Discovery of motion based communication between the unicellular micro-organisms "Paramecium" by using artificial intelligence techniques

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Abstract

The study presents an interdisciplinary approach to investigate the motion based electromagnetic (*EM*) communication activities between the unicellular micro-organisms *"paramecium"* by their behaviour analysis using microscopic video interpretation techniques and Bayesian networks. Otherwise such communication could not be detected or interpreted easily by a conventional microscopic observation with a naked eye. The suggested model is also based on the theory of *EM* cellular interactions, stating that electromagnetic field can be generated by moving charged particles (e.g. protein molecules) in a cell which enables cells to interact with each other. Hence in our work each micro-organism is naturally assumed to contain electrical charged particles which help convert any physical motion to electromagnetic signals to interact and communicate with each other.

Keywords - Communication, Bayesian networks, optical flow, microscopy, video analysis

1 . INTRODUCTION

Many investigations have been carried out in last few decades to discover the communications between unicellular micro-organisms and cells (Tsong, 1989; Tsong and Gross, 1994; Fels, 2009; Cifra et al., 2011) either in visible or non-visible parts of electromagnetic spectrum. But none of these studies has focused on the motion based (body language) inter-cellular communications between the micro-organisms by interpreting and quantifying their behaviours which may have direct physical link with their *EM* signal generation over the specific part of electromagnetic spectrum. This sort of communication (based on non-visible part of electromagnetic spectrum) will be called "electromagnetic (*EM*) communication" in this work, which is produced by the continuous motions of micro-organisms.

The proposed method aims to investigate the motion based electromagnetic (*EM*) communication activities between the unicellular micro-organisms by using microscopic video interpretation methods and artificial intelligence techniques (Bayesian inference methods and classifiers). This sort of motional communication can not be interpreted by a naked eye (with a microscopic magnification) but could only be detected by an analysis of motional behaviours of micro-organisms by using artificial intelligence techniques like Bayesian networks (Orun et al., 2012). The suggested model is based on an electromagnetic theory of cellular interactions stating that an electromagnetic field can be generated by moving charged particles (e.g. protein molecules) in a cell which enables cells to interact with each others (Cifra et al.,2011). Similarly the micro-organisms (e.g. *Paramecium*) used in our work naturally contain charged particles and they are supposed to convert any physical motion to electromagnetic signals to interact and communicate with each other. In many previous works the electric potential fluctuations in the body of *Paramecium* (Figure 1a) were investigated (Oosawa, 1971; Majima, 1980; Oosawa, 2007). In some research particularly its membrane of cilia (5000 hair-like organelles arrayed around the body) and cell membrane are mentioned as the origins of electric potential fluctuation whose amplitude varies between 0.5 – 5 mV. This sort of electric potential fluctuation of the charged components in *Paramecium* or other micro-organisms may possibly lead to EM field generation during their motions. Ogawa et al. (2006) indicates that Paramecium's ciliary motion is stimulated by the progressive shifts of body membrane's electrical potential. Majima (1980) also states that the average electric potential of Paramecium is (-)30 mV.

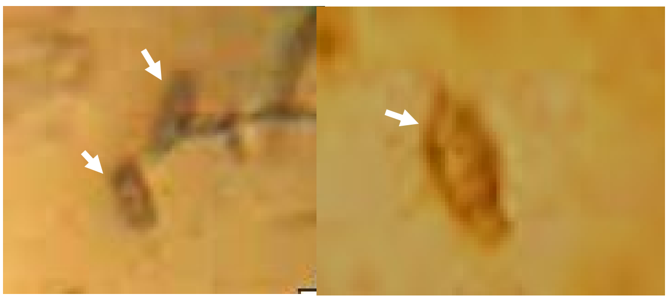

Figure 1a – Microscopic view of *paramecium* (indicated by white arrows) under different *(300-600X)* magnifications

