Thermal FEM Simulation of a Multilevel Lab on Chip Device for Genetic Analysis

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Abstract: In this work, time dependent thermal analyses, performed on the 3D FE model of a multilevel Lab on Chip (LOC) platform are executed in order to gain insight into the temperature distribution within the device.
By means of the Comsol Multiphysics CAD import module, an extremely close 3D reproduction of the actual device, allowing to probe temperatures in those regions where an experimental measurement appears difficult or even impossible, is obtained. Different microfluidic chip materials (silicon/Pyrex®, polycarbonate, polymethylmethacrylate and cyclo-olefin copolymer) are virtually tested. Furthermore the close connection between the FE model and the actual device provides an immediate outcome on its fabrication steps.

Keywords: LOC, Microfluidic chip, FEM Simulation, polymeric materials.

1. Introduction

Lab-on-chips (LOCs) are microfluidic-based devices with the aim of speed up and reduce the costs of traditional, laborious and extensive analyses, ordinarily carried out in a biological laboratory.
LOCs and Micro-Total Analysis Systems (μ-TAS) often require critical elements such as microfluidic channels, packaging [1], fluidic and thermal controller and so on, that are a function of the biological or chemical protocol to implement.
Very important issues connected with LOCs and in general with μ-TAS are therefore the miniaturization and automation of biological assays, chemical analysis procedures, low reagent volume, high speed and throughput screening.
In order to gain such goals, critical issues should be solved and this fact is strictly connected with a proper design of the device.
To achieve an high reproducibility of biological protocol, a fact which determines the success of a LOC and μ-TAS, the design should be carefully optimized, so that each component of the device performs at its best.
It is clear therefore that modeling software are more and more needed in the field of miniaturized fluidic devices [2, 3, 4]. The capability of numerical tools to explore possible technological solutions, through a "what if" study, or determine physical quantities, almost inaccessible through experiments, makes them extremely attractive for the scientific community.
In this work it is shown how a Finite Element (FE) model of LOC device can be employed to explore some innovative technological solutions involving a new generation of polymeric chips made of PolyCarbonate (PC) PolyMethylMethAcrylate (PMMA) and Cyclo-Olefin Copolymer (COC). Polymeric based microfluidic chips represent indeed a strategic evolution, with respect to Silicon/Pyrex® devices, to reach a high disposable device thanks to their reduced cost.
The paper is organized as follows: section 2 contains a brief description of the LOCs design and fabrication, section 3 the experimental set-up and finally, section 4 a detailed description of the 3D FE model of the LOC, realized within Comsol Multiphysics 3.5a.
The DNA denaturation is considered as case study for all the analyzed devices being this step common to the genetic protocols.

2. LOC Design and Fabrication

This section reports a description of the LOC design.
The LOC platform has a multilevel structure which enables the integration of DNA microarray technology [5] with the microfluidic chip. The whole multilevel structure is composed of the following elements (Fig. 1): the microfluidic chip which vehicles the reagents and enables their mixing, the PDMS interconnections that connect the external tubes to the microfluidic inlets, a microarray glass slide containing the DNA probes for the genetic detection, the PDMS reaction chamber that both

ensures a reversible gasket seal with the microarray glass slide and integrates the outlet tube, and finally the clamping system that allows for the fixing of all these elements. Clearly, these last are made of different materials and should be properly connected and interfaced.
As reported elsewhere in details [6], all the components are fabricated and assembled employing standard Computer Numerical Control (CNC) techniques and optimized Micro Electro-Mechanical Systems (MEMS) processes.
Such a LOC platform has been successfully tested [6] by implementing a colorimetric genetic protocol employing a Silicon/Pyrex® microfluidic chip. This experiment represents the starting point for the advanced system calibration reported in this work.

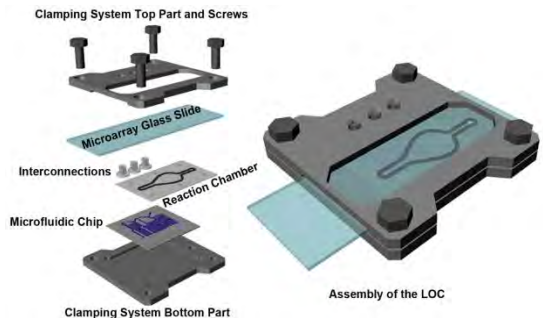

Figure 1. Exploded (left) and Assembly (right) view of the multilevel LOC platform CAD with the clamping system.

