Modeling and Simulation of a Manycore PCR-CE Lab-on-Chip for DNA Sequencing using SystemC/SystemC-AMS

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ABSTRACT

The paper details the systemC/systemC-AMS model of a bio-compatible heterogeneous system, a lab-on-chip that encompasses several disciplines such as analog, digital, chemical kinetic reactions, optics and embedded software. The corresponding system virtual prototype takes as input an initial DNA concentration as well as the expression of the gene as a DNA string, and is able to compare the input string to a huge database of reference samples, and this detects mutation and pathologies. The model is composed of three parts: (1) DNA amplification by Polymerase Chain Reaction (PCR), (2) molecular separation by Capillary Electrophoresis (CE) and optical detection of fluorescently labeled molecules, (3) automated DNA sequencing on a digital manycore architecture running a highly multi-threaded software. The platform also digitally assists the two former analog parts which allows performance in complete autonomy. The simulated model can be used as a simulatable specification at a very high level of abstraction and can be seen as the first refinement step towards the design of a complex heterogeneous and bio-compatible system.

Keywords

system modeling, simulation, lab-on-chip, DNA, PCR, capillary electrophoresis, SystemC, SystemC-AMS

1. INTRODUCTION

Lab-on-Chip (LOC) is a rapidly expanding area of science. The technological progress has made it possible to perform many complex analyses on a few square millimeters chip. The modeled LOC is composed of a chamber for PCR reaction, a separation channel for CE, optical excitation and detection and finally embedded many-core architecture for system control and information processing. First, the DNA sample, as well as some reagents are introduced into the PCR chamber. Polymerase Chain Reaction (PCR) is a technique for DNA amplification introduced in 1985 [1] that relies mainly on thermal cycling. The first phase of a PCR cycle consists in heating the double stranded DNA (dsDNA) input sample till its melting point, at which it is decomposed into two single stranded DNA (ssDNA), corresponding to the Denaturation phase. The second phase of PCR cools down the mixture to a temperature that allows the (previously synthesized) primers to anneal to a specific place in the ssDNA, delimiting the target sequence to be amplified (Annealing phase). The third phase consists in heating the mixture up to the optimal temperature for polymerase enzyme activation, which uses dNTPs (Deoxynucleoside Triphosphates) as building blocks to synthesize new strands of DNA (Extension step). Ideally, the number of copies of dsDNA molecules is doubled every cycle, leading to an exponential growth of DNA molar concentration. PCR phases are repeated for as many cycles as needed, until the required target DNA concentration is obtained, i.e. sufficient for detection.

The chain termination method, or Sanger method [2], is one of the most used techniques for automated DNA sequencing. It consists in initiating the synthesis of new strands of DNA using one set of primers, dNTPs and thermostable polymerase enzyme, in addition to fluorescently labeled chain terminators or ddNTPs (dideoxynucleotide triphosphates). This reaction results in the generation of DNA molecules of variable lengths, each of which being terminated by one of the four terminators corresponding to one of the four DNA bases (Adenine (A),Cytosine (C),Guanine (G) or Thymine (T)). It is based on statistical functions relying on the DNA sequence, and reagents concentrations.

DNA molecules are then separated by capillary electrophoresis, by means of an electric field applied across the separation channel. The negatively charged molecules start migrating towards the anode with different velocities, according to their molecular complexity, DNA length and electric charge.

The separation channel ends with an optical detection system, which is responsible for translating the fluorescence intensity and frequency of the labeled molecules into electric signals. These analog signals are converted to digital and are further filtered/processed in order to determine the

target DNA sequence.

The digital subsystem is composed of a shared-memory compliant 2D mesh of processors and memories interconnected via a Network on Chip (NoC). It is responsible for the control of the two former subsystems (PCR and CE). It also performs the comparison between the target sample coming from CE against a library of reference samples located in an internal or external memory. The local alignment of DNA sequences is computed by a parallel version of the dynamic programming Smith-Waterman algorithm.