1.1 Previous works on cellular communication

In previous studies some investigations on different types of cellular communication were carried out by using different methods. The detection of a cellular electromagnetic field was first explained by Scheminzky (1916) for the visible spectrum in 1916. The electromagnetic field generation by the cells were first detected by a few number of researchers in the past (Jafary and Smith, 1983; Del Giudice et al., 1989). Fels (2009) conducted experiments by a special test configuration which separates between the micro-organisms by a glass barrier to avoid chemical communication to prove a sole photonic (light based) communication activity between the unicellular micro-organisms. He did not use any artificial intelligence based inference techniques to unveil communication between micro-organisms but rather correlated their communication activities with their cellular division rate and population growth. Cifra et al. (2011) reviewed the evidence of alternative communications other than chemical or electrical ones which require synchronous behaviour among physically independent units.

2. METHODS AND MATERIALS

2.1. Utilities and software used

The proposed system consists of different components such as video recording unit (facilitated with a light microscope) with zooming capability, Bayesian software utilities (PowerConstructor™ and PowerPredictor™)(Cheng et al., 2002), set of micro-organisms and video image analysis tools (e.g. optical flow). The microscopic video recorder utility (*Bio-recorder shown in Figure 1b*) records the video sequences at *400x300* pixel resolution, 27 frames/sec and 24 bit sample size and each lasts about 1-2 minutes length. A short video duration for each recording is chosen intentionally, because all observed moving micro-organisms can not be kept together in the microscopic field-of-view for a long period of time. The optical magnification is optional and can be set to *100X – 600X* but the lower magnification provides more global view and provides a longer observation period of micro-organisms which are in continuous motion. The full image size approximately corresponds to 0.7mm on the microscopic object stage. The image of trajectory map (Figure 2) refers to *100X* magnification which provides sufficient field-of-view (FOV) to follow all characteristic motional behaviours of micro-organisms on their long trajectories. The vectorial x,y image coordinates of the micro-organisms in motion along their traces are specified by screen display utility (Serif PhotoPlus™) manually by naked eye, which is sampled once in every 3-5 frames. But the ultimate data analysis is made by an artificial intelligence utility called Bayesian networks.

2.2. Video image analysis technique

In our work a quantitative video analysis technique called "*optical flow*", which yields a total value of motion vectors for whole micro-organism population that randomly move within any area of observation is used. The optical flow (1) is defined by its general formula :

$$I'(x, y, t) = I(x + \Delta x, y + \Delta y, t + \Delta t) \qquad (1)$$

For successive video frames (I' and I) each 3D voxel (2D pixel + t) at location *(x,y,t)* with intensity *I(x,y,t)* will be moved by Δx, Δy, and Δt between the two video frames. We adopted well-known Lucas-Kanade method for the optical flow (Lucas and Kanade, 1981). This method has two basic assumptions which are reasonably valid in our application: brightness is constant over motion and the motion is constant in a small neighborhood. The optical flow values (Δx, Δy) are included into the data set (Table 2). In experiment II, the numerical results calculated by optical flow for different micro-organism groups (utilized in different times and tests) are used in Bayesian networks to discover vectorial motion links between the micro-organisms (Figure 3).

2.3. Bayesian Networks

The motion based behavioural analysis of micro-organisms is made by Bayesian network inference and classification utilities which are known as very robust against data sets which may contain substantial level of uncertainty (Orun and Seker, 2012). Bayesian Networks (BN) are known as "directed acyclic graphs" (DAG) which perform knowledge representation and reasoning even under uncertainty. They are also called directed Markov fields, belief networks, or causal probabilistic networks (Jensen, 1998). Bayesian networks are the probabilistic models which graphically encode and represent the conditional independence (CI) relationships among a set of data. In Bayesian networks each node represents a data attribute and is called a variable (it refers to type of micro-organism in this work). The connections (arcs) between the nodes represent the dependency relationships of variables. Bayesian networks are very efficient tools for modelling the joint probability distributions of variables. For example, if X = {*X*1,.., *Xn*} is a random variable which denotes patterns spanning the *n* = *N* by *M* dimensional vector space, the joint probability distribution *P* =(*X*1,…, *Xn*) is then a product of all conditional probabilities and may be represented as

