Studying the Chlorophyll Fluorescence in Cyanobacteria with Membrane Computing Techniques

Ioan Ardelean[1], Daniel Díaz-Pernil[2], Miguel A. Gutiérrez-Naranjo[3], Francisco Peña-Cantillana[3], Iris Sarchizian[4]

[1]Institute of Biology Bucharest, Romanian Academy, 060031 Bucharest, Romania
`ioan.ardelean57@yahoo.com`

[2]Research Group on Computational Topology and Applied Mathematics
Department of Applied Mathematics - University of Sevilla, 41012, Spain
`sbdani@us.es`

[3]Research Group on Natural Computing
Department of Computer Science and Artificial Intelligence
University of Sevilla, 41012, Spain
`magutier@us.es, frapencan@gmail.com`

[4]Scoala Gimnaziala nr.38 Dimitrie Cantemir, Constanta, Romania
`irissarchizian@yahoo.com`

Summary. In this paper, we report a pioneer study of the decrease in chlorophyll fluorescence produced by the reduction of MTT (a dimethyl thiazolyl diphenyl tetrazolium salt) monitored using an epifluorescence microscope coupled to automate image analysis in the framework of P systems. Such analysis has been performed by a family of tissue P systems working on the images as data input.

1 Introduction

Membrane Computing has many features that makes it suitable for the study and the implementation of algorithms of digital images. One of them is that, usually, these algorithms can be parallelized and locally solved. Regardless how large the picture is, many algorithmic processes can be performed in parallel in different local areas. Another interesting feature is that the local information needed for a pixel transformation can also be easily encoded in the data structures used in Membrane Computing.

Recently, a new research line has been open by applying well-known Membrane Computing techniques for solving problems from digital images. For example, segmentation is a well-known problem in the process of digital images which tries to

assign a label to every pixel in an image in such way that pixels with the same label share certain visual characteristics. Segmentation has shown its utility, for example, in bordering tumors and other pathologies or computer-guided surgery. In [11, 12, 13, 15, 31] we can find several approaches to this problem with Membrane Computing techniques. Other problems as *thresholding* [10] or *smoothing* [25] has also been considered in the framework of membrane computing. Special attention deserves [18], where the *symmetric dynamic programming stereo* (SDPS) algorithm [19] for stereo matching was implemented by using simple P modules with duplex channels or [33], where the authors combine Membrane Computing and quantum-inspired algorithms for image processing.

In [2], a first approach of the application of Membrane Computing techniques to the study of images from Microbiology was presented. Automated image analysis is increasingly used in Microbiology to quantify important parameters for research and application. The most studied so far are the cell numbers, cell volumes, frequencies of dividing cells, *in situ* classification of bacteria, enumeration of actively respiring bacteria, characterization of bacterial growth on solid medium, viability and physiological activity in biofilms (e.g. [9, 17, 29, 30]).

In [2], the focus was the study of the application of Membrane Computing techniques to the problem of counting cells. The whole process is a combination of different techniques of processing images (binarization, segmentation, noise reduction . . .) which can be performed by different families of P systems. The final algorithm is a sequence of partial processes which can be performed by Membrane Computing techniques, and the application of such processes can be seen as a global machine which takes as input a digital image showing a biological entity (usually, a photograph taken with a microscopy in a *wet lab*) and the output is the number of cells in the picture.

In this paper, we focus on the problem of considering the intensity of color in cyanobacteria, a phylum of bacteria that obtain their energy through photosynthesis, by using algorithms based on Membrane Computing techniques. We report a new study of the decrease in chlorophyll fluorescence produced by the reduction of MTT (a dimethyl thiazolyl diphenyl tetrazolium salt) monitored using an epifluorescence microscope coupled to automate image analysis in the framework of P systems The different stages of the analysis have been performed by a family of tissue P systems working on the images as data input.

Such families of P systems used in the stages of the process have inspired parallel software programs which have been developed by using a device architecture called CUDA™, (Compute Unified Device Architecture). CUDA™ is a general purpose parallel computing architecture that allows the parallel NVIDIA[1] Graphics Processors Units (GPUs) to solve many complex computational problems in a more efficient way than on a CPU. GPUs constitute nowadays a solid alternative for high performance computing, and the advent of CUDA allows programmers a friendly model to accelerate a broad range of applications. This novel architecture has been previously used to implement parallel software that simulates the behav-

---

[1] http://www.nvidia.com.

ior of P systems [5, 6, 7, 8, 24, 25], and, in a similar way to other implementations, the obtained results in the problem of detecting cyanobacteria are quite promising.

The paper is organized as follows: Next, we recall the computational model used to design the different families of P systems that performs the stages of the algorithm. In Section 3, a short presentation of the biological experiment on cyanobacteria is presented. Section 4 outlines the steps of the analysis via a family of P systems with takes as input data the images taken in the wet lab. Section 5 provides some details of the implementation of such families on CUDA and finally, the paper ends with some conclusions and open lines for future research.

