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Development of an Instrumentation System for Behavior Analysis of Organisms

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Abstract. The present study approaches the creation of a system that allows a controlled environment and equipped with filming resources to monitor biologic movement. The system uses electronic resources of temperature control, air humidity monitoring and a monitoring camera. With the intention of isolating the variables that might interfere on biologic behavior and the possibility of monitoring the organism's movement, the system becomes an excellent tool for behavior studies, allowing the adding of stressing compounds.

Keywords. Instrumentation, Environment control, Humidity monitoring, OpenCV.

1 Introduction

The use of controlled environment is extremely important to control the environmental conditions in tests with organisms. This environment can be controlled using equipments as incubation chambers as, for example, the ones used for BOD (Biochemical Oxygen Demand), such chambers allow to measure the oxygen consumption of microorganisms [1], needing controlled conditions, on controlled temperature and humidity conditions.

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The use of living organisms, to evaluate the substances' toxicology is a widely used tool and allows to obtain and evaluate the answers on organisms' behavior when exposed to hostil environments [2]. Among this organisms, we can cite *Folsomia candida*, which has been used to evaluate soil toxicity [3].

There is a wide application of using cameras for biologic movement detection. In [4], the author presents the use of cameras for human faces detection. In [5], a human emotions detector device was created through the facial expressions in real time. In [6], a biological application was performed with the development of a system for speed detection on aquatic organisms, such as *Daphnia magna*. As well as aquatic organisms in [7], a study was performed with the *Folsomia* spp organism, among the results, movement and population dynamics were studied.

The programming using the OpenCV library [8] allows to perform applications with the image use in a faster and practical way along the C, C++, Python programming languages, among others. The use of these tools allows to improve the biological studies the reliability, accuracy and precision on the results.

The proposed system presents the building of an incubator with temperature control, humidity monitoring, equipped with biologic movement monitoring through microscopic USB (Universal Serial Bus) camera, with a movement detection software, that allows the use in environmental monitoring.

2 Materials and Methods

The construction of a setup for organism analysis is based on eliminating the natural variables that may correspond to a behavioral variation. In this constant and controlled environment, it is possible to affirm by repetitive tests that such behaviors are due to the application of possible stressors. There are a number of stressing elements that can be analyzed through living organisms. For the initial test the control and monitoring of the thermal quantities, relative humidity of the air and mechanical vibration was performed.

2.1 Controlled Environment

Biological tests generally make use of BOD (Biochemical Oxygen Demand) chambers, such equipment is built using a chamber with temperature controller with the aid of heating units and allow temperature control in the order of +/- 1 ℃. For the project presented, a refrigerator of the type Minibar was used. Its reduced size was easy automation and installation of electronics. The electronics employed are shown in the diagram in Fig. 1.

The microcontrolled system starts the motor-compressor, the PWM (Pulse Width Modulation) control of the heating elements and sensor reading: LM35 analogic and SHT11 digital. Has a man-machine interface with buttend and 16x2 Liquid crystal-display. The operation flowchart is shown on Figure 2.

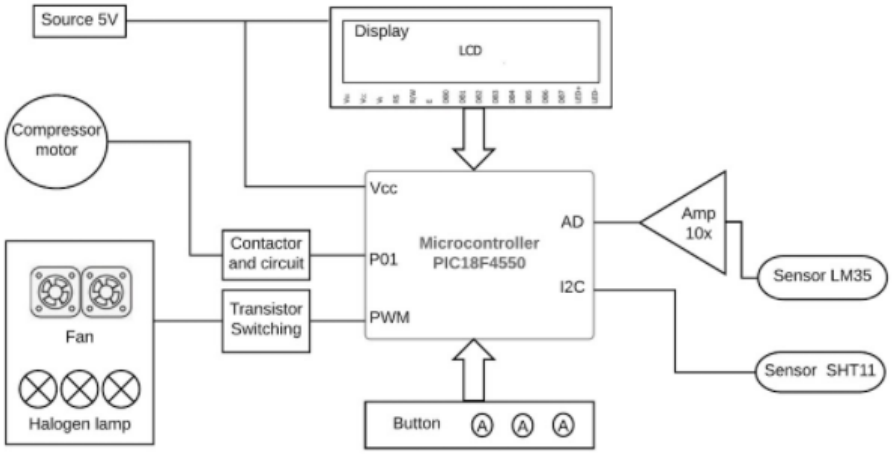

Figure 1: System of temperature control and humidity monitoring.

2.2 Temperature Control

For the temperature control a sensor LM35 was used. It is a low cost sensor, has the accuracy of +/-0,5 ℃, although there is a certain thermal inertia despised in the application. The sensor installation was performed on the chamber's central position so that the sensors temperature corresponds to the average temperature of all area.