$$P(X) = \prod_i P(X_i \mid pa(X_i)) \qquad (2)$$

In Equation 2, *pa*(*Xi*) is the parent set of *Xi*. Structural learning is one of the major specifications of Bayesian networks. This is based on constructing relationships among the variables and is similar to the data mining principle. In this work the

the utility (PowerConstructor©) is used (Cheng et al.,2002). In this work, the Bayesian network software packages (PowerConstructor™ and PowerPredictor™) are used for network construction and classification processes respectively, where both utilities are based on "dependency analysis" (Cheng at al.,2002). In the software set up utility, threshold value (*t*) is set to 0.1 (minimum) in order to establish maximum number of links within the network. In addition, *Receiver Operating Characteristic* (area under the ROC curve) is chosen as an option to determine the optimum models.

3. EXPERIMENTS CARRIED OUT TO INVESTIGATE ANY POSSIBLE INTER-CELLULAR COMMUNICATION

3.1. ***Quantising the micro-organism behavioural characteristics***

In this part of work the video based data are used to calculate behavioural (vectorial) data of micro-organisms which is similar to one of our previous works (Orun et al., 2012). These data are used to specify the characteristics of the micro-organisms and then extracting the behavioural attributes to configure a Bayesian Network. In this experiment the tracking (*x,y*) coordinates of each selected micro-organism is recorded by using a special utility (Serif™) along its trajectory. The data set contains the cases where each case indicates the vectorial motion parameters (e.g. velocity change) of a micro-organism. The selected length of each step of motion depends on micro-organism's velocity along its trajectory. Meanwhile each case refers to vectorial velocity value of micro-organism in the data set. The trajectories of micro-organisms' motions can not easily be interpreted by a naked eye to find the links of synchronization between each other because the synchronized velocity changes of numerous micro-organisms are not visible. These invisible parameters can only be revealed and linked by an artificial intelligence technique (like Bayesian networks). The data extracted from the trajectories of micro-organisms contain large number of cases where each case corresponds to each micro-organism's step of motion calculated by the formula 4 (here *d* corresponds to a distance that the micro-organism moves on its trajectory between the two sequential frames).

$$d_i = \sqrt{(\Delta x_i^{\ 2} + \Delta y_i^{\ 2})} \tag{4}$$

The observed trajectories of micro-organisms are shown in Figure 2 (Orun et al., 2012)

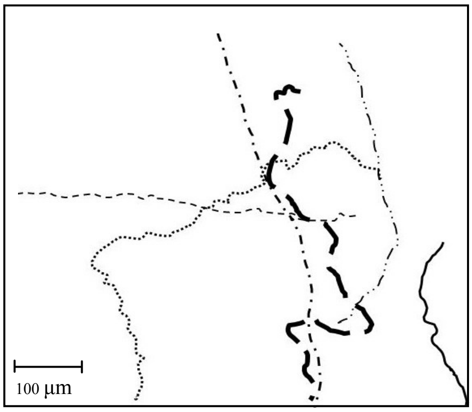

Figure 2. a trajectory map of micro-organisms

3.2. ***The communication analysis***

The type of micro-organisms used in this experiment is selected as ideal one in terms of communication ability. Referring to previous studies (Fels, 2009) particularly Paramecium was used in a similar light based communication experiments. He used a test domain in which a molecular diffusion between micro-organisms is disabled by a glass separator allowing them to communicate only through the light. In our research we rather focus on non-visible part of electromagnetic (EM) spectrum based on the theory that "a magnetic field can be generated by a moving charged particle" (Cifra et al.,2011) establishing a direct link between the physical motion of a micro-organism and its electromagnetic signal generation. In this experiment only one type of micro-organism (*Paramecium*) is used with two separated test groups.

As is shown in Figure 1b, two groups of micro-organism populations are separated by a light shield made of a light-resistant material to avoid any light based communication between two populations. The shield material should also be non-metallic to allow EM signal transmission. Then the population (B) at the right-hand side of chamber (Figure 1b) is terminated by acetic acid solvent to force them to emit electromagnetic radiation at least for a limited time period just before they die. (A similar observation is also made in a previous research (Slawinski, 2005) for "ultra weak light photon emission" by cells which are damaged). At this stage the population (A) is observed and recorded by microscopic video recorder utility.