The paper is composed of six sections. Section two details the related work and presents the various models found in the literature. Section three thoroughly describes the complete case study and the associated mathematical models. Section four presents the implementation and explains how systemC and systemC-AMS can be used to model and simulate the system as a netlist of communicating multi-disciplinary modules. Section five gives some simulation results. In the final section, a conclusion is given that shows how systemC and systemC-AMS can be used with success to design high-level executable specifications of complex biocompatible systems.

2. RELATED WORK

James T. Hsu et al. [3] developed a mathematical model for polymerase chain reaction, taking into account the three steps in this process: denaturation, annealing and extension. Computer simulation of the model was carried out to determine the effect of various parameters such as initial DNA concentration, number of cycle, temperature and enzyme deactivation on DNA generation.

Ji Youn Lee et al. [4] developed a more complex mathematical model for PCR, and performed computerized simulation with real time monitoring of the amplification profile, mainly to investigate the cause of the plateau phenomenon where the output of the PCR saturates.

Dieter Schmalzing et al.[5] presented a model that quantitatively describes the performance of micro-fabricated electrophoretic devices. The dependence of resolution on various separation parameters such as selectivity, diffusion, injector size, device length, and channel folding was investigated. The model was used to develop and optimize microfabricated electrophoretic devices for DNA analyses.

An extremely sensitive tool to monitor the interaction of fluorescently labeled molecules or other micro-particles with their respective biological counterparts is presented by Peet Kask et al.[6]. They developed fluorescence-intensity distribution analysis based on adjustable formula and the technique of generating functions for calculation of theoretical photon count number distributions, and their method permits the simultaneous determination of concentrations and specific brightness values of a number of individual fluorescent species in solution.

Additionally, the modeling of electropherogram data [7] [8] has also been investigated with the development of an algorithm for data interpretation or "base-calling", framed mainly within a Bayesian probabilistic framework. Besides, computer programs, like Phred, have been developed to perform automated base calling [9].

All these works, as well as many others, aim at modeling physical, chemical or biological phenomena, in order to have a better understanding of the facts. As a result, more optimization can be applied to the process, yielding more efficiency with accurate results. However, the modeling of interaction between processes of different natures, within a system, is less obvious.

Consequently, our goal is to model and simulate a complete heterogeneous system as a single simulation platform, which gives global view of the system, with its interacting parts, opening the gate for global optimization, as well as refinement of sub-systems.

3. CASE STUDY: PCR, CE AND DNA SEQUENCING ENGINE ON A SINGLE CHIP

3.1 System Overview

Figure(1) gives a general description of the system, and shows the different sub-systems interact together under the control of the digital manycore subsystem. The first part of our system is a chamber where the PCR takes place. First, a DNA sample is introduced, represented by the couple (initial concentration, sequence of characters referring to the acid bases (A,C,G,T)). Then all the necessary reagents are mixed together, each with a given concentration. The reaction is heated up and cooled down by means of a Peltier device, a solid state active heat pump used for heating and cooling that is controlled by an input DC current. The temperature of the reactor is sensed by a Resistance Temperature Detector (RTD), that is normally made of platinum which resistive value depends on temperature. This variation in resistance is translated into electric voltage and is fed to the positive input of three comparators. On the negative input of each comparator is a reference voltage, set by the digital control, corresponding to one of the three temperatures for the denaturation, annealing or extension phase. The thermocycling is carried on for as many cycles as decided by digital control. After the target DNA sequence has been amplified by PCR, DNA sequencing occurs using the Sanger method. The fluorescently labeled chain terminators or ddNTPs are introduced into the mixture. New strands of DNA are synthesized, resulting in DNA fragments of variable lengths. The next step consists in molecular separation by capillary electrophoresis. The control valve is opened, and an electric potential is applied across the capillary separation channel. The negatively charged DNA molecules start migrating towards the anode, with velocity approximately inversely proportional to their length.