3. Experimental Set-up

The experimental set-up for controlling reagents motion and thermal cycles is here presented and discussed.
In order to handle the reagents and to control their position in the LOC, a good sealing and a driving system are necessary. A syringe pump system (Fig. 2) connected to the microfluidic chip allows for a fine flow rate control. In particular, the Syringe Pump Model 33 by Harvard Apparatus, working with Hamilton precision syringes, (250 µl gas-tight type) has been employed. As shown in figure 2, polyurethane tubes (outer diameter 2 mm, inner diameter 1.2 mm) are connected directly to the syringes via luer-lock connectors.

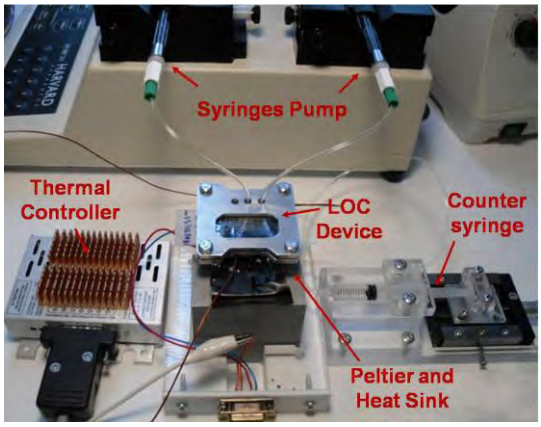

Figure 2 Experimental set-up for liquid handling and thermal control.

A typical DNA denaturation protocol is considered, a process which requires a temperature of 95°C. To this aim, the temperature has been increased from its room value (RT) to 95°C and kept constant for about 5 minutes.
To control the temperature, the whole clamping apparatus is fixed onto the hot face of a Peltier module (HT type - Supercool). The hot face temperature is controlled by a Wavelength MPT-5000 module connected to a thermistor probe for the feedback signal. In this way, the temperature is measured with a precision of ± 0.1°C.
Finally, in order to monitor the time evolution of the temperature on the different LOC levels two thermocouples (0.7 x 1 mm T type) named TC1 and TC2 respectively, are employed.
The first thermocouple (TC1), positioned in a proper groove on the bottom part of the clamping in contact with the hot face of the peltier module is employed to obtain the temperature vs time curve named T_1. This temperature profile corresponds to the above mentioned DNA denaturation step (Fig. 3) and represents also the temperature profile ideally needed into the reaction chamber.
On the other hand, the TC2 thermocouple is positioned in the top part of the clamping system, in a second proper groove fabricated via CNC machine. As shown in section 5, the same two thermocouples are also employed to calibrate the FE model with respect to the experimental data.

4. Fem Simulation Details

The reaction chamber is undoubtedly the most critical part of the LOC, since it represents the portion of the device where the genetic protocol is actually implemented. The temperature of the DNA probe on the microarray glass slide is a key parameter with respect to this detection method and it should be known with an high degree of accuracy. Since the reversible sealing between the microarray glass slide and the reaction chamber does not allow for an experimental probe of this temperature, an alternative way should be provided to access this kind of information. A viable solution is the realization of an accurate 3D FE model, able to reproduce the actual temperature distribution inside the reaction chamber.
With this objective in mind, a FE model of the whole LOC device has been built up. Since the aim of the model is to keep track of the time variation of the temperature within the device, all the analyses performed are of "time dependent" type.
In this way it is possible to virtually reproduce all the steps of the DNA analysis protocol and therefore to calibrate the numerical model on the experimental data.
This immediately leads to the possibility of correct the input temperature according to the genetic protocol needs and, overall, to have an accurate numerical tool able to test the influence of different materials on the efficiency of the genetic protocol . This last represents the final goal of the FE model, since, in this way, a new generation of LOCs made of polymeric material can be easily designed and tested for future realization.
In what follows therefore it is reported a detailed description of the FE model realized with Comsol Multiphyisics 3.5a.