2 Formal Framework

Next, we recall some basics on the P system model chosen for implementing the solution described below. The model is tissue-like P systems with promoters. Promoters are usually defined on cell-like models [20] and its extension to tissue-like is quite natural. Next, we recall the formal definition.

Definition 1. *A tissue-like P system with promoters of degree $q \geq 1$ is a tuple of the form*

$$\Pi = (\Gamma, \Sigma, \mathcal{E}, w_1, \ldots, w_q, \mathcal{R}, i_{in}, i_{out})$$

where

1. *Γ is a finite alphabet, whose symbols will be called objects;*
2. *$\Sigma \subseteq \Gamma$ is the input alphabet;*
3. *$\mathcal{E} \subseteq \Gamma$ is a finite alphabet representing the set of the objects in the environment available in an arbitrary large amount of copies;*
4. *$w_1, \ldots, w_q$ are strings over Γ representing the multisets of objects associated with the cells in the initial configuration;*
5. *$\mathcal{R}$ is a finite set of rules of the following form:*

$$(pro \,|\, i, u/v, j), \quad for\ 0 \leq i \neq j \leq q,\ pro, u, v \in \Gamma^*$$

   In these rules, the labels $1, \ldots, q$ correspond to the q cells and the label 0 corresponds to the environment;
6. *$i_{in} \in \{1, 2, \ldots, q\}$ denotes the input region;*
7. *$i_{out} \in \{1, 2, \ldots, q\}$ denotes the output region.*

The rule $(pro \,|\, i, u/v, j)$ can be applied over two cells (or a cell and the environment) i and j such that u (contained in cell i) is traded against v (contained in cell j). The rule is applied if in i the objects of the promoter pro are present. The promoter is not modified by the application of the rule. If the promoter is empty, we will write $(i, u/v, j)$ instead of $(\emptyset \,|\, i, u/v, j)$.

Rules are used as usual in the framework of membrane computing, that is, in a maximally parallel way (a universal clock is considered). In one step, each

object in a membrane can only be used for one rule (non-deterministically chosen when there are several possibilities), but any object which can participate in a rule of any form must do it, viz., in each step we apply a maximal multiset of rules. A *configuration* is an instantaneous description of the system Π, and it is represented as a tuple $(w_0, w_1, \ldots, w_q)$, where $w_1, \ldots, w_q$, where represent the multiset of objects contained in the q cells and w_0 represent the multiset of objects from $\Gamma - \mathcal{E}$ placed in the environment (initially $w_0 = \emptyset$). Given a configuration, we can perform a computation step and obtain a new configuration by applying the rules in a parallel manner as it is shown above. A sequence of computation steps is called a *computation*. A configuration is *halting* when no rules can be applied to it.

3 Cyanobacteria

The object of study of our research are cyanobacteria. It is a phylum of bacteria that obtain their energy through photosynthesis. The ability of cyanobacteria to perform oxygenic photosynthesis is the reason why the primitive reducing atmosphere has became an oxidizing one. This new atmosphere sustained the emergence of living beings depending of oxygen, and changed the face of the Earth. It is thought that chloroplasts in plants and eukaryotic algae evolved from cyanobacterial ancestors.

Cyanobacteria are the most diversified, ecologically most successful and evolutionary most important group of prokaryotes [27] clearly defined by the ability to carry out oxygenic photosynthesis in the thylakoid membranes and respiration both in plasma membrane and thylakoid membrane [26].

Oxygenic photosynthesis, the ability to use the light energy to synthetize glucides from carbon dioxide and water, and to evolve oxygen from water molecules is essential for all the other forms of life on Earth. Historically, cyanobacteria were the first organisms to perform oxygenic photosynthesis and this metabolic ability of early cyanobacteria have converted the early reducing atmosphere of Earth (when no free molecular oxygen was available) into an oxidizing one. This process emerged approximately 3.5 billion years and had an essential effect on the evolution of life on our planet. There is a general agreement that the oxic atmosphere allowed the emergence and evolution of aerobic microorganisms, this is the occurrence of one of the greatest evolutive events on Earth, the emergence of eukaryotic cells most probably by endosymbiotic association between different types of prokaryotic cells.

The early cyanobacteria participated to this endosymbiosis thus all photosynthetic organisms on Earth have some cyanobacteria as ancestors; together with cyanobacteria (50 % contribution at planetary level) all these photosynthetic eukaryotes, including higher plants, contribute today to the synthesis of organic matter and oxygen production, the basis of all life forms here.

As an example of the importance of cyanobacteria for the life on our planet, one can remember that *Prochorococcus* -the most abundant cyanobacterium on

Earth- is responsible for 20 % of the molecular oxygen evolved (and, corresponding for 20 % of the consumed carbon dioxide and 20 % organic matter synthetized during oxygenic photosynthesis). Some cyanobacteria have also the ability to use atmospheric nitrogen as nitrogen source for growth, thus being able to live in environments where the concentrations of organic or inorganic nitrogen are very low. Cyanobacteria being very versatile microorganisms can live in very different environments for example from warmer springs to many cold sites, including glaciers. The important functions in Nature make cyanobacteria very strong candidates for the development of bio(nano)technologies the most known topics being the photoproduction of molecular hydrogen or electricity, biomass (and related processes, including valuable products) production and removal of different pollutants (petroleum hydrocarbon, heavy metals, nitrogen and phosphorus etc.,) from the environment.

