Dispensing of Polymeric Fluids for BIO-MEMS Applications

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Abstract

Microsystems have been recently introduced as tools for screening in the chemistry, biochemistry and biological fields. More precisely, it has been shown that non-contact micro-dispensing systems could be a viable and interesting technology for accurate, high throughput deposition of biological fluids.

The present study considers the ejection and the impact of polymeric fluids onto a silicon wafer having a number of pits covered by functionalized surfaces. The polymeric fluid is used to protect the constituent bases of DNA during reactive processes using a proprietary technology. It is shown here that the film constituted after drying should have a given thickness in order to be able to fulfill the masking role. Now, it is well known that the jetting of a polymeric fluid gives longer filaments which may be detrimental for accurate deposition and process reproducibility. The deposition process is rendered more difficult by the fact that the drops should land in well defined pits of different diameters with straight walls. We give here the methodology used to overcome these difficulties.

After giving the basics of the oligonucleotides in-situ synthesis process with special emphasis on the microfluidics aspect, we conduct extensive jetting of some polymeric fluids in order to choose the appropriate polymer and concentration for masking purposes and to ascertain their behavior in an industrial environment. Concerning the impact process itself, we characterize the surface of the silicon biochip at each step of the process and follow the thin film formation of single and multiple drops using a number of elaborate techniques. To conclude, we demonstrate that the combination of tailored operating conditions leads to satisfactory synthesis performance.

Introduction

A large number of engineering applications are concerned with the dispense of minute quantities of fluid. While it is familiar to use ink-jet printheads for printing with ink in the graphics industry, this method is also becoming a viable one in areas where there is either a need to control accurately the structure of material deposited onto a variety of surfaces and/or to overprint different materials on one another. The capability of ink jet printing to accurately dispense a wide range of materials such as light emitting polymers for OLED display fabrication, waxes, adhesives, solders and biologically active fluids have been recently demonstrated.[1-4] In this work, we focus on the use of fluid jetting in the area of biotechnology.

The DNA chip technology has found large application in the following domains: gene expression analysis, detection of new point mutations, insertions or deletions, detection of single nucleotide polymorphism. Working with DNA chips requires combining different components: the chip itself with its special surface, the device for producing them by spotting the nucleic acids onto the chip or for their in situ synthesis, a fluidic system for hybridization to target DNA, a scanner to read the chips and sophisticated software programs to quantify and interpret the results. Special equipment is now commercially available for each of these components. Moreover, in the future, complete systems should move this technology from its current standing as a laboratory-based research tool towards becoming an analytical method for clinical use. A number of different approaches have been developed to allow in-situ DNA synthesis on oligonucleotide arrays. The Affymetrix® photolithography method[5] uses a nucleotide to construct the oligonucleotide chain via successive photoexposure steps. One of the advantages of this method is that the number or density of oligonucleotides on the chip can be very high. However a disadvantage is that the quality of the oligonucleotides on the chip is poor due to the low overall efficiency in each cycle. Another method is the ink-jet in-situ synthesis method[6] developed by Rosetta® and Agilent®. In this method, standard dimethoxytrityl blocked phosphoramidites are used to construct oligonucleotides. Compared to the Affymetrix® photolithography method, the step wise coupling efficiency is higher and therefore the quality of the oligonucleotides produced on the chips is better. Also, this methodology is much more flexible than the Affymetrix® method, so that it is more useful for the researcher who needs to frequently change the design of the

oligonucleotides on the chips which is greatly needed in combinatorial synthesis experiments. Finally, the LETI has also developed its own principle of in situ synthesis[7]. It is based on the strategy of a selective protective polymer deposition by a microfluidic dispenser. The polymer film formed after baking constitutes a protective barrier during the synthesis cycle. After each cycle of nucleotide unit coupling, the protective polymer film is stripped in a rinse step with a suitable solvent. In the next cycle, a subsequent deposition of polymer on other selected sites is performed, and the cycle is repeated until all the desired sequences are obtained[8]. The laboratory results obtained at the LETI have been advanced to an industrial stage by Yamatake®.

