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DEVELOPMENT OF OPEN-SOURCE ROBOTIC COMPUTER-BASED SYSTEM FOR AUTOMATIC COUNTING OF LACTIC ACID BACTERIAL COLONIES

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Abstract

Counting bacterial colonies is an important step in performing a variety of biotechnological analyses. There are several approaches to counting bacterial colonies according to the types of growing medium and all of them can be classified into three main groups - manual, semi-automatic, and automatic. Manual approaches to counting bacterial colonies are time-consuming and subjective. This method requires manually labeling and counting each colony using a marker on the Petri dish. Semi-automatic methods are faster because a specially developed device is used which uses a digital pointer and a special touch display to mark each colony and count it automatically. Although counting error is minimized with this method, manual marking of each colony remains a time-consuming operation. This research aims to develop an open-source robotic computer-based system that provides techniques for automatic positioning of Petri dishes using a robotic arm, scanning the surface of each Petri dish, and counting bacterial colonies based on object recognition methods.

For this research, an open-source Selective Compliance Assembly Robot Arm (SCARA) with 4 degrees of freedom is used and several sets of Petri dishes with fully grown lactic acid bacterial colonies are analyzed using digital image processing methods implemented in a specially developed standalone software with graphical user interface. The body of the robotic arm is printed using a 3D printer and assembled with all mechanical and electrical parts. The control and training of the robotic arm are conducted using Arduino Uno microcontroller and open-source software with GUI. To test the efficiency of the proposed system the time required for performing the two approaches - manual counting and automatic counting is measured. In addition, the accuracy of counting bacterial colonies of both approaches is examined.

A set of agar plates is analyzed using two approaches - an automatic computer-based with a robotic arm and a manual one. Each agar plate contains a different number of colonies with varying sizes. The results show a significant improvement in the time needed to count the bacterial colonies using the developed automatic system. In addition, an absolute error ΔA is calculated, and after processing the data a relative error is calculated too. The results show that the errors calculated after performing the automatic counting process are close to the ones obtained with manual counting.

In conclusion, an open-source robotic computer-based system for the automatic counting of bacterial colonies has been developed. The proposed system shortens the time required for colony counting and improves efficiency in performing biotechnological analyses.

***Key words**: Robotics, Digital image processing, Colony counting, SCARA robot.*

1. Introduction

Microbiological and biotechnological research often relies on the process of accurate determination of so-called Colony Forming Units (CFU). This process is performed by sampling and separating a small amount of liquid culture and subsequently inoculating the sample into already prepared Petri dishes containing a semi-solid growing medium, for ex.: De Man-Rogosa-Sharpe (MRS) agar [1 - 3]. The process of identification, classification, and counting of the microorganisms developed in a given sample, occupy an important part of the control procedures in the food industry and the quality of raw materials and foods [4]. Nowadays, there are several specialized devices and technical means for carrying out microbiological analyses. Their main

disadvantage is the high cost and the impossibility of using them freely for scientific and research purposes, except in specialized laboratories.

For conducting a microbiological analysis, it is necessary to know the exact number of developed colonies of a specific type of bacteria. For this purpose, special devices have been designed and created, which enable specialists to distinguish and count the colonies that have developed in the nutrient medium. Such colony-counting devices are called colony counters in the field of microbiology and biotechnology. Their main drawback lies in the process of counting colonies. First, the laboratory technician places the examined Petri dish in a special section in the apparatus, and then manually counts each of the colonies. Using appropriate sample illumination, a magnifying glass, and a purpose-built pen or other type of marking device, the laboratory technician touches a transparent and gridded screen, each touch of the screen is detected and counted as a dot corresponding to a given colony. Special software implemented in the device takes care of the reading and counting of the points (colonies). The problem with this approach is that it is easy to make a mistake in counting colonies and the process itself is very subjective. This approach is called semi-automatic because the observer does not have to remember the current amount of counted colonies and the counting process can be stopped at any time and continued in the future. Another significant disadvantage of this approach and such devices is the high market price, which is a prerequisite for conducting microbiological analyses of this type to be carried out only in specially equipped laboratories.

There is another approach of manually counting each colony in the examined Petri dish. This method is based on manually marking each colony directly on the surface of the Petri dish using a felt-tipped pen. A main disadvantage of this method is that the person who conducts the analysis (laboratory assistant) has to remember the current amount of colonies and there is a high probability that a counting error will be made. This approach is efficient when there is a small amount of colonies in the examined Petri dish.

There are computer-based approaches for automatic detection, measurement, and counting of bacterial colonies which rely on a specially developed experimental setting and a software for detecting and counting the colonies [5 - 9].

This research aims to propose a fully automated system based on a computer-controlled robotic arm to position the examined Petri dishes and assist the process of detecting and counting lactic acid bacterial colonies.

2. Materials and Methods

2.1 Object of analysis

A randomly selected group of Petri dishes with fully developed lactic acid bacterial colonies of different sizes and different concentrations was used to conduct the experiments. A semi-solid MRS agar with an average layer thickness of about 5 mm was used as the culture medium. Each Petri dish contained a different number of colonies of different sizes, and the Petri dishes used in this work are a sample of batches used for conducting microbiological analyses. The lactic acid bacterial colonies were insulated and kept in the Department of Biotechnology at the University of Food Technologies - Plovdiv, Bulgaria. For this research, a set of sixteen Petri dishes was analyzed. The lactic acid bacteria form round-shaped white colonies by their growing. Each of the agar plates is captured by a digital web camera in a laboratory setting with controlled artificial lighting placed below the Petri dishes. Figure 1 shows the images of all Petri dishes.