The concentration of metalimnetic populations of *Planktothrix* sp. can be measured by epifluorescence microscopy of filaments collected on membrane filters. Computer image analysis is used to determine the length of filaments whose phycoerythrin fluoresces strongly in green light [32]. Similar methods have been used for enumeration of picoplanktonic cyanobacteria [1]. Image analysis was used in a previous work done on color analysis of cyanobacteria under labelling with quantum dots [3] and the cells within filamentous cyanobacteria were counted with tissue-like P systems [2].

Sarchizian *et al.* [28] investigated the ability of a cyanobacterial strain- isolate IS-H- to reduce MTT [(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)], an artificial electron acceptor, with special emphasis on quantitative determinations at single cell level using automated image analysis for precise color measurement of cells within the filaments of this strain. Up to our best knowledge this is the first report on the use of automated image analysis for the measurement of reduction of artificial redox carriers at single cell level in cyanobacteria or any other levels. The results show a strong decrease in the blue signal during MTT reductions by each individual analyzed cell, as a consequence of orange light absorption by reduced MTT.

Cyanobacterial filaments (actually each filament is one biological specimen) contains chlorophyll a which has a characteristic red fluorescence. This red fluorescence can be seen using different physical instruments, as fluorescence microscopes. This fluorescence is related to the light initially absorbed by the cell. In constant experimental conditions the fluorescence as one can be seen using a fluorescence microscope is practically constant. In our experiments cyanobacterial culture were challenged with a special chemical, namely MTT. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole), a chemical belonging to tetrazolium salts that is largely used to measure the metabolic activity in living cells (see, e.g., [4] or [28]).

The rationale design of our experiments is the following: the interaction of living cyanobacteria with MTT causes the reduction of MTT with electrons coming from cyanobacterial metabolism (photosynthesis, respiration and intermedi-
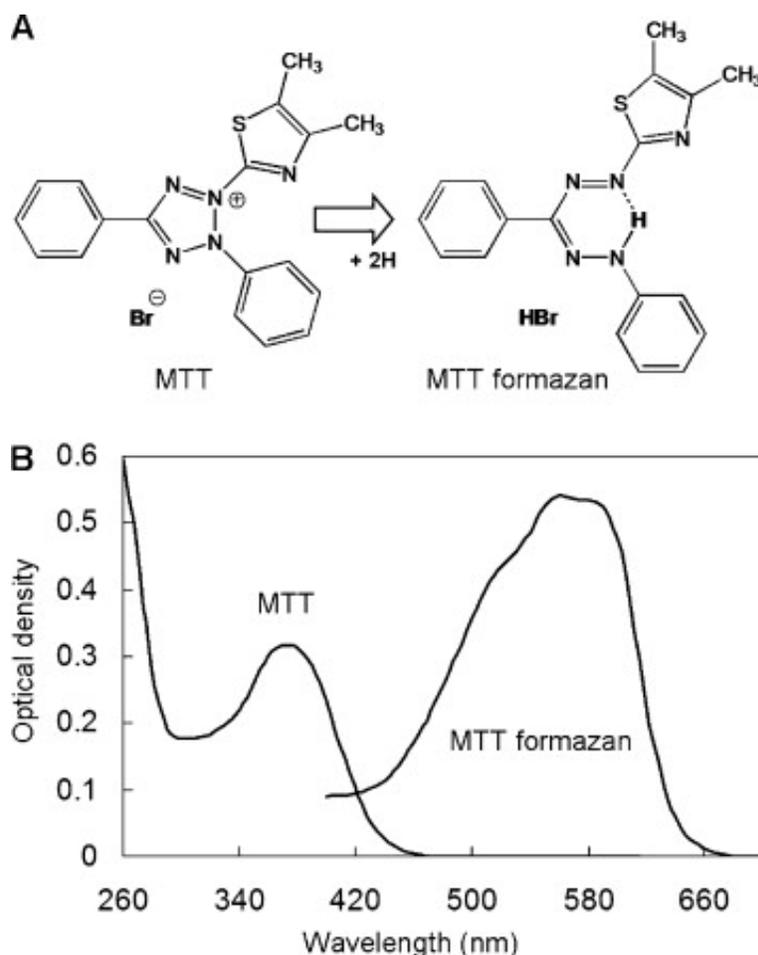
Fig. 1. The decrease of chlorophyll red fluorescence as a consequence of the accumulation inside the cell of MTT formazans crystal can be used to measure the intensity of MTT metabolic, light -dependent, reduction by cyanobacteria

ary metabolism). The reduction of MTT further decrease the intensity of chlorophyll fluorescence. The chemical reduction of MTT changes some of its properties, namely the color and the physical state of the molecule. The oxidized molecule is yellowish and water soluble whereas the reduced molecule (the so-called formazan) is dark brown, having a specific absorption spectrum, and it is insoluble in water (Fig. 1 (A)).