The work which is presented here, besides the introduction of a novel in-situ DNA synthesis prototype, is connected to the ejection, impact, spreading of drops and polymer film formation which are of paramount importance in the process. The emphasis is first on the characterization of phenomena related to the formation of polymeric drops and the low speed landing of single and multiple drops into a silicon pit. Through these experiments we investigate not only the effects of the type and concentration of polymer used but also the spreading process of the drops and the deposition of the polymer film which occurs in the pit after final drying of several drops. Finally, with those different results in hand we discuss the overall performance of the in-situ synthesis prototype and we indicate the work which still needs to be done for improving the masking process.

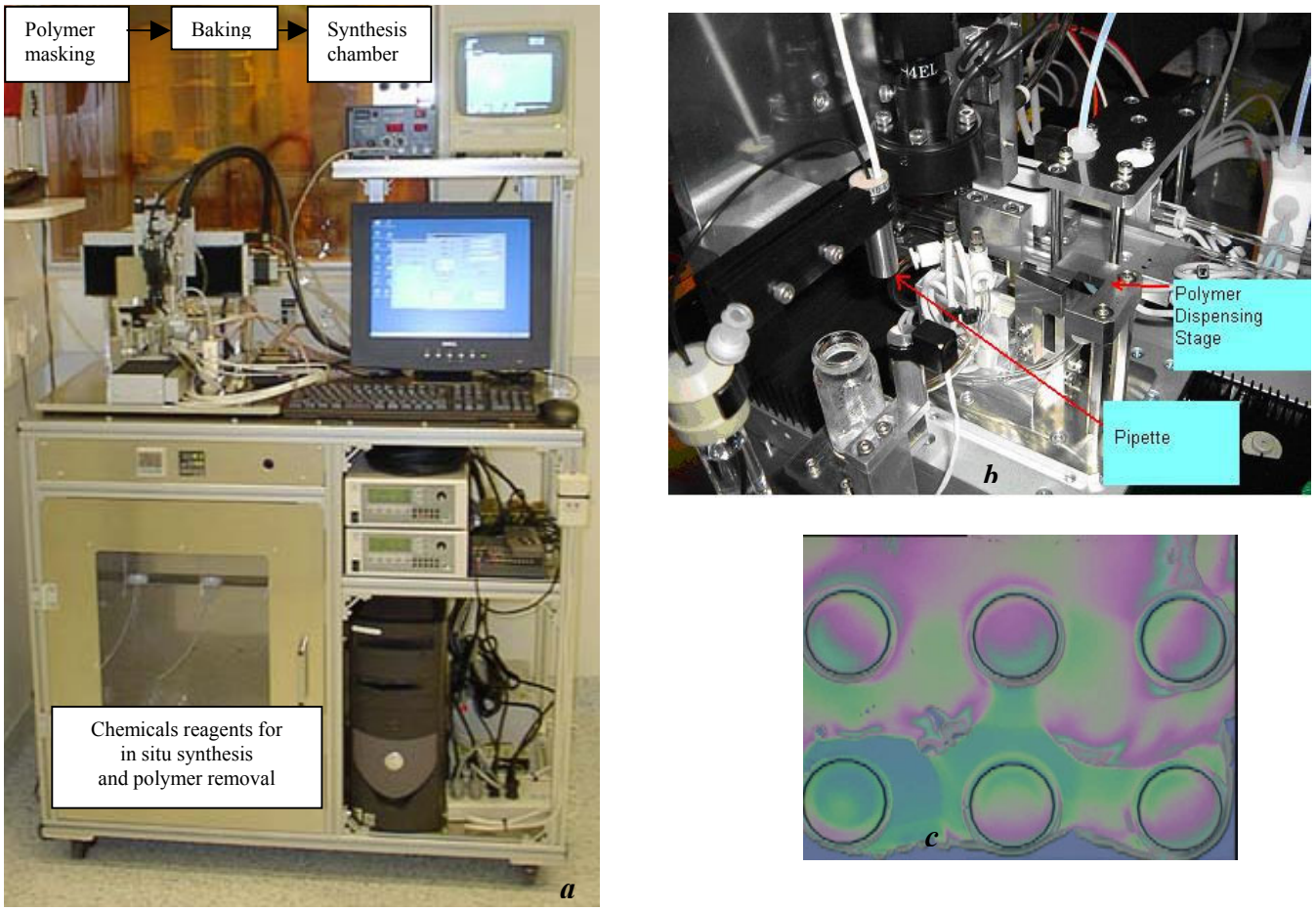

Figure 1. (a) General View of the Yamatake prototype, (b) Polymer dispensing unit, (c) biochip

Experimental

Here, we will first describe the prototype with the different parts and their interaction during the process which protocol is detailed. This is followed by the materials sub-section explaining the choice of the polymer.

Prototype

The Yamatake® prototype (figure 1a) used in this work is capable of performing four basic operations in polymer masking and removal of the protection. These are the following:

- Polymer dispensing
- Baking of polymer
- Chemical deprotection (consists in rendering the sites receptive for synthesis)
- Nucleotide coupling
- Polymer removal

In Figure 1a which gives the general view of the prototype, the bottom stage with the transparent cover lodges the reservoirs containing the chemical reagents for the in-situ synthesis and polymer removal. Also at the bottom, on the right side, one can note the electrical controllers and the mainframe. Just above in the upper part is the PC used for the control as well as the monitor which is useful for checking the chip alignment. The heart of the system is situated on the left side of the upper part where one can find the polymer masking unit which essentially consists of a MicroDrop® single nozzle piezoelectric drop on demand ink-jet system with a specified nozzle diameter of 70 μm (pipette on figure 1b). The ejector is mounted on a x-y displacement stage which moves the head from the maintenance station to the chip with an accuracy of +/-1 μm. The head ejects the polymer drops into the biochip wells (figure 1c), and it is followed by a baking of the polymer which consists in evaporating the solvent and in obtaining a protective polymer film. A chip pickup unit is responsible for the retrieval and placement of the chip, from the dispensing stage to the baking stage. The nucleotide coupling then takes place inside the synthesis chamber. Once the reaction is finished, the polymer film is stripped by the chemicals. In order to obtain a better evaluation of the ejection, impact and spreading processes, the visualization system initially available on the prototype has been recently replaced with a new optical system coupled to an image recording system. Special triggering electronics combined with appropriate software allow to perform the pseudo-cinematography process[9] and some selected results using the system are shown in the next section.

The prototype follows a well defined protocol to perform the whole process of DNA in situ synthesis. A brief outline of the steps is as follows:

1. The chip is set on the deposition stage manually
2. Click the start button
3. XY-Axis moves to the chip recognition position
4. Automatic checking of the chip item and recognition of the chip position
5. Polymer deposition
6. After completion of polymer deposition, pick up unit will retrieve the chip and place the chip on the bake stage automatically
7. Once baking is completed, pick up unit will retrieve the chip and place it onto the synthesis stage
8. Synthesis chamber is closed automatically and synthesis begins
9. Steps 2 to 4 are repeated automatically
10. Operations 2 to 9 can be repeated as many times as necessary.

Materials

As said above, the process consists in having a selective deposition of a polymer protective film in a structured silicon substrate having functionalized pits. The mechanical addressing using the ejection of polymer drops replaces the photochemical or electrochemical ways of addressing used in other processes. It consists in fabricating a solid impervious polymer cover accurately adjusted on the appropriate pits and which helps to select, during the in-situ synthesis, the active zones vis à vis the chemical reagents. The polymer is then eliminated by dissolution in a solvent after each cycle of the synthesis or, according to the properties of the polymer after the detritylation step. The stripping of the polymer film is either included in synthesis cycle or could be an additional step using a solvent which is compatible with the experimental procedure.