At the end of the capillary channel, optical excitation is done with a Light-Emitting Diode (LED) at a carefully chosen center wavelength. Fluorescently labeled molecule, absorb the optical power and begin to fluoresce at different wavelengths. Four photodetectors with optical filters are used to detect the fluorescence. Each photodetector is responsible for detecting light at a specific wavelength, corresponding to fluorescence frequency of molecules ending with a specific base (A,C,G or T). Photocurrent is then amplified, converted to digital, and further processed.

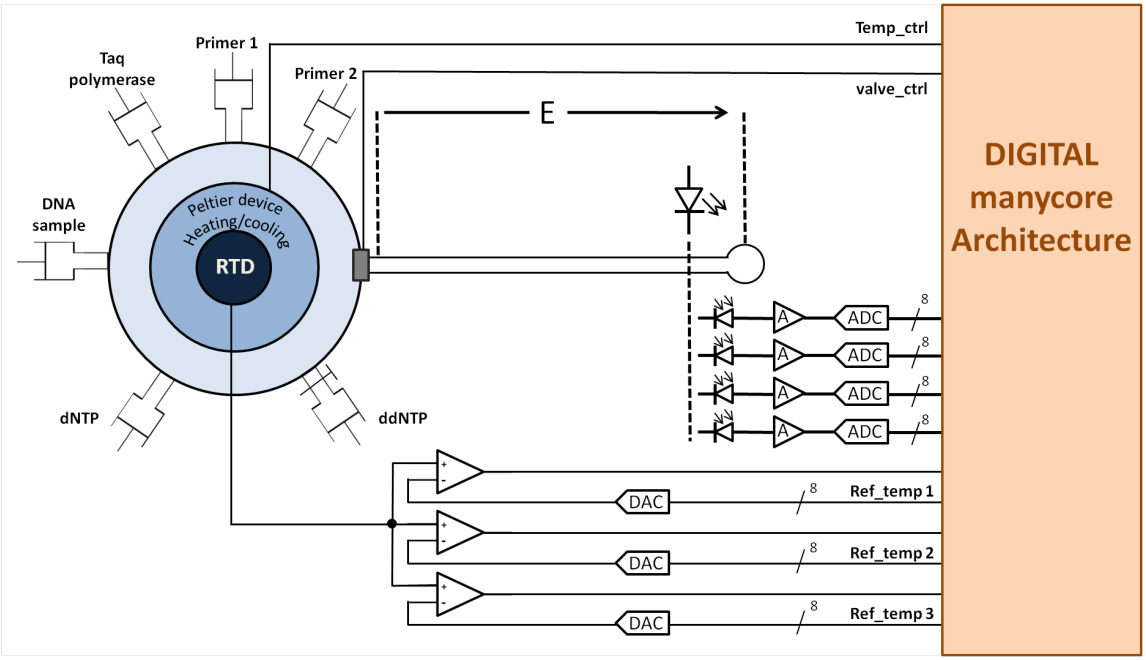

Figure 1: System overview

The digital platform has two main functions: PCR and CE control on one hand, and data processing on the other. First, it controls the thermocycling of the PCR by means of control signals sent to the Peltier device, causing the heating or cooling, and signals from the comparators connected to the RTD indicating the current temperature range. Then it sends control signal to open the valve allowing the fluid to flow into the capillary channel. Its second role is to process the signals coming from the photodetectors, in order to identify the unknown sequence of DNA. Furthermore, the digital subsystem executes a complex sequence alignment algorithm (Smith-Waterman) to identify the best match of the detected sequence within a set of database stored on a local memory of large capacity. Next section discusses how all the different parts of the system are modeled.