The second temperature sensor SHT11 was installed on the test environment beside the cuvette (see Subsection 2.5). It is possible to follow the temperature variation in the place under test.

The cooling unit is made through the original Minibar motor-compressor control. The microcontroller starts the motor-compressor using a contactor, this drive is made in a temporal way due the current chamber temperature.

The heating unit is composed by 12V incandescent lamps and coolers for thermal transference. The lamps and coolers are connected in parallel and it is fed and controlled by one pulse of PWM.

The graphic of Figure 3, shows the temperature behavior next to the sample in a 24h total using the sensor (SHT11). As it starts, the temperature is not stabilized due the chamber opening. Next, the thermal stabilization is noticeable.

2.3 Humidity Monitoring

The air humidity control is more complex, there are techniques for its application, therefore for this project only the monitoring was made.

The abrupt change in air humidity can cause a change in the behavior of the organism. Therefore, it is interesting to monitor the humidity changes over the test, and when abrupt changes shows the test must be repeated.

The humidity monitoring is shown on Figure 4. The SHT11 sensor, has the accuracy of 3,0% in humidity and +/-0,4 ℃in temperature.

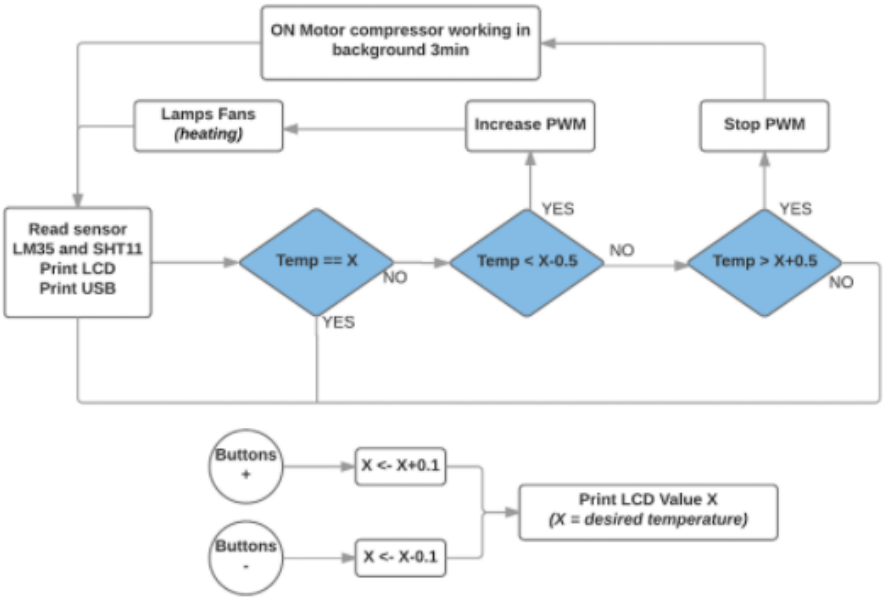

Figure 2: Operation flowchart of the microcontrolled system.

2.4 Mechanical Vibration

The monitoring of organism used for movement detection does not tolerate mechanical vibrations. Once they are detected, the mechanical vibrations may be classified as noise and its values must be disconsider with the help of after processing filters.

The vibrations may occur from external environment or even from on/off the motor-compressor. In order to avoid mechanical movement, the mechanical apparatus (micro-scope, cuvette and support) was over a piece of granite sustained by a medium density foam.

2.5 Anti-glare Cuvette

One of the issues on the experiment, is due to the glare on the cuvette wall, which was used to place the organism for testing. Unsatisfactory test were made using glass cuvettes. The organism image reflected on the glass cuvette wall, so the organism seemed to be duplicated. The camera along with the movement monitoring software was detecting two organisms at the same time, producing wrong data.

To eliminate the glare, a personalized cuvette was created by 3D printer. The cuvette was printed using PLA material in the colours black and red. The printed cuvette didn't show glare at any moment, even when the organism was near the walls.

A square flap was also created intending to erase the images outside the cuvette's circumference.

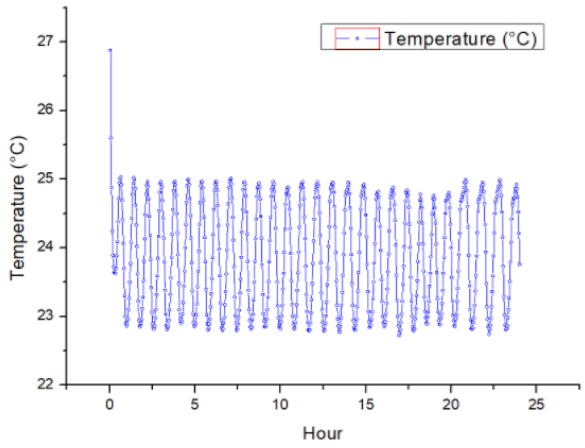

Figure 3: 24h temperature monitoring.