Table 1. Properties of different polymers

Polymer	CH_2Cl_2	CH_3CN	Protection step
PolyVinylAlcohol	Insoluble	Insoluble	All
PolyStyrene	Soluble	Insoluble	Coupling
PolyvinylCarbazole	Soluble	Insoluble	Coupling
PolyImide XU 5218	Soluble	Insoluble	Coupling
PolyHdroxyStyrene	Insoluble	Soluble	Detritylation
Photoresist XP 8843	Insoluble	Soluble	Detritylation
PolyEthyleneOxide	Soluble	Soluble	None

Concerning the choice of the polymer itself, it is necessary to consider the whole process where three specific solvents: dichloromethane (CH_2Cl_2), acetonitrile (CH_3CN) and tetrahydrofuran (THF) are used. We give in Table 1, the different polymers which have been studied indicating their solubility properties with respect to the solvents used in the synthesis cycle so as to state their potentiality for future use. As can be seen below, various cases are possible:

- The polymer is insoluble in all the solvents intervening in the synthesis cycle.
- The polymer is insoluble in one of the solvents.
- The polymer is soluble in all three solvents.

We have particularly considered the case of PolyVinylAlcohol (PVA) insoluble in all solvents and PolyHydroxyStyrene (PHS) a polymer which is highly

soluble in CH_3CN. Preliminary testing with PVA proved to be unsatisfactory due probably to an high extensional viscosity which limits its use in the jetting process.[10] Thus we have chosen to work with PolyHydroxyStyrene having a molecular weight of 20,000 g/mol.

Results and Discussion

To fulfill the objectives of successful polymer masking, we first performed jetting experiments with the above chosen polymer using the MicroDrop® piezoelectric pipette. We then studied the impact of one or several drops into the pits of the biochip while characterizing carefully the thickness of the obtained film using a number of sophisticated techniques. Finally, we examine the overall performance of the synthesis using a matching test. These different steps are described in some detail below.

Drop Formation

Once the polymer is chosen, the next step is to find the adequate concentration. From the standpoint of film thickness, it is advisable to choose, of course, the highest concentration with witch it is possible to eject. The experimental observations and analysis procedures we have designed allow to obtain both qualitative and quantitative information on the transient shapes of the ejection of the liquid filament which then forms a drop. It is essential to recall here that because of the very different physical processes involved in the ejection of the filament and the retraction into a drop, the time scales which are involved in these processes cover several orders of magnitude.[9]

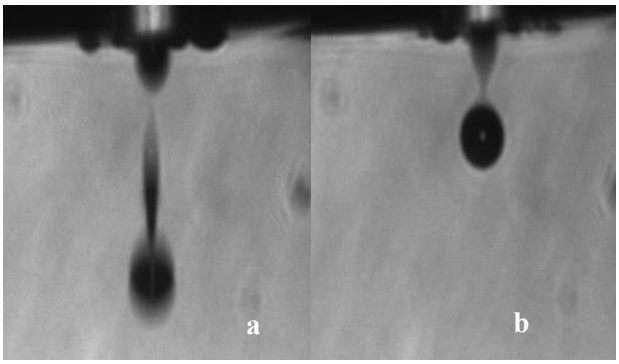

Figure 2. Snap shots of drop formation

The preliminary jetting experiments carried out with the chosen polymer showed us that a concentration of 3% in dimethylsulphoxide (DMSO) was too low for a constituting an appropriate polymer cover and that the 10% concentration lead to many jetting problems and was not feasible in an industrial unattended environment. Fluids with polymer concentrations of 5 and 7% proved to be successful.

We show in figure 2a and 2b the drop obtained for two different waveforms It is important to note that these photographs have been obtained using the pseudo-cinematography technique and thus represent an average of many pictures taken at well defined strobe delays.[9] The sequence shows that the drop formation takes place without a satellite drop. These low resolution pictures not only allow a rapid qualitative evaluation of drop formation but are also helpful for calculating an average velocity of the drop. Figure 2a for example has been obtained with a voltage of 144V with a pulse width of 90 μs and at a frequency of 200 Hz. The length of the filament tail, in this case, is close to 500 μm while if one keeps all other specifications the same and just changes the voltage to about 120 Volts then the length of the filament is very much reduced (figure 2b) and that situation is favorable for suppressing satellites.