3.2 Amplification by PCR

The modeling of the amplification by PCR is based upon the mathematical model developed by James T. Hsu, Simantini Das and Satish Mophapatra [3]. According to their work, The most important component in PCR is the Taq DNA polymerase enzyme. The first phase of PCR cycle, the denaturation phase, lasts from 15 to 60 seconds depending on the exact nature of the DNA strand composition. Although the enzyme is added in excess at the beginning of a PCR process, repeated exposure at a temperature of 94°C results in enzyme deactivation, which can hamper product generation in later cycles. The rate of deactivation of the enzyme can be represented as:

$$(-r_A) = (-\frac{da}{dt}) = K_d\, a^{\alpha} \tag{1}$$

where K_d is the deactivation rate constant of the enzyme (s^{-1}); $a = \frac{E(t)}{E_0}$ is the ratio of the activity of enzyme at any time "t" E(t), to initial activity E_0; and α is the reaction order of thermal deactivation.
K_d can be expressed in Arrhenius [10] form as:

$$K_d = K_{do}\, e^{-\frac{E_d}{RT}} \tag{2}$$

E_d is the energy of deactivation (Joules/mol); K_{do} is the Arrhenius constant for deactivation; R is the universal gas constant ($Joules/mol\,°\mathrm{K}$); and T is temperature (in Kelvin). For the annealing phase, it has been proposed an empirical relationship between the optimum annealing temperature and the normalized length of the primer. In this model, we have assumed an intermediate annealing temperature where non-specific annealing is minimum and the reaction is stable. In the extension phase, the polymerase enzyme joins the nucleotides together and completes primer extension to synthesize a double-stranded DNA. The rate of generation of DNA in the first cycle is given by:

$$\frac{dC_D}{dt} = K_1^* \cdot \frac{C_{D0}}{y} \tag{3}$$

C_{D0} being the initial concentration of target template DNA present in the medium in M; C_D the concentration of DNA after time "t" in M; K_1^* the rate of incorporation of nucleotides by the polymerase enzyme in one DNA strand in the first cycle; and y the number of nucleotides that constitute one target sequence. From one cycle to another, the number of ssDNA available for reaction doubles, and so doubles the reaction rate. Hence, the rate of amplification of DNA is given by:

$$\frac{dN}{dt} = 2^{n-1}\, K1 \tag{4}$$

where $N = \frac{C_D}{C_{D0}}$, $K_1 = \frac{K_1^*}{y}$, n= number of cycles and K_1 could be represented as:

$$K_1 = K_0\, e^{-\frac{E_a}{RT}} \tag{5}$$

where K_0 is the Arrhenius constant for the reaction; and E_a is the energy of activation for synthesis. The above equations hold true if the alloted time is sufficient for the extension process and the ratio of active enzyme to ssDNA concentration (β) is >1.0. Throughout the early cycles, the active enzyme is in excess, and the number of molecules of target DNA doubles from cycle to another, showing an exponential behavior. At some point, due to the enzyme deactivation and product accumulation, the available amount of active enzyme is not sufficient to extend all the available ssDNA molecules within the alloted time. From this point on, the amplification rate is defined as:

$$\frac{dN}{dt} = K_r \,.\, \beta_r \tag{6}$$

where $K_r = 2^{r-1}K_1$ and

$$\beta_n = \frac{E_0\, a_n}{C_{D0}\, 2^n} \tag{7}$$

where E_0 is the initial concentration of the active enzyme in M; and a_n is fraction of enzyme activity remaining after n cycles. And from this cycle on, the PCR product accumulates in an approximately linear function of time, rather than exponential.