A few minute video sequences are then processed to calculate the motion vectors along the micro-organisms' trajectories.

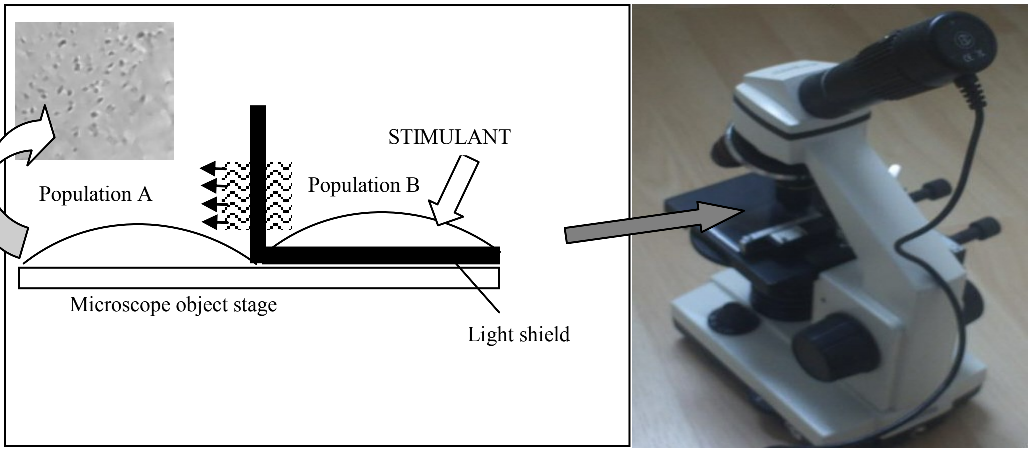

Figure 1b. microscopic video recording utility (on the right) at different magnifications (100-600X), with the specifically designed object stage (on the left) to separate two different groups of micro-organisms in droplets : Population A (EM signal receiving) and Population B (EM signal emitting. The test chamber is specifically designed so that, the electromagnetic radiation which is generated by Population B (micro-organisms) during the stimulation stage can diffuse through the light shield and reaches Population A to effect their motion behaviours. A sample of video recording is shown at top-left corner.

For the object tracking task an optical flow algorithm and manual trajectory tracking techniques are used. The optical flow algorithm, having a global view, yields a total value of micro-organism vectorial motions for each video sequence. In this experiment only a restricted group of 14 micro-organisms is used. because of the difficulty of simultaneous object tracking as well as poor visibility of micro-organisms along their tracks due to optical focusing problem originated from their vertical motions. Also only a small amount of micro-organisms remain in microscopic field of view within the entire observation period.

The data set consists of 14 attributes each refers to specifically observed micro-organism and 33 cases which correspond to vectorial values of their horizontal continuous motion. The data set format is shown in Table 1 which is particularly configured to be used for training/test purposes in Bayesian networks. The class node includes Boolean values which refer to EM radiation free period (1) and radiation emitting period (2). They correspond to first half (where no acidic stimulant applied on population B) and second half (acidic stimulant applied on population B) of the video recordings respectively. The data set contains "0" regions where there is no any vectorial value included.

TABLE 2. Data set of vectorial motions of micro-organisms used in experiment 2. Here Period 1 (non-EM radiation emission before the stimulation) and Period 2 (EM radiation emission during stimulation) correspond to OP_A (class 1) and OP_B (class 2) respectively

Case No.	Vectorial motions of micro-organisms *(in pixel unit)* ***A_1 receivers (period 1)***	***A_2 receivers (period 2)***	**Optical flow**	**Class node**
1	.	.	.	1
2	.	.	.	1
3	v_1 . . . V_A . . v_7	. . 0 . . .	OP_A	.
.		.	.	.
.		.	.	.
19				1
20	.	.	.	2
.	.	.	.	2
.	.	.	.	.
.	. . 0 . . .	v_1 . . V_B . . . v_7	OP_B	.
.	.	.	.	.
33	.	.	.	2

Results : The data set is analysed by Bayesian inference (ImageConstructor™) utility to discover any possible connection between the attributes. Figure 3 indicates that the class node (EM radiation mission or non-emission) has link to micro-organism A2 and also all nodes (micro-organisms) are linked to optical flow node.