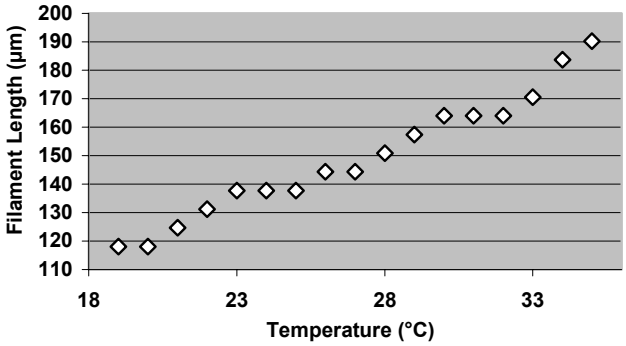

Figure 3. Evolution of filament length versus temperature

Another important thing to remind here is that the bottom of the pits of the biochip are at a distance of about 500 μm from the tip of the pipette. Knowing that the velocity of the flying drop is close to 1.4 m/s, it takes hardly 360 μs for the drop to impact into the well and it is important that the filament has coalesced with the main drop before the latter reaches the pit. We have carried out a simple calculation which gives the retraction velocity as[11]

$$\sqrt{\frac{\sigma}{\rho R}}$$

where σ is the surface tension for the fluid, ρ is the density of the liquid and R the average radius of the filament. The comparison between calculated and experimental results are fair (discrepancy of about 20%) with the measured retraction velocity being close to 4.8 m/s. The filament joins the main drop in about 100 μs which then gives ample time to the drop to stabilize before landing into the pit. If these precautions are not taken then the biochip may be wetted in an unwanted manner as shown in figure 1c.

As noted earlier, it then becomes important to know the length of the filament under different operating conditions. This is done for example in figure 3 versus temperature and it shows that it is best to keep the operating temperature between 20 and 25°C, for the selected voltage, in order to avoid having long filaments, eventual satellites and above all misplacement of the drop.

Impact of Drops

When a drop having a finite velocity impacts on a solid substrate, it spreads radially in the form of a "pancake" shape. The rate of spreading depends on a combination of parameters and is essentially driven by the inertia of the drop and is slowed by viscous and surface tension effects. When the inertial energy is dissipated, the drop reaches its maximum diameter. A rough model for the prediction of this maximum diameter has been worked out from a simple energy balance equation[12] and reads:

$$\beta_{max}^2 = \frac{-(1-\cos\theta)+\sqrt{(1-\cos\theta)^2+6\alpha Ca\left\{\frac{We}{3}+4\right\}}}{3\alpha Ca} \qquad (1)$$

where β_{max}, equal to d_{max}/D, is the maximum spreadfactor, θ is the contact angle, We is the Weber number, Ca is the capillary number equal to the ratio of viscous forces over surface tension forces with the viscous forces being represented by the Reynolds number (Re). The semi-empirical factor α accounts for the uncertainty in the dissipation function and probably depends both on fluid and operating characteristics. We have found recently that α equal to 1.5 gives the best agreement with our other experimental results.[13] We have also demonstrated elsewhere[13] that the wetting angle at maximum spreading is very close to 80° and is almost independent of the fluid and of the substrate.

Taking into account the characteristics of the fluid and the operating conditions we obtain in the experiments performed here a Weber number close to 3.5 which is quite a low value. It is useful to recall that this number should be larger than one in order to have a successful ejection of the drop. With such a low value for the Weber number, the maximum spreadfactor is close to 2, if we refer to our previous experiments.[14] Most importantly this means that the size of the impact for one single drop is about 140 μm which is almost half the size of the pit.