4.1 Cad Model Description

A 3D CAD model of the whole structure (Fig. 1) has been realized through proper CAD tools and then employed both for the fabrication and modeling steps.
As for the modeling steps, by means of the Comsol Multiphysics CAD import module, the same model has been imported in the Comsol pre-processor, to obtain the corresponding FE model. This approach resulted very useful, both for the fabrication and the simulation purpose of the device.
It is worth to note that the FE model has been created in the assembly mode in the Comsol pre-processor, in order to gain more freedom about boundary conditions on the interfaces within the various layers (see section 4.3).

4.2 Materials Properties of the Model

Table 1 reports the physical properties assigned, within the thermal analyses, to the aluminum and silicon parts of the LOC device. The properties of the other assembly parts, being temperature dependent, were instead directly taken from Comsol Multiphysics material database, loaded from web resources (www.matweb.com) or from materials data sheet.
As for the reagents, thermal conductivity, density and heat capacity values are referred to water, being these parameters difficult to know.

	Density ρ [kg/m^3]	Thermal Conductivity k [W/(m*K)]	Heat Capacity at constant pressure C_p [J/(kg*K)]
Clamping System (Aluminium)	2700	160	900
Chip bottom layer (Silicon)	2330	163	703

Table 1: Materials properties.

4.3 Equations and Boundary Conditions

In absence of volume sources/sinks, the Comsol Heat Transfer module, in the time dependent case, solves for the following equation:

$$\rho C_p \frac{\partial T}{\partial t} + \nabla \cdot (-k\nabla T) = 0 \qquad (1)$$

The quantities ρ , C_p and k represent respectively the material density, heat capacity at constant pressure and thermal conductivity.
For what concerns the boundary conditions, on the external surfaces ($\partial\Omega$) of the model both Dirichlet and Neumann conditions are employed. They are respectively:

$$T = T_1 \text{ on } \partial\Omega_1 \quad (2a)$$

$$-\bar{n} \cdot k\nabla T = h(T_{inf} - T) \text{ on } \partial\Omega_2 \quad (2b)$$

where $\bar{n}$ is the normal unit vector, h the heat transfer coefficient and T_{inf} the reference bulk temperature.
In particular, Eq. (2a) is imposed on the bottom boundary of the clamping, that is, the part of the LOC which is in contact with the Peltier (Fig. 3). The values of input temperature T_1 are function of time and they have been obtained experimentally through the thermocouple TC1 (see section 3).
On the other hand, Eq. (2b) is applied to all the boundaries of the model in contact with air, to model natural air convective cooling, setting $h = 2$ [$Wm^{-2}K^{-1}$] and $T_{inf} = T_{amb} = 19.6$ [°C].

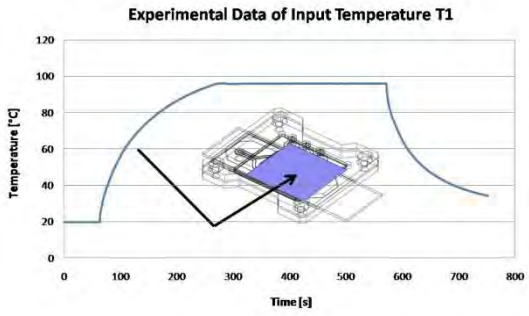

Figure 3. Input temperature curve T_1 (experimentally measured) applied on the surface of the bottom part of the clamping system.

Particular care is dedicated, furthermore, to interior boundaries i.e. boundaries between two subdomains in contact, where the heat transfer is active.
The contact surface, between the microfluidic chip and the bottom part of the clamping system is extremely important, being a place where, because of a non-optimal contact quality, a discontinuity in the heat flux may exist (Fig. 4).