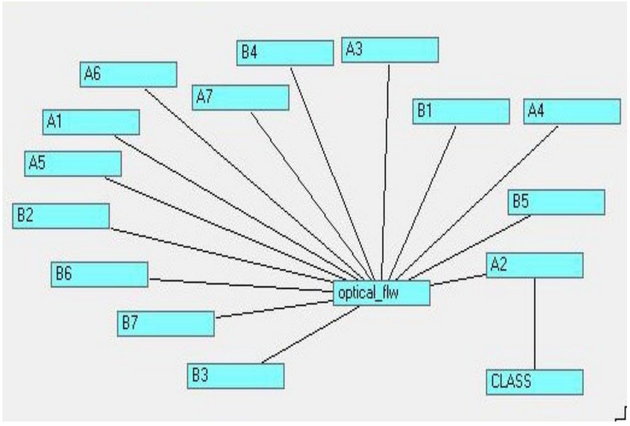

Figure 3 - Bayesian network is constructed automatically by using PowerConstructer utility by use of 33x14 dimension data set where Class node is linked to group of micro-organism (A2) indicating that it enables network to distinguish between EM radiation emitting and non-emitting groups, which also proves the EM radiation emission. We have to note that all nodes refer to same types of micro-organisms used in different sub-tests at different times. Attribute of optical flow is shown by optical flow node.

Data set matrix of vectoral motions which belong to micro-organisms and their optical flow values (in image pixel unit) are shown in Table 2 where the columns A_1 and A_2 correspond to the groups of micro-organisms used in two different modes of stimulation periods.

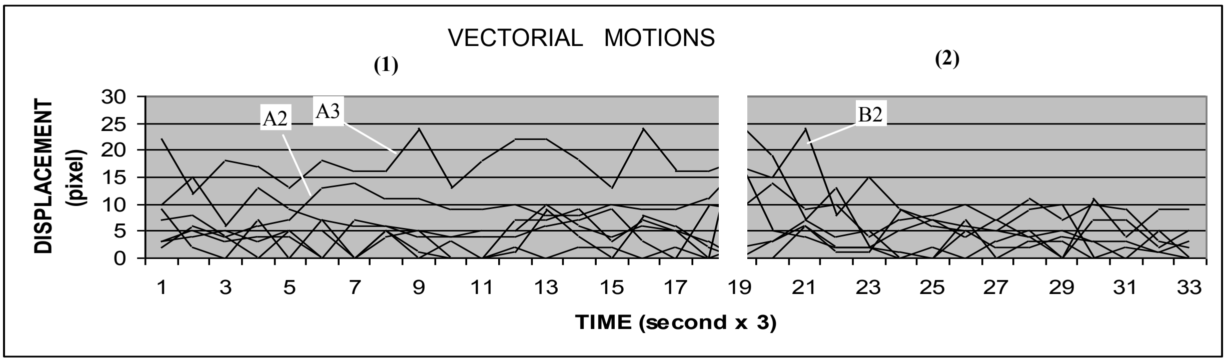

Figure 4 – The difference between period 1 (non-EM radiation emission) and period 2 (radiation emission) can be seen through the graphs each belongs to micro-organism's vectorial motions along the time sequence. In period 1 and 2 different populations of micro-organisms are observed in the same domain (droplet) assuming same type of micro-organisms behave similarly in the same conditions

In the second stage of experiment a classification procedure has been followed by using ImagePredictor™ with the training and test set (approximately the ratio of 18/15) and the results of classification procedure proceeded by different Bayesian networks are shown in Figure 5.