3.3 Sanger chain termination

The Sanger method for DNA sequencing is based on the use of ddNTPs, as chain terminators, producing DNA fragments of varying lengths to be separated by CE. The used mathematical model relies on the one that was developed by Linda G.Lee et al. [11] to predict the pattern of termination of DNA molecules based on enzyme activity and ratios of ddNTP/dNTP. The probability of termination at a given base "p_n" is given by:

$$p_n = R_N \frac{[ddNTP]}{[dNTP]} \tag{8}$$

where R_N is an enzyme-dependent discrimination constant. The molar amount terminating at base number "i" is given by:

$$d[i] = X[i]D_0 \tag{9}$$

where D_0 is the initial molar amount of DNA, i.e. the result of the PCR stage; and X[i] is a factor dependent on the probability of termination as well as the previous bases in the sequence, and is calculated by the following recursive expression:

$$X[i] = X[i-1]\frac{(1-p_{i-1})p_i}{p_{i-1}} \tag{10}$$

3.4 Separation by capillary electrophoresis

For molecular separation by CE, an electric field 'E' is applied to the separation channel, causing the DNA fragments to migrat towards the anode with a velocity given by:

$$v = \mu_e E \tag{11}$$

where μ_e is the electrophoretic mobility, which varies according to the molecule length, resulting in different velocities for the DNA fragments. Several authors have assumed that one-dimensional diffusion is the major contributor to peak broadening in CE [12]. If the sample plug that was injected were a true δ-function, that plug would spread in time to a Gaussian peak with a variance equal to [13] :

$$\sigma^2 = 2\,D\,t_m \tag{12}$$

where D is the diffusion coefficient, and t_m is the migration time of a particular zone, $t_m = L/v$, L being the separation channel length. The temporal width of the peak could be calculated by dividing the spatial width by the zone velocity.

3.5 Optical detection

For the optical detection, a LED serves as excitation source at a center wavelength of 488nm. The four fluorescent dyes attached to the the four chain terminators (ddATP, ddCTP, ddGTP, ddTTP) absorb the light, and each starts fluorescing at a different wavelength (500, 540, 570, 590 nm). Four photodiodes generate photocurrent proportional to the detected optical power, with a proportionality constant equivalent to the photodiode responsivity. Each photodiode is preceded by a dichroic filter, allowing one of the desired wavelength, and attenuating all the others. A dichroic filter is a very thin sequential array of Fabry-Pérot interferometers and is therefore characterized and modeled by:

$$T = \frac{(1-R)^2}{1+R^2-2R\cos\delta} \tag{13}$$

$$\delta = (\frac{2\pi}{\lambda})\,2\,n\,l\cos\theta \tag{14}$$

Where 'T' is the transmittance function, 'R' the surface reflectance, 'λ' the incident wavelength, 'θ' the angle of incidence of light, 'l' the thickness of the etalon and 'n' the refractive index of the material between the reflective surfaces.

The analog photocurrent is filtered, amplified and then converted using a successive approximation analog to digital converter (SAR ADC), which is suitable thanks to its low power consumption, and since no high resolution or fast conversion is required. The converted signal is then used by the digital platform for the base-calling and identification of the target DNA sequence.

3.6 Control and processing

The digital hardware architecture that is responsible for PCR-CE control and intensive calculations follows a NUMA shared-memory paradigm, i.e. any initiator (processor) can access any target (RAM) in the chip provided it knows its address. This manycore architecture is a 2D-mesh of computing tiles interconnected via a global asynchronous NoC. The NoC can be seen as a collection of communicating routers that collaborate to forward packets coming from one of the five directions (N, S, E, W and local) to the appropriate destination, using the X-first routing algorithm. In practice, and to avoid deadlocks, there are two meshes in the NoC, one for the requests and one for the responses. A tile contains one to 4 32-bit simple-scalar low-power processors such as MIPS32 with their associated instruction and data caches, local memory banks and peripherals. All the components of a tile are connected to a local interconnect which is in turn connected to the global mesh interconnect through the use of a synchronous/asynchronous interface network controller.