It is also interesting to know, at this stage, what would be the final size of one single drop sitting in the well. For that purpose it is important to measure the wetting angle at different stages of the process. Contact angle measurements on different flat surfaces were performed with a Digidrop® tester and the results are plotted in figure 4 as a function of type of the substrate. The process may begin either with bare silicon with a contact angle of 10° or with a hydroxylated thermal oxidized surface having a contact angle of about 22°. The process proceeds with a silanization step noted "Sil" in the figure. Then comes the oligonucleotide spacer noted "5T"and other various reactions up to completion of the process with 21 DNA constituents coupled in the pit and a final wetting angle of about 23°. It is important to note that along the process the contact angle only varies between 10 and 23°. All the contact angle measurements reported here are for DMSO/PHS as the test fluid so the results may somewhat differ from that usually given in the literature which are generally for water.

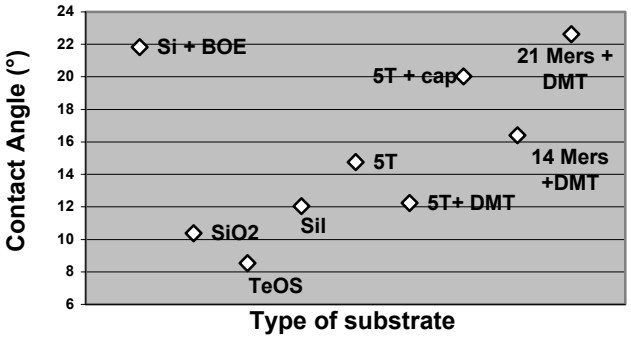

Figure 4. Wetting angle versus the stages of the process

Having these results in hand it is possible to calculate the diameter of a drop at equilibrium provided that we assume that it has a form of a truncated sphere. In this case, one obtains from geometrical considerations:

$$R = \left(\frac{3V\sin^3\theta}{\pi(1-\cos\theta)^2(2+\cos\theta)}\right)^{1/3} \qquad (2)$$

where R is the radius of the drop, V the volume of the drop and θ is as usual the contact angle. In our case with the above given wetting angles (figure 4), we obtain a diameter which is about 2.3 times the diameter of the flying drop i.e. a little bit higher than β_{max}, the value obtained at maximum spreading during the inertial regime Top view measurements of a single sitting drop, using the prototype, have allowed to confirm these calculations.

The in-situ synthesis process should allow to build a chain of about 50 DNA constituents and the theoretical length of this chain is calculated to be around 20 nanometers. One single drop should then be sufficient to provide appropriate covering although the dried extract is only 5% of the total volume of a drop. Nevertheless, independent measurements have shown that a polymer film of at least 0.5 μm is necessary to have an efficient coverage and this not obtainable with one single drop. We have then chosen to spot 5 drops into one pit with the pipette working at a frequency of 200 Hz. The pit has a diameter of about 320 μm and a height close to 10 μm so that 5 drops are just sufficient to fill it. This number of drops should also be sufficient to give almost 0.5 μm thickness if an homogeneous coverage is obtained after baking of the polymer. It is important to note here that while the second drop lands on a sitting drop in conditions quite similar to what has been studied elsewhere[15] the fourth and fifth drops land on a thin liquid film for which some hydrodynamic peculiarities do exist[16]. We have checked that the

dimensionless Sommerfeld parameter $K = We^{1/2}Re^{1/4}$, for our experiments; is much smaller than a critical value of 50 from which there is a cross-over between spreading and splashing.[17] This should allow us to guarantee that there will be no unwanted wetting on the top of the biochip.

Characterization of the Polymer Film

To check if the polymer deposition, after the drying process, gives rise to flat uniform thick film we have used quite a number of techniques. We show in figure 5 below a three dimensional picture obtained by Laser Scanning Confocal Microscopy (LSCM). The instrument used in our analysis is a LEICA® TCS-SP2 system.