Fig. 1 (B) shows how MTT is reduced by enzymes called reductase (acting in photosynthesis, respiration and intermediary metabolism) to formazan. MTT is yellowish, soluble in water, and reduced MTT (MTT formazan) is insoluble (not

shown); when an appropriate chemical is added to dissolve the insoluble purple formazan product into a colored solution, this colored solution can be quantified by measuring the optical density at a certain wavelength (usually between 500 and 600 nm, as one can see, by a spectrophotometer).

Thus the reduction of MTT generate crystals of formazan which remain inside the cyanobacterial cell covering the intracellular structures of cyanobacteria. The most important intracellular structures of cyanobacteria involved in the reduction of MTT (as well as in the reduction of other artificial electron acceptors) are the thylakoides. Thylakoides are the sites where some of the light energy absorbed by thylakoids is converted in chemical stable energy found in molecules such as ATP and reduced form of chemical compounds (e.g NADPH etc.,); other part of some of the light energy absorbed by thylakoids is re-emitted as fluorescence.

During the process of MTT reduction by living cyanobacteria in light, the reduced MTT (formazan) accumulate inside the cell; this accumulation can be seen microscopically using light microscopy and even quantified by automated image analysis [28]. This accumulation of reduced MTT inside the cell physically covers intracellular structures, including thylakoides thus acting as a shield which blocks the access of light to thylakoides, thus decreasing the intensity of chlorophyll fluorescence.

Up to our best knowledge, this is for the first time when the decrease in chlorophyll fluorescence produced by the reduction of MTT is monitored using an epifluorescence microscope. However, the inhibition of important metabolic processes in cyanobacteria during tetrazolim salt reduction is documented in literature. Paerl and Bland [23] show the effects of localized reduction of five tetrazolium salts has strong negative impact on three important metabolic processes in cyanobacteria : N_2 fixation (acetylene reduction), CO_2 fixation, and H_2 consumption.

During short-term (within 30 min) exposures in the cyanobacterium *A. oscillarioides*, salt reduction in heterocysts occurred simultaneously with inhibition of acetylene reduction

Conversely, when salts failed to either penetrate or be reduced in heterocysts, no inhibition of acetylene reduction occurred. When salts were rapidly reduced in vegetative cells, $^{14}CO_2$ fixation and $^{3}H_2$ utilization rates decreased [23] .

The type of experiment presented in this paper has a deeper biological theoretical significance and a stronger practical application than our previous work done on color analysis of cyanobacteria under labelling with quantum dots [3] because of metabolic background. The decrease of chlorophyll fluorescence as a consequence of the accumulation inside the cell of MTT formazans crystal can be used to (indirectly) measure the intensity of MTT reduction at the level of filaments or even at the level of individual cells within each filament, cells which are subcomponents of the biological individual (the filament in the case of filamentous cyanobacteria).

4 Analyzing the Images

The study of the chlorophyll fluorescence in cyanobacteria has been split in several stages. In each stage, an image is provided as input and it is processed by a tissue P system with promoters described above. The result is an automatized image process performed by a sequence of P systems.

The target is to obtain information about the central cyanobacteria of the image of Fig. 2 (a). To do that, the following stages are performed:

Stage 1: Grey Scale. The image is transformed into a grey scale one (Fig. 2 (b)). We only keep the information on the red plane to do this.

Stage 2: Sampling (Fig. 3 (a)). Before being processed by a computer, the images greater than an specific size must be sampled. The aim of this is a basic process is to obtain images of the same size before comparing them.

Stage 3: Dynamical AGP Segmentator (First threshold, Fig 3 (b)). This is an iterative stage. We apply, in each iteration, a variant of the AGP segmentator (See [14]). In order to blur the image, each pixel on the boundary turns on white or it takes the smallest gray value of their neighbours. As usual in P systems, this process finishes when no more segmentation rules can be applied.

Stage 4: Iterative Edge Erosion Fig 3 (c). In each iteration, rules of the following type are applied:

$$\begin{pmatrix} & K_1\ K_2\ K_3 & & K_1\ K_2\ K_3 & \\ 1, & K_8\ B\ K_4 & / & K_8\ K'\ K_4 & ,0 \\ & K_7\ K_6\ K_5 & & K_7\ K_6\ K_5 & \end{pmatrix}$$

where $K' = min\{K_i\ :\ i = 1, \ldots, 8 \wedge K_i \neq B\}$

Stage 5: Segmentation (Second threshold, Fig 4 (a)). Again, the P system implementation of the Sobel segmentator is used, but in this stage it is combined with the AGP segmentator [14] in order to obtain a sharper definition of the boundary.