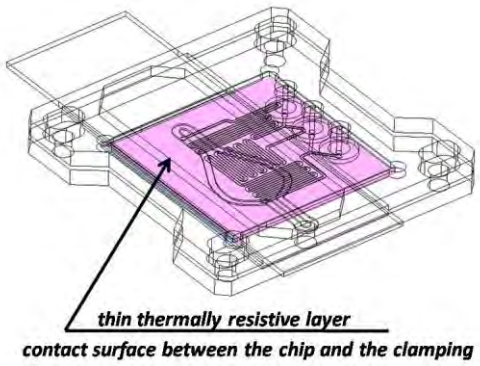

Figure 4. Contact surface, between the microfluidic chip and the bottom part of the clamping system for the modeling of thin thermally resistive layer.

In correspondence of this contact surface, the actual device contains a thermal grease employed in order to increase the thermal conductivity at the interface. Its main goal is indeed to fill air gaps that occur when the microfluidic chip is pressed against the irregular surface of the clamping system (bottom part), being the air thermal conductivity respectively about 9000 times less then aluminum (i.e. the material used for the clamping) and 6000 times less than silicon (i.e. the material used for the microfluidic chip).
Surface imperfections, on the other hand, arise from limitations in CNC manufacturing technology, used to obtain the clamping system. Both thermal conductivity and the "conformability" (i.e., the ability of the material to conform to irregular surfaces) are therefore crucial characteristics of thermal grease.
To model such a non-trivial contact condition within the computational model, a special boundary pair condition[1] which mimics a thin thermally resistive layer has been employed.
This condition can be expressed as two separate but symmetric heat flux boundary conditions:

$$-\bar{n}_{down} \cdot (-k\nabla T)_{down} = \frac{k_{res}}{d_{res}}(T_{up} - T_{down}) \text{ on } \partial\Omega_{down} \quad (3a)$$

[1] the assembly mode requires pair boundary conditions wherever the parts of the assembly are in contact with each other

$$-\bar{n}_{up} \cdot (-k\nabla T)_{up} = \frac{k_{res}}{d_{res}}(T_{down} - T_{up})$$

on $\partial\Omega_{up}$ (3b)

where k_{res} and d_{res} are respectively the thermal conductivity and the thickness of the thin resistive layer, modeling the thermal grease. The "up" and "down" subscripts are referred to the two boundaries making up the pair.
Clearly Eqs. 3a, b hold if the layer is sufficiently thin and the tangential heat flux is negligible, thus making the temperature jump across the boundary proportional to the normal heat flux.
Such an approach allows to model the presence of the thermal grease just through an equation, thus avoiding all the problems concerning the meshing of a thin layer. This fact leads to a considerable saving of the overall computational cost.

4.4 Mesh Settings

Tetrahedral lagrangian linear elements were used for the discretization of the model.
Mesh convergence study determined that a mesh involving about 86000 tetrahedral elements corresponding about 30000 degree of freedom was sufficient for obtaining accurate results.

4.5 Solver Settings

Time dependent analyses were performed to track the time evolution of the thermal cycles of the genetic protocols. The default BDF solver was employed to solve the numerical problem in the time range (0,723s) with steps of 1s.

5. FE Model Validation: Experimental Measurement vs Fem Results

In order to obtain a FE model that predicts the temperature values in the reaction chamber, a set of validation analyses has been carried out and compared to the experimental measurements.
It is worth to note that, to reproduce the presence of the TC2 thermocouple, a point probe has been located, within FE model, in the corresponding position (Figs. 5, 6).
The calibration curve reported in Fig. 7, shows a good agreement between the experimental and numerical data, especially for what concerns the b zone.

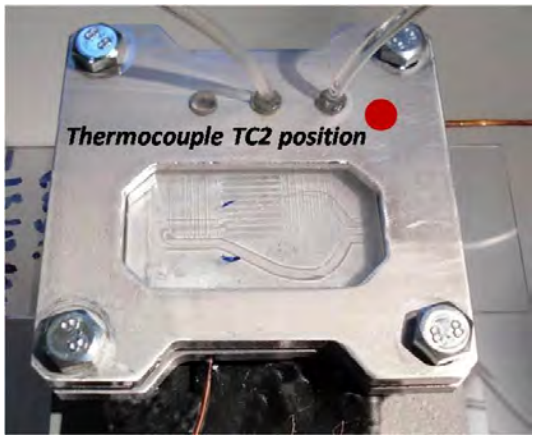

Figure 5. Thermocouple position TC2 in the LOC.