The embedded application running on the digital platform is an integer implementation of the Smith-Waterman (SW) algorithm [14] that performs local sequence alignment of DNA strings. In the paper, Smith-Waterman is used to compare the DNA string coming from the detection sub-system against a library of reference samples. Comparing the target sample with any of the reference samples can be seen as an autonomous task, and a parallel, multi-threaded implementation of this dynamic programming algorithm, written in C language, can be mapped onto the described hardware architecture. The embedded application follows the standard Kahn Process Network model, i.e. an application is composed of a finite number of collaborative Posix threads that exchange data through the use of non-blocking software fifos located in the local memory banks of the tiles. The quality of the mapping of software tasks onto physical processors and fifos on memory banks has a great impact on the overall performance. Pthread scheduling for each processor is managed by means of a dedicated operating system microkernel implementing all the required Pthread system calls. According to the computation power needed, and using the task farm principles, any number of "comparing" tasks can be mapped onto the processors.

4. SYSTEMC / SYSTEMC-AMS IMPLEMENTATION

The analog subsystems have been modeled with the recent SystemC-AMS standard [15][16][17][18][19], using the Timed DataFlow (TDF) non conservative model of computation. TDF is a discrete-time modeling style which considers data as signals sampled in time. These signals are updated at discrete points in time and carry discrete or continuous values, like amplitudes.

The set of connected TDF modules with input and output ports forms a directed graph, called a TDF cluster. TDF modules are the vertices of the graph, and TDF signals correspond to edges. Each TDF Module involved in the cluster contains a C++ member method, named **processing()**, that computes a mathematical function f (i.e. $f_A()$, $f_B()$, and $f_C()$) that depends only on its direct inputs. The overall equation computed by the cluster is therefore defined as the mathematical composition of the functions of the involved TDF modules in the appropriate order, i.e. $f_C(f_B(f_A()))$.

The power of the TDF MoC comes from the ability to modify the characteristics of an instantiated TDF module or one of its constitutive ports. In TDF, it is possible to associate a particular timestep to a TDF module (module timestep association, needed to set the operating cluster sampling frequency).

Provided the associations performed on the ports or the modules of a TDF graph are compatible, the order and number of samples (sampling rate) in a TDF cluster is known for each calculation. This order can be statically determined before the simulation starts and corresponds to a static schedule of the TDF cluster. This static schedule, computed once for all during the so-called simulator elaboration is at the origin of the simulation speedup over traditional simulation kernels.

The digital subsystem has been modeled in SystemC, using an existing library of simulation models dedicated to the modeling [20] of multiprocessor architectures. The library contains hundreds of cycle-accurate bit-accurate IP models ranging from processors to peripherals and NoC, and is OCP-compliant for interoperability. All the models are described as Finite State Machines, with a state register, a transition function, and a Moore output generation function. By means of this description, the elaborated (in the VHDL sense) digital platform can be seen as a collection of communicating synchronous finite state machines which can efficiently be simulated with a canonical simulation loop (call all the transition functions in any order, then call all the Moore output functions in any order) instead of the traditional but slow discrete event simulation paradigm.

5. SIMULATION RESULTS

During a PCR cycle, the mixture is heated up to 94°C for 1 minute (Denaturation), then it is cooled down to 37 °C for 1 minute (Annealing) and then re-heated up to 72°C for 2 minutes (extension). PCR is carried on for 25 cycles, and the mixture temperature is monitored by means of the RTD. Figure(2) shows the temperature variation throughout the first 10 cycles. Repetitive exposure to high temperature

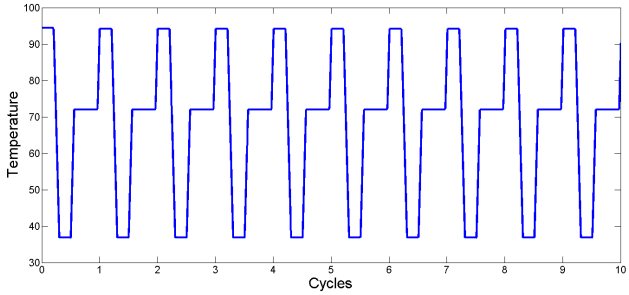

Figure 2: Temperature variation

causes polymerase enzyme deactivation. Figure(3) shows the ratio of the activity of enzyme at time 't', E(t) to initial activity E_0. It can be noticed that enzyme deactivation