Stage 6: Quantization (Third threshold, Fig 4 (b)) Quantization is a lossy compression technique achieved by compressing a range of values to a single quantum value. In our study we apply the tissue P system implementation presented in
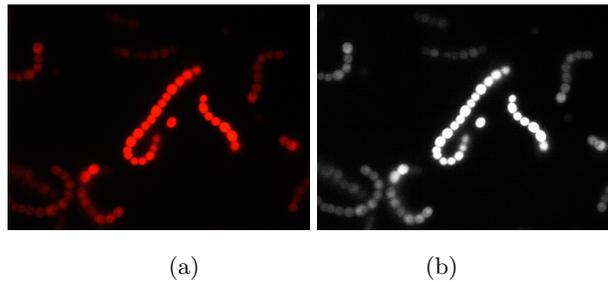

(a)   (b)

Fig. 2. (a) An example image (b) Grey Scale Stage

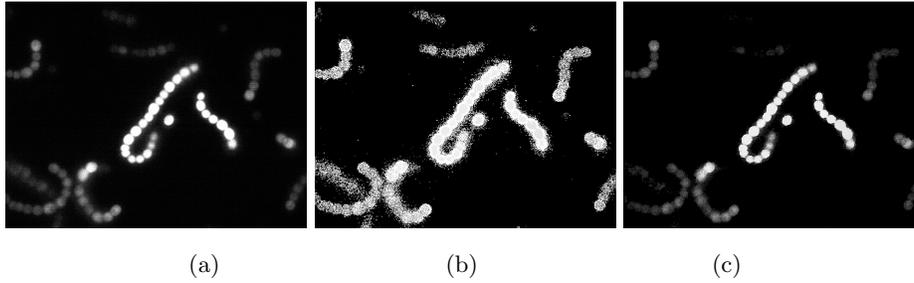<center>(a)   (b)   (c)</center>

Fig. 3. (a) Sampling Stage (b) Dynamical AGP Segmentator Stage (c) Iterative Edge Erosion Stage

[24]. So, we propagate the background, black pixels, between the dark areas (with color early to black).

Stage 7: Spot Erasing: In this stage, two copies of the image provided as output of the previous stage are taken as input. This new stage is split into two steps:

- *White Marking:* Rules of type $(1, KN/BN, 0)$ are applied. Eight rules are defined, one for each neighbour of the pixel (K). (Fig 4 (c)).
- *White Unmarking:* Rules of the same type as the used ones in the iterative edge erosion perform this step, but in this case, the neighbours are taken from the second copy of the image. (Fig 5 (a)).

Stage 8: Edge Erasing

- *Edge Erasing type 1:* The white edges adjacent to pixels with a associated color different to black are deleted. Rules as the ones in the iterative edge erosion stage are used (Fig 5 (b)).
- *Edge Erasing type 2:* Edges are eliminated by using the same type of rules of the iterative edge erosion stage. (Fig 5 (c)).
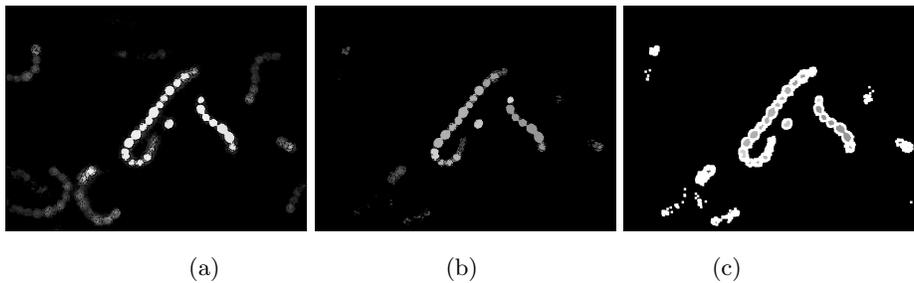

<center>(a)   (b)   (c)</center>

Fig. 4. (a) Segmentation Stage (b) Quantization Stage (c) Spot Erasing Stage: White Marking

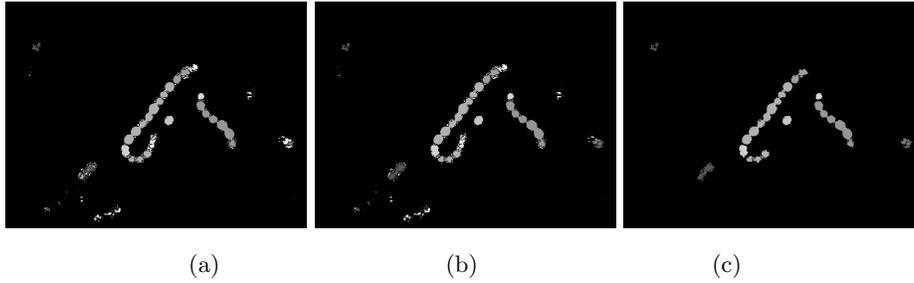(a)   (b)   (c)

Fig. 5. (a) Spot Erasing Stage: White Unmarking (b) Edge Erasing Stage: type 1 (c) Edge Erasing Stage: type 2

Stage 9: Dealing with Connected Components. Labelled connected components are seeked (Fig 6). In this case, two pixels are considered *connected* when their distance is less than 3. See [16] for a detailed description of a family of tissue P systems for finding connected components in a binary image.