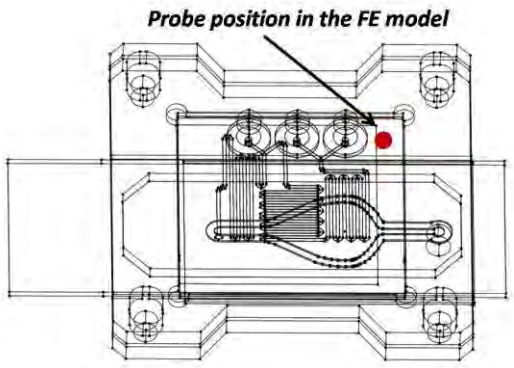

Figure 6. Probe position in the FE model.

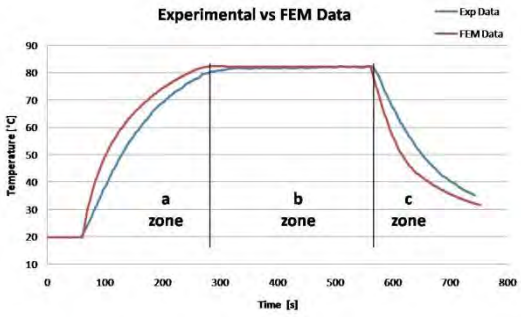

Figure 7. Calibration curve of the FE model.

The discrepancy in the a and c zone is probably due to values of the heat capacity employed in FE model, which may slightly differ from their experimental counterparts. On the other hand, it is worth to note that heat capacity influences the

results just when time variation of temperature are present (see Eq. 1), that is, actually in a and c zones.
The best results of the calibration curve reported in the figure 7 have been obtained for a value of d_{res}=100 µm and k_{res}=10 W/(m·K).

6. Results

Figs. 8-11 contain the results about the thermal analyses. In particular, for each material of the microfluidic chip, the corrections for the input curve T_1 are reported. These correction curves represent the temperature profile to be applied to the bottom part of the clamping system , in order to obtain the DNA denaturation temperature into the reaction chamber.
The correction is defined as $T_{1\_corr} = T_1 + \Delta T$ where the correction factor ΔT depends on the particular material that composes the microfluidic chip.
The temperatures into the reaction chamber before (T_RC) and after (T_RC_corr) the correction are calculated as the average over the water domain. This was obtained through integration coupling variables in the FE model.
To test the overall accuracy of the FE model, the T_RC_corr curve and the DNA denaturation temperature profile T_1 have to be compared.
The best agreement is obtained for the Silicon/Pyrex® microfluidic chip (Fig. 8). As expected, this is due to the good thermal properties of the silicon in terms both of the heat capacity and thermal conductivity.
As for the polymeric materials, the best results is obtained for the PC materials. This is particularly evident in the ―plateau" of the curve, where a temperature of 95 °C is necessary for the DNA denaturation (Fig. 9).
These results demonstrate that, in order to switch from Silicon/Pyrex® to the polymeric based microfluidic chips, the PC is the best choice. This polymeric materials indeed ensures, into the reaction chamber, a thermal curve which is closer to the DNA denaturation one than those of PMMA and COC (Figs 10, 11).

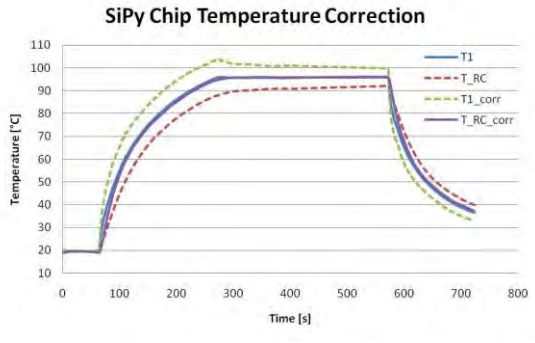

Figure 8. Comparison between the temperature correction curve into the reaction chamber ―T_RC_corr" and the DNA denaturation curve ―T1" for the Silicon/Pyrex® microfluidic chip. The other two curves ―T1_corr" and ―T_RC" represent respectively the temperature correction for the input temperature and the temperature into the reaction chamber before the correction.