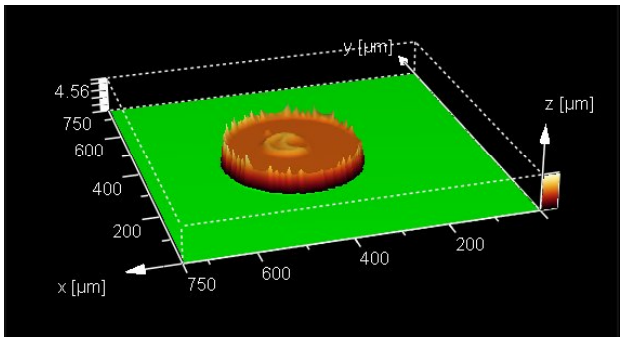

Figure 5. LSCM of the dried polymer film in the pit

For the past five years, LSCM has become a technique of choice for biological research, chemical analysis and materials testing and we use it here to obtain a 3-D view of the baked film in the well. The baking has been carried out for two minutes on a hot plate at 110°C. In order to obtain a good signal over noise ratio we have added fluorescent colorant to the polymer solution. The picture shows us that the film thickness is much more higher near to the walls than in the middle of the well where polymer coverage is the most needed.

To ascertain the results obtained with LSCM, we have used a Alpha Step 500® profile meter and the results are shown in figure 6. This device helps to obtain the roughness and undulations of a solid substrate thanks to the scanning of the surface with a very accurate mechanical probe. Different resolutions are possible and in figure 6 the cross-section taken in the middle of the pit is represented. At least three cross-sections are performed on a single pit in order to obtain an average value. The results again show that there is significant mass transport to the wall of the pit since the thickness of the film obtained in the middle of the pit for five drops is only of the order of 0.11 µm whilst a thickness of 0.49 µm was expected had the polymer thickness been uniform.

Although the picture given here is a bit different, because of other boundary conditions, the trends seen in this study are very similar to the results obtained in the drying of polymer inks used for Polymer Light-Emitting Displays (PLEDs) production by ink-jet.[18]

Figure 6. Middle cross-section of the polymer film

In order to obtain the 0.5 µm polymer thick film that we estimated to be necessary, because of some consumption of polymer during the intermediate steps, we need to carry out a 25 drops spotting and baking process which is done by sequences of ejection of 5 drops and baking. The results obtained by fluorescence testing at the end of the process show that the protective polymer technique works well although its efficiency, essentially in the middle of the wells, could probably be enhanced by using other strategies during drop deposition.

Conclusions

In this paper, after giving the basics of the oligonucleotides in-situ synthesis process with special emphasis on the microfluidics aspect, we have performed jetting and impact experiments using a recently built laboratory prototype. The jetting experiments have allowed to pinpoint the main problems occurring polymer ejection and to remedy to them in a limited proportion. To better understand the impact and spreading process, we have carefully characterized the surface of the biochip at each stage of the in-situ synthesis process. We have then shown that very different hydrodynamic phenomena are at work according to the number of ejected drops. We have then checked the film formation of single and multiple drops using a number of elaborate techniques which all show that a large mass transfer occurs during the drying process probably favored by Marangoni effects since the middle of the well, during the baking process, can be considered to be a hot spot compared to the walls. Nevertheless, final fluorescent matching test carried out at the end of the process shows that the polymer film is effective in preventing unwanted oligonucleotide coupling. We expect that some of the techniques that we have put forth in this study on microfluidic biochip technology will lead not only to the miniaturization of devices for highly sensitive analysis but also will help to resolve some of the issues in other areas where fluid jetting is a critical process.

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Biography

Takaaki Kuroiwa graduated in chemical engineering from the Osaka University in 1978. While working with Yamatake Honeywell as a research scientist he obtained his Ph.D. in materials science from the Ehime University in 1994. Dr. Takaaki Kuroiwa is since 1998, Director of the Yamatake Research and Development Center in Kanagawa where he has developed with his team a large expertise in the field of bio-informatics and microfluidic MEMS. taki@ssac.yamatake.co.jp