After finishing all the stages of the previous algorithm, some information of each connected component, i.e., each cyanobacteria, like the area, medium intensity, etc. can be obtained, and, of course, the number of studied Cyanobacteria too.

For example, the algorithm can be applied to study the medium intensity in two images where the same Cyanobacteria appears with a little difference of time (See Fig 7 (a) and (b)). After applying the algorithm, the images in Fig 7 (c) and (d) are obtained, where the different connected components are shown.

Finally, some statistical information is obtained. On one hand, for the first image, we have detected 6 connected components. We have kept the greatest of them (the chosen cyanobacterium for the study) whose area has 6375 pixels and a size of 1570244. Moreover, its average intensity is 246, 31. On the other hand, the image taken in the second place has the same 6 connected components. The greater
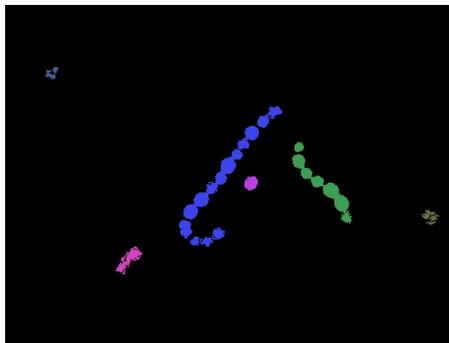

Fig. 6. Dealing with Connected Components Stage

(a)   (b)

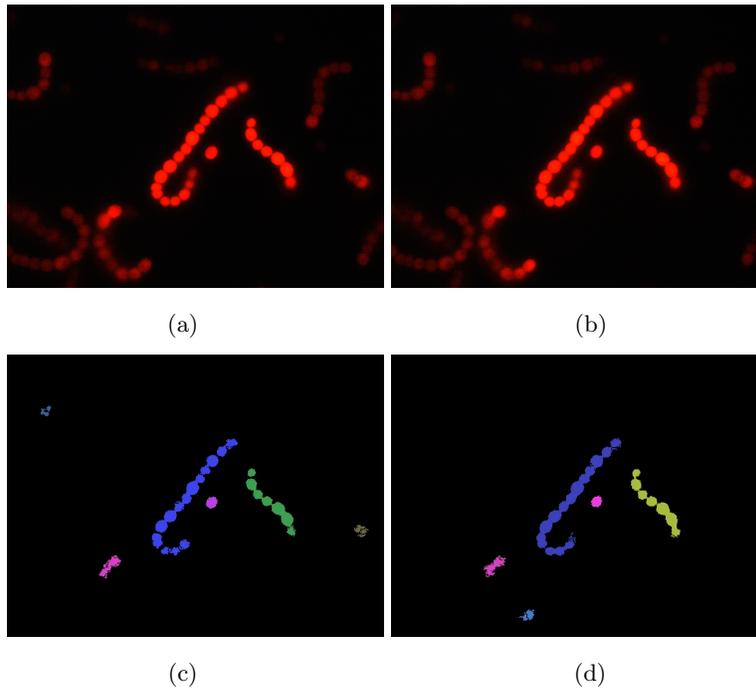(c)   (d)

Fig. 7. (a) Original Images of the previous example (b) Image taken an briefly later instant (c) Different Cyanobacteria detected by our software in the first image (d) Different Cyanobacteria detected by our software in the second image

of them (our cyanobacterium) has a greater area with respect to the previous. In this case it has 7227 pixels and a size of 1784567 and the average intensity is similar or lightly greater to the cyanobacteria of the previous image. In this case, it is 246, 93.

5 Implementation

Inspired in the families of tissue-like P systems that perform the stages of the process of counting cells, a software tool has been implemented by using CUDA™, (Compute Unified Device Architecture) [21, 22]. CUDA™ is a general purpose parallel computing architecture that allows the parallel NVIDIA Graphics Processors Units (GPUs) to solve many complex computational problems in a more efficient way than on a CPU.

The experiments have been performed on a computer with a CPU AMD Athlon II x4 645, which allows to work with four cores of 64 bits to 3.1 GHz. The computer has four blocks of 512KB of L2 cache memory and 4 GB DDR3 to 1600 MHz of

main memory. The used graphical card (GPU) is an NVIDIA Geforce GT240 composed by 12 *Stream Processors* with a total of 96 cores to 1340 MHz. It has 1 GB DDR3 main memory in a 128 bits bus to 700 MHz. So, the transfer rate obtained is by 54.4 Gbps. The used Constant Memory is 64 KB and the Shared Memory is 16 KB. Its Compute Capability level is 1.2 (from 1.0 to 2.1). The implementation deals with N blocks of threads for the complete image in our GPU of 96 cores.

6 Conclusions

The discovery of new application areas of Membrane Computing is a powerful engine for future research. In parallel, the new hardware architectures, as CUDA, allows a real implementation of the inherent parallelism of P systems. In this paper, we report a new step in the applications of Membrane Computing techniques to Digital Images. As pointed above, Membrane Computing techniques allow a natural treatment of the parallelism of the flow of information in Digital Images algorithms where the information can be encoded with simple data structures.