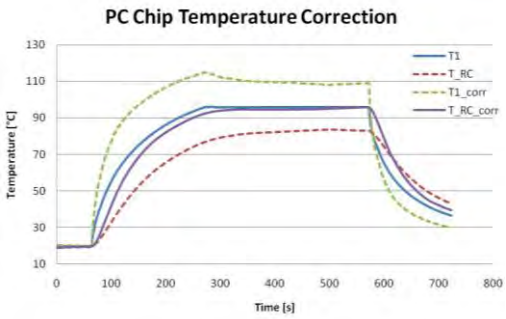

Figure 9. Comparison between the temperature correction into the reaction chamber ―T_RC_corr" curve and the DNA denaturation curve ―T1" for the PC microfluidic chip. The other two curves ―T1_corr" and ―T_RC" represent respectively the temperature correction for the input temperature and the temperature into the reaction chamber before the correction.

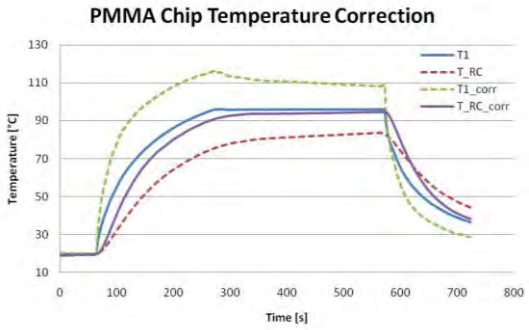

Figure 10. Comparison between the temperature correction into the reaction chamber ―T_RC_corr" curve and the DNA denaturation curve ―T1" for the PMMA microfluidic chip. The other two curves ―T1_corr" and ―T_RC" represent respectively the temperature correction for the input temperature and the temperature into the reaction chamber before the correction.

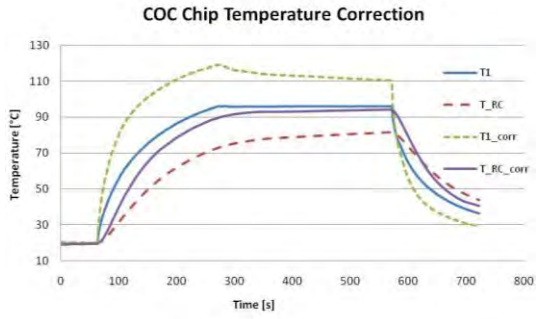

Figure 11 Comparison between the temperature correction into the reaction chamber ―T_RC_corr" curve and the DNA denaturation curve ―T1" for the COC microfluidic chip. The other two curves ―T1_corr" and ―T_RC" represent respectively the temperature correction for the input temperature and the temperature into the reaction chamber before the correction.

7. Conclusion and Future Work

In this work, a Finite Element model which allows to monitor the temperature values into the reaction chamber of a LOC device is illustrated and applied to some innovative technological solutions involving different chip materials.

Thanks to the numerical results, it has been possible to establish that, among all the polymers analyzed, the PolyCarbonate (PC) is the one which performs better, from a thermal point of view. Furthermore it is shown how to correct the temperature profile to be applied to the bottom part of the clamping system, to obtain, into the reaction chamber, the DNA denaturation temperature.

Future work will be focused on implementing the FE model on the new version 4.0a of Comsol Multiphysics by adding the Structural Mechanics module to explore the mechanical stress and strain of the whole device. In this way it will be possible to further optimize the geometry of the device in the region of the contact area between the reaction chamber and the microarray glass slide, where a reversible sealing is necessary.

With the new release of Comsol Multiphysics it will be also possible to perform parametric time dependent analyses to determine, through a ―one-step" calculation, the minimum thickness of the chip that both maximizes the heat transfer and also allows to obtain the required temperature into the reaction chamber.

It is clear that this approach will allow to gain also considerable information about the technological steps (i.e the masters design and the hot embossing procedures for their fabrication) involving the polymeric chip.

8. References

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