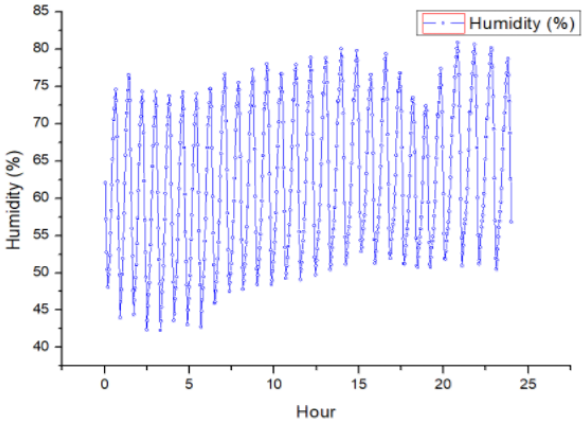

Figure 4: 24h humidity monitoring.

3 Motion Caption

As it is a visual analysis of organisms, a digital 200X model AVEN 26700-300 microscope was used. An enlargement is needed and it is limited to the monitored area. The crops' height must be constant from one test to another, to eliminate optical displacement errors.

The presented microscope is USB connected and it is drive is executed as a webcam. This way, it was possible to monitor the biologic organisms using OpenCV library with a Python software in Linux environment. Figure 5 shows the monitoring moving software of the organism. On the left the registration data are shown containing the coordinates in pixels of (x,y), date, hour, minutes and seconds. On the right the image of the organism detected in real time. The movement detection is made by the creation of a yellow circle that surrounds the alternate pixels, and a red dot on the center defines the position considered read by the software.

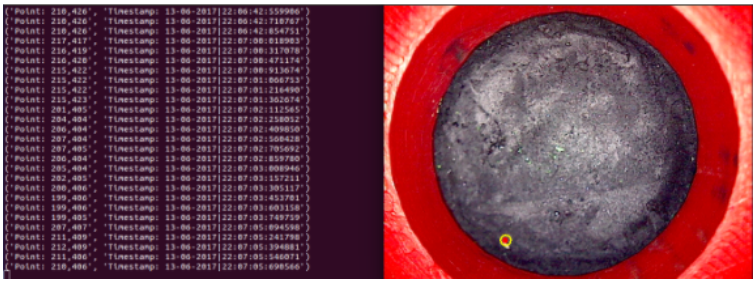

Figure 5: Organism's movement detection software.

The tests with the *Folsomia* spp organism, allowed until 7 captures per second in a moment of great movement. Obviously this rate can be despised because it is a slow organism.

4 Results

Tests were made with controlled temperature, monitored humidity, and special attention to the mechanical vibrations. The graphic of the x,y coordinates of the collected data of movement using the *Folsomia* spp organism is shown on Figure 6. It is possible to conclude that the organism has a preference for the corners of the cuvette. Which is a plausible fact, due the instinctive seek of protection.

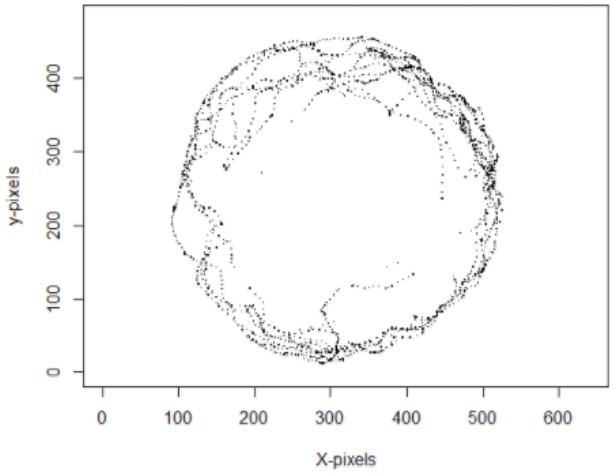

Figure 6: X,Y coordinates graphic (pixels).

For the test a camera with 640x480 pixels resolution was used, enough to validate the system. The collected data can be treated once there is the movement location (coordinate) and the moment (Time) After processing the data it is possible to determine: speed; movement over time; resting time; stress behavior.

5 Conclusion

The presented project is easy implemented, the hardware can be easily built because, there is a 8 bits microcontroller, commercial use sensors and diverse electronic components. The image capture software is open sourced and is possible to use higher resolution microscopes and/or cameras, depending on the application. To extract information thru data must, necessarily, use the treatment software as MatLab, StudioR, Mathematics, because the filter algorithm, calculus and conversions are necessary.

For the biological organism analysis, the presented technique could be a great tool, and with low investments.

It is possible to affirm that the developed system is of great value for analysis of organims' behavior, being an interesting option to biological behavior study.

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