From a practical point of view, such techniques are a real innovation in the study of biological images. In this paper, the case study has been the chlorophyll fluorescence in cyanobacteria and its use for computing their density. A deep study of these cyanobacteria can contribute for the development of future bio(nano)technologies as the production of electricity or pollutants removal.

In near future we intend to simultaneously measure on the same sample both the formation of MTT formazan (using bright field microscopy, as in [28]) and the decrease in chlorophyll fluorescence (using epifluorescence microscopy, as in this report) and to quantitatively analyse the correlation between the two processes.

Acknowledgements

References

1. Albertano, P., Somma, D.D., Capucci, E.: Cyanobacterial picoplankton from the central baltic sea: cell size classification by image-analyzed fluorescence microscopy. Journal of Plankton Research 19(10), 1405–1416 (1997)
2. Ardelean, I., Díaz-Pernil, D., Gutiérrez-Naranjo, M.A., Peña-Cantillana, F., Reina-Molina, R., Sarchizian, I.: Counting cells with tissue-like P systems. In: Martínez-del-Amor, M.A., Păun, G., Pérez-Hurtado, I., Romero-Campero, F.J. (eds.) Tenth

Brainstorming Week on Membrane Computing. vol. I, pp. 69–78. Fénix Editora, Sevilla, Spain (2012)

3. Armăşelu, A., Popescu, A., Apostol, I., Ardellean, I., Damian, V., Iordache, I., Sarchizian, I., Apostol, D.: Passive nonspecific labeling of cyanobacteria in natural samples using quantum dots. Optoelectronics and Advanced Materials - Rapid Communications 5(10), 1084 – 1090 (October 2011)

4. Berridge, M.V., Herst, P.M., Tan, A.S.: Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction. Biotechnology Annual Review 11, 127 – 152 (2005)

5. Carnero, J., Díaz-Pernil, D., Gutiérrez-Naranjo, M.A.: Designing tissue-like P systems for image segmentation on parallel architectures. In: Martínez-del-Amor, M.A., Păun, G., Pérez-Hurtado, I., Romero-Campero, F.J., Cabrera, L.V. (eds.) Ninth Brainstorming Week on Membrane Computing. pp. 43–62. Fénix Editora, Sevilla, Spain (2011)

6. Cecilia, J.M., García, J.M., Guerrero, G.D., Martínez-del-Amor, M.A., Pérez-Hurtado, I., Pérez-Jiménez, M.J.: Implementing P systems parallelism by means of GPUs. In: Păun, G., Pérez-Jiménez, M.J., Riscos-Núñez, A., Rozenberg, G., Salomaa, A. (eds.) Workshop on Membrane Computing. Lecture Notes in Computer Science, vol. 5957, pp. 227–241. Springer, Berlin Heidelberg (2009)

7. Cecilia, J.M., García, J.M., Guerrero, G.D., Martínez-del-Amor, M.A., Pérez-Hurtado, I., Pérez-Jiménez, M.J.: Simulating a P system based efficient solution to SAT by using GPUs. Journal of Logic and Algebraic Programming 79(6), 317–325 (2010)

8. Cecilia, J.M., García, J.M., Guerrero, G.D., Martínez-del-Amor, M.A., Pérez-Hurtado, I., Pérez-Jiménez, M.J.: Simulation of P systems with active membranes on CUDA. Briefings in Bioinformatics 11(3), 313–322 (2010)

9. Chávez de Paz, L.E.: Image analysis software based on color segmentation for characterization of viability and physiological activity of biofilms. Applied and Environmental Microbiology 75(6), 1734–9 (2009)

10. Christinal, H.A., Díaz-Pernil, D., Gutiérrez-Naranjo, M.A., Pérez-Jiménez, M.J.: Thresholding of 2D images with cell-like P systems. Romanian Journal of Information Science and Technology (ROMJIST) 13(2), 131–140 (2010)

11. Christinal, H.A., Díaz-Pernil, D., Real, P.: Segmentation in 2D and 3D image using tissue-like P system. In: Bayro-Corrochano, E., Eklundh, J.O. (eds.) Progress in Pattern Recognition, Image Analysis, Computer Vision, and Applications 14th Iberoamerican Conference on Pattern Recognition, CIARP 2009, Guadalajara, Jalisco, Mexico, November 15-18, 2009. Proceedings. Lecture Notes in Computer Science, vol. 5856, pp. 169–176. Springer, Berlin Heidelberg (2009)

12. Christinal, H.A., Díaz-Pernil, D., Real, P.: Region-based segmentation of 2D and 3D images with tissue-like P systems. Pattern Recognition Letters 32(16), 2206 – 2212 (2011)

13. Díaz-Pernil, D., Gutiérrez-Naranjo, M.A., Molina-Abril, H., Real, P.: A bio-inspired software for segmenting digital images. In: Nagar, A.K., Thamburaj, R., Li, K., Tang, Z., Li, R. (eds.) Proceedings of the 2010 IEEE Fifth International Conference on Bio-Inspired Computing: Theories and Applications BIC-TA. vol. 2, pp. 1377 – 1381. IEEE Computer Society, Beijing, China (2010)

