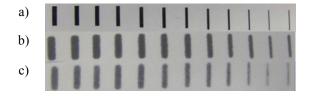


Fig. 1: a) Printed surface before melting, b) printed surface after melting, c) bottom surface after melting

#### III. RESULTS AND DISCUSSION

## A. Determination of optimum printed line width

Line width was found to significantly influence the formation of effective barriers. Wider lines have more wax available on the printed surface to melt into the depth of the paper and form a barrier on the melted surface. Fig. 2 shows grey scale measurements of the melted surface of a paper strip printed with wax lines. The crests of the curve represent grey scale values of the white background paper, while the troughs represent those of the melted lines. Lines decrease in width from left to right of the curve, starting from 1000 µm and decreasing in 100 µm increments to 100 μm. The two remaining troughs represent lines of width 50 μm and 30 μm respectively. The same line widths are used for all grey scale value charts hereafter. According to Fig. 2, wider lines (1000 µm to 300 µm) give grey scale values between 98 and 103. These values indicate the formation of effective barriers. Narrower lines, having lower grey scale values, penetrate the paper to a lesser extent. This results in

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poor hydrophobic barriers. This is observed by the similar grey scale values of the 30  $\mu m$  wide line and the background paper. Hence lines  $\geq \! 300 \ \mu m$  create effective barriers. The optimal line width should lie midway of the operational range. Selecting a width of 300  $\mu m$ , which lies at the lower end of the operational range would lower fabrication costs, but may be more prone to leakages. The 500  $\mu m$  line has almost the same grey scale value, and hence barrier effectiveness, as that of the 1mm line. Therefore the selection of the 500  $\mu m$  line forms the correct balance between operational effectiveness and cost.

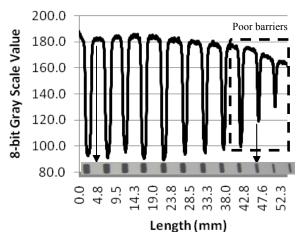


Fig. 2: Effect of line width on melting effectiveness

A cross section analysis of the paper was performed and observations agree with the grey scale measurements (Fig. 3). The location of the melted wax front is better observed using Image J. For all line widths, the software uses a straight line marker to indicate the location of the wax front. In Fig. 3, all the markers are located less than <sup>3</sup>/<sub>4</sub> way through the paper cross section for lines ≤300µm. This indicates why narrower lines have higher grey scale values on the melted surface. For lines ≥300µm in width, the markers are able to reach the opposite end of the paper. The widest lines (900 µm and 1mm) display a bulge at the melted surface. This indicates that these lines not only penetrate the cross section of the paper, but also form an additional wax layer on the melted surface. The cross section analysis confirms that lines greater than 300 µm in width offer better functionality.