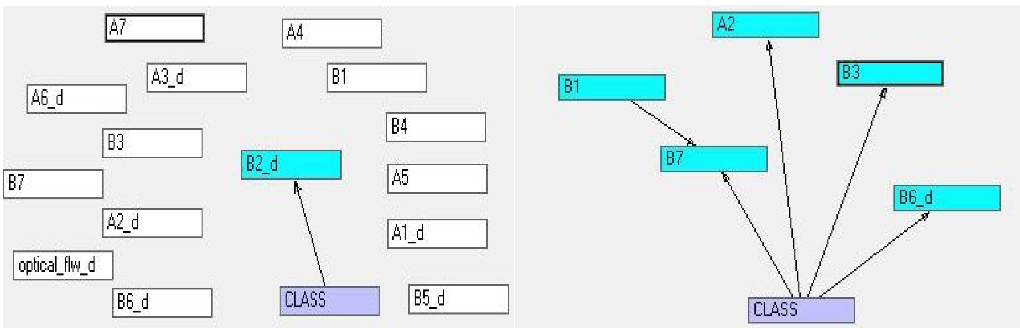

Figure 5 – Left image : 1. Network automatically configured by PowerPredictor utility during classification (by data set shown in Table 1). The link between the Class node and B2-d indicates that the attribute B2-d is sufficient to achieve a classification between EM radiation emitting and non-emitting groups. Right image : By using different parameters than 1. Network , the 2. Network configuration is made by PowerPredictor™ using data set presented in Table2. The links between the class node and the others indicate that the classification of EM radiation emitting and non-emitting groups is achieved via attributes, each refers to different groups of micro-organisms' vectorial motions. (The disconnected nodes are not shown in 2. Network).

By the selection of different parameter options in PowerPredictor classification utility (e.g. threshold, discretization method, etc.) the network structure and classification accuracy may change. For example if threshold is selected too low (t = 0.1) then more nodes are connected in the network (Figure 5) but the classification accuracy may decrease. Different ratios of training/test data sets may also effect the network configuration. Unlike to PowerPredictor, the PowerConstructor (inference utility) algorithms work differently where all related nodes are connected.

The classification was achieved with 78.95% accuracy at 95% confidence level by the best combination of training/test data set (18/15 ratio). The ROC (area Under ROC Curve) matrix values are ;

$$\begin{vmatrix} 0.0 & 0.911 \\ 0.688 & 0.00 \end{vmatrix} \Rightarrow \{\text{ area under ROC Curve}\}$$

The values of SPECIFICITY = 0.91 and SENSITIVITY = 0.69 specify the classification accuracy, as they correspond to the percentages of correctly classified EM non-emission and EM emission cases.

The results belong to the classification whose network is shown in Figure 5. We have to note that the nodes automatically left disconnected are not necessarily useless for classification but their inclusion does not improve the results of classification.

4. CONCLUSION AND FUTURE WORK

In this paper we studied the exploitation of General Bayesian classifier and Bayesian inference method to unveil invisible connection between different types of micro-organisms. The classification results and network configurations indicate that there is a high possibility of electromagnetic based communication (approx. 80%) between the micro-organisms produced by their characteristic motional behaviours. This conclusion has been made by our proposed interdisciplinary methods and also confirmed by vectorial motion graph (Figure 4). The results of this research would lead to some further development in the future works such as :

1) Decoding the Electro-magnetic communication signals between the micro-organisms for different stimulant domains like "pleasure case" by feeding them by a nutrition (e.g. glucose, protein, etc.) or "hazardous case" (e.g. poisoning by chemicals) or more advance cases which correspond to different medical substances, would help develop a communication ontology to interpret the advance level language of micro-organisms. It helps better understand their behaviours linked to medical substance and may provide more accurate models for some of the diseases caused by micro-organisms and drug based treatments.

2) The work may help avoid the harmful radiation effects of electromagnetic (EM) devices (e.g. mobile phones, wireless systems, etc.) (Verschaeve, 2005) by observing the extraordinary behaviours of micro-organisms. This sort of research may lead to designing more healthy EM devices.

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