14. Díaz-Pernil, D., Berciano, A., Peña-Cantillana, F., Gutiérrez Naranjo, M.A.: Segmenting Images with Gradient-based Edge Detection Using Membrane Computing. Pattern Recognition Letters 34(8), 846–855 (2013)

15. Díaz-Pernil, D., Gutiérrez-Naranjo, M.A., Molina-Abril, H., Real, P.: Designing a new software tool for digital imagery based on P systems. Natural Computing pp. 1–6 (2011)
16. Díaz-Pernil, D., Gutiérrez-Naranjo, M.A., Real, P., Sánchez-Canales, V.: Computing homology groups in binary 2D imagery by tissue-like P systems. Romanian Journal of Information Science and Technology 13(2), 141–152 (2010)
17. Fero, M., Pogliano, K.: Automated quantitative live cell fluorescence microscopy. Cold Spring Harb Perspectives in Biology 2(8), a000455 (2010)
18. Gimel'farb, G., Nicolescu, R., Ragavan, S.: P systems in stereo matching. In: Real, P., Díaz-Pernil, D., Molina-Abril, H., Berciano, A., Kropatsch, W. (eds.) Computer Analysis of Images and Patterns, Lecture Notes in Computer Science, vol. 6855, pp. 285–292. Springer Berlin / Heidelberg (2011)
19. Gimel'farb, G.L.: Probabilistic regularisation and symmetry in binocular dynamic programming stereo. Pattern Recognition Letters 23(4), 431–442 (2002)
20. Ionescu, M., Sburlan, D.: On P systems with promoters/inhibitors. Journal of Universal Computer Science 10(5), 581–599 (may 2004)
21. Nickolls, J., Buck, I., Garland, M., Skadron, K.: Scalable parallel programming with CUDA. Queue 6, 40–53 (March 2008)
22. Owens, J.D., Houston, M., Luebke, D., Green, S., Stone, J.E., Phillips, J.C.: GPU Computing. Proceedings of the IEEE 96(5), 879–899 (May 2008)
23. Paerl, H.W., Bland, P.T.: Localized tetrazolium reduction in relation to N_2 fixation, CO_2 fixation, and H_2 uptake in aquatic filamentous cyanobacteria. Applied and Environmental Microbiology 43(1), 218–226 (January 1982)
24. Peña-Cantillana, F., Díaz-Pernil, D., Berciano, A., Gutiérrez-Naranjo, M.A.: A parallel implementation of the thresholding problem by using tissue-like P systems. In: Real, P., Díaz-Pernil, D., Molina-Abril, H., Berciano, A., Kropatsch, W.G. (eds.) CAIP (2). Lecture Notes in Computer Science, vol. 6855, pp. 277–284. Springer (2011)
25. Peña-Cantillana, F., Díaz-Pernil, D., Christinal, H.A., Gutiérrez-Naranjo, M.A.: Implementation on CUDA of the smoothing problem with tissue-like P systems. International Journal of Natural Computing Research 2(3), 25–34 (2011)
26. Peschek, G.A.: Structure-function relationships in the dual-function photosynthetic-respiratory electron-transport assembly of cyanobacteria (blue-green algae). Biochemical Society Transactions 24(3), 729–733 (August 1996)
27. Peschek, G.A., Obinger, C., Fromwald, S., Bergman, B.: Correlation between immuno-gold labels and activities of the cytochrome-c oxidase (aa_3-type) in membranes of salt stressed cyanobactria. FEMS Microbiology Letters 124(3), 431–437 (1994)
28. Sarchizian, I., Cîrnu, M., Ardelean, I.I.: Isolation of a heterocyts - forming cyanobacterium and quantification of its biotechnological potential with respect to redox properties at single cell level. Romanian Biotechnological Letters 16(6), 3–9 (2011)
29. Selinummi, J., Ruusuvuori, P., Podolsky, I., Ozinsky, A., Gold, E., Yli-Harja, O., Aderem, A., Shmulevich, I.: Bright field microscopy as an alternative to whole cell fluorescence in automated analysis of macrophage images. PLoS ONE 4(10), 1 9 (2009)
30. Selinummi, J., Seppälä, J., Yli-Harja, O., Puhakka, J.A.: Software for quantification of labeled bacteria from digital microscope images by automated image analysis. Biotechniques 39(6), 859–863 (2005)

31. Sheeba, F., Thamburaj, R., Nagar, A.K., Mammen, J.J.: Segmentation of peripheral blood smear images using tissue-like P systems. Sixth International Conference on Bio-Inspired Computing: Theories and Applications (BIC-TA), 2011, 257–261 (sept 2011)
32. Walsby, A.E., Avery, A.: Measurement of filamentous cyanobacteria by image analysis. Journal of Microbiological Methods 26(1-2), 11 – 20 (1996)
33. Zhang, G.X., Gheorghe, M., Li, Y.: A membrane algorithm with quantum-inspired subalgorithms and its application to image processing. Natural Computing 11(4), 701–717 (2012)