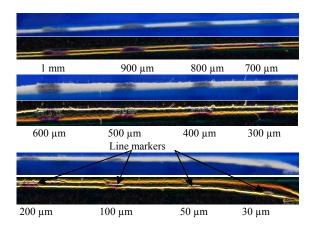


Fig. 3: Cross sectional view of paper

B. Comparison of the melting effectiveness between an oven and a hot plate

For simplicity, Fig. 4 shows only the grey scale values measured at 100°C and 250°C., However temperatures ranging from 50°C to 250°C were investigated. As wax is stable up to 60°C [5], no penetration of the wax was visible at 50°C. Wax penetrates the paper from 100°C upwards when using both the oven and hot plate (HP). The extent of penetration however, is different between the two methods. Fig. 4, as with Fig 2, displays the trend of increasing grey scale values with decreasing line widths. This indicates that wax penetration is still largely influenced by the original line width rather than changes in temperature. Melting in the oven at 100°C resulted in the poorest penetration of wax over the range of line widths investigated. This is indicated by high grey scale values. At 250°C, lower grey scale values observed, indicating an improvement in wax penetration. The extent of melting at 250°C in the oven appeared similar to that obtained using a hot plate at the same temperature. However, as the line width decreases, an increasing difference in melting effectiveness between both heating sources is observed. Grey scale values for lines melted using a hot plate at 100°C, an oven at 250°C, and a hot plate at 250°C were similar for line widths ≥400 μm. For lines narrower than 400 µm, the grey scale values start to differ. This is observed for each line width by the increasing vertical distance between the troughs of each curve. When using an oven at 250°C, the grey scale values for the 300  $\mu$ m line (encircled) is  $\pm 15$  units higher than that obtained using a hot plate at the same temperature. A similar increase is observed for the 200 µm and 100 µm lines. For lines narrower than 100 µm, poor wax penetration was seen. This is due to an insufficient amount of wax on the printed surface. For lines narrower than 400 µm, melting on a hot plate at 100°C gave lower grey scale values than when melting in the oven at 250°C. This indicates that the hot plate enables better wax penetration than the oven, even at lower temperatures. The hot plate enables direct contact between the wax and heating plate allowing for more efficient melting.

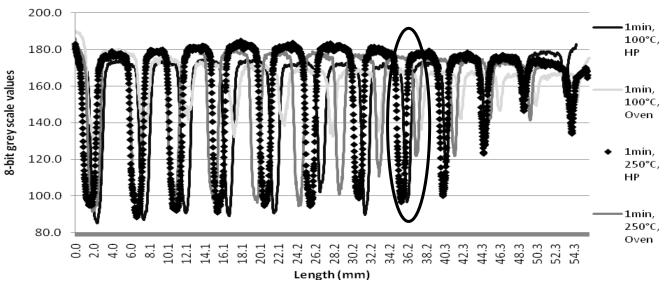


Fig. 4: Comparison between the melting effectiveness of oven and hot plate (HP)

#### C. Determination of the optimum melting temperature

Fig. 6 shows the effect of temperature on melting effectiveness For lines widths ranging from 1000 µm to 300 um, similar grey scale values were obtained for each temperature where melting was observed. As the line widths decrease below 300 µm, the influence of temperature becomes more apparent. Higher temperatures result in better penetration of wax, as observed by the low grey scale values obtained at 200°C and 250°C. At 100°C and 150°C, grey scale values were  $\pm 20$  units to 30 units higher than that obtained at the higher temperatures. The optimum melting temperature will be selected by examining the influence of temperature on the melting effectiveness of narrower lines (30  $\mu$ m to 300  $\mu$ m). This is because no affect of temperature is observed on lines larger than 300 µm, since the extent of penetration is predominantly influenced by the original line width. Melting at temperatures of 100°C and 200°C results in similar grey scale values for line widths between 1000 μm and 300 μm. Selecting a melting temperature of 100°C for lines  $\geq 300 \, \mu m$  will provide the same melting effectiveness as melting at 200°C, but will prove more economical. For narrower lines, melting at 200°C appears optimal, as it gives the lowest grey scale value. One may have expected to obtain the lowest grey scale value at the highest temperature (250°C). The reason for this not occurring might be due to a preference in lateral spreading over vertical spreading at such high temperatures.

# D. Determination of the optimum melting time

Fig. 7 shows how different melting times influence the extent of wax penetration. In the figure, all curves display similar grey scale values per line width. This indicates that increasing the melting time above 1 min, when melting at 200°C, does not significantly improve wax penetration. Furthermore, increased melting times can reduce fabrication throughput and increase fabrication costs. Based on the grey scale values, maximum wax penetration is achieved after melting for 1 min at 200°C. Increasing the melting period to

7 min does not improve the extent of wax penetration, even for the narrower lines. At lower melting temperatures, melting time would have a more significant influence on wax penetration. While increasing the melting time above 1 min has no affect on melting effectiveness, decreasing the melting time does. Printed lines were melted at 200°C for 30s (data not shown). An unclear, lightly shaded image formed on the melted surface.