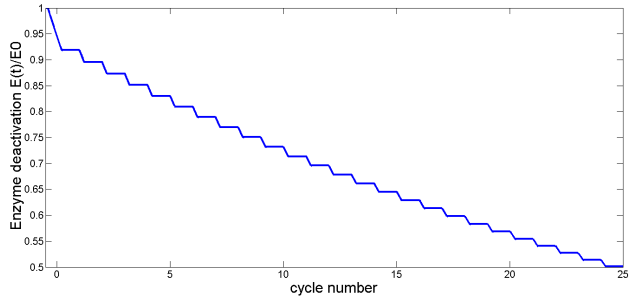

Figure 3: Enzyme deactivation

occurs mainly during the first minute of each cycle, corresponding to denaturation phase, due the high temperature. Figure(4) shows the DNA amplification profile with respect to the cycle number. During the first two minutes of each cycle the DNA molecules are heat denaturated, and then hybridized with primers, however, there is no increase in the

number of dsDNA molecules. It is only in the third phase (extension), that the concentration of dsDNA increases, as shown in the graph. It is also be noticed that until cycle 16, the simulated amplification matches the exponential theoretical maximum, equivalent to 2^n, 'n' being the number of cycles. From cycle 17 onwards, amplification becomes linear as the enzyme and reaction time limite the kinetic reactions and all the ssDNA-primer complexes are not extended to dsDNA. These results match with those published in [3], validating the results. Figure(5) shows the output signals

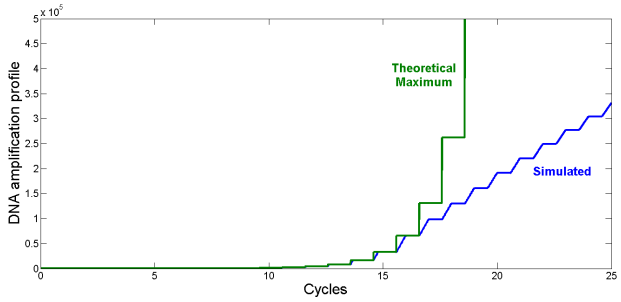

Figure 4: DNA amplification

of the four photodetectors, after amplification. They correspond to the relative fluorescence of the four dye-labeled chain terminators. These signals, after conversion, are used for DNA base-calling, to identify the target sequence.

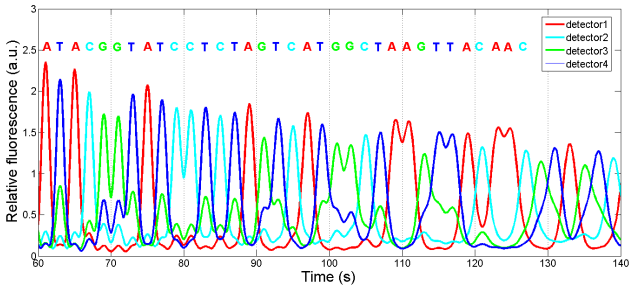

Figure 5: Analyzed four color DNA sequencing data

6. CONCLUSION

The virtual prototype of the PCR-CE lab-on-Chip has been successfully designed and is able to perform PCR, CE, detection and calculation in less than 10 minutes (real time) for an input sample of 100 bases, and a database of 100 reference samples. Using the TDF modeling style it is possible to refine any submodel of PCR, CE or optics and nothing prevents the designer to replace a component with ideal behavior by a more accurate model taking into account higher order transfer functions. As is, the model can act as simulatable specification or executable specification and the value of any digital or analog signal can be extracted. The use of SystemC/SystemC-AMS and freely available models allows for the design of tunable and flexible simulator that can be modified as needed. From the simulation viewpoint, once the TDF cluster has completed the amplification and detection of the DNA sample, it would be effective to deactivate the scheduling of the TDF cluster as it is no more needed. The current version of SystemC-AMS does not provide this feature yet.

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