#### E. Determining the effectiveness of barriers

To test the effectiveness of barriers food dye was pipetted into melted square chambers. Each square had different barrier line widths. Besides the 50  $\mu m$  and 100  $\mu m$  squares, all others were able to contain the fluid for longer than 10 minutes, as shown in Fig. 5. This confirms that lines  $\geq\!300$   $\mu m$  form good quality barriers. The 50  $\mu m$  barrier leaked rapidly. Although fluid was able to seep across the 100  $\mu m$  barrier, it did not do so as rapidly. This indicates the presence of fewer leakage points along the 100  $\mu m$  barrier than the 50  $\mu m$  barrier.

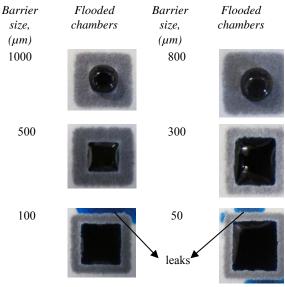


Fig. 5: Hydrophobic barrier effectiveness squares

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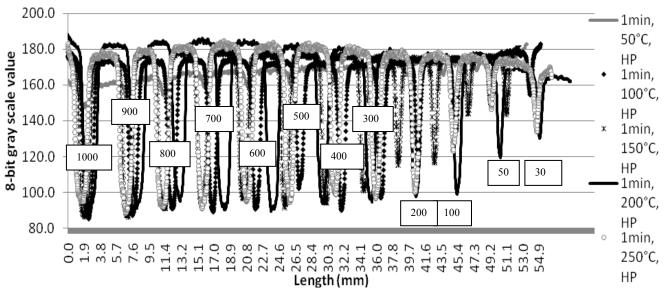


Fig. 6: Effect of temperature on melting effectiveness (text boxes indicate the line width  $(\mu m)$ )

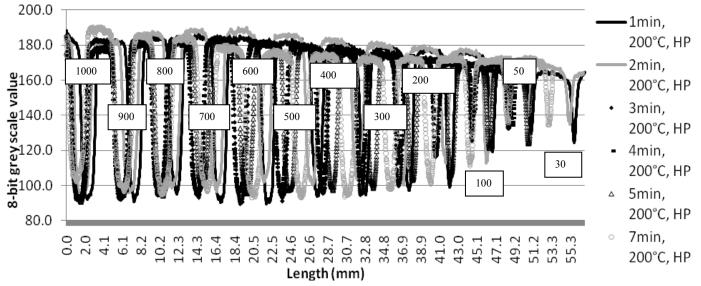


Fig. 7: Effect of melting time on melting effectivness (text boxes indicate the line width  $(\mu m)$ )

# IV. CONCLUSION

These studies show that the original line width is the most important fabrication parameter in creating effective wax barriers. All studies performed show that grey scale values increased with a decrease in line width, regardless of the melting times and melting temperatures used. An optimal line width of 500 µm was selected, however any line width above 300 µm is capable of forming impermeable wax barriers. The effect of temperature on melting effectiveness was only observed for lines narrower than 300 µm. These line widths have insufficient wax on the printed surface to penetrate the cross section of the paper. An optimal melting temperature of 200°C was selected, as it gave the best wax penetration for narrow lines. However, should lines wider than 300 µm be used, a lower melting temperature is recommended to help reduce costs. Optimal melting times depend largely on the melting temperature. An optimal time

of 1 minute was selected when using a melting temperature of 200°C. Melting times above 1 minute did not improve wax penetration, however shorter melting periods do significantly decrease the extent of wax penetration. A hot plate serves as a more effective heating source. At 100°C the hot plate is able to provide the same extent of wax penetration as the oven at 250°C (for line widths below 400  $\mu m$ ). The barrier effectiveness test confirms that line widths below 300  $\mu m$  cannot form impermeable hydrophobic barriers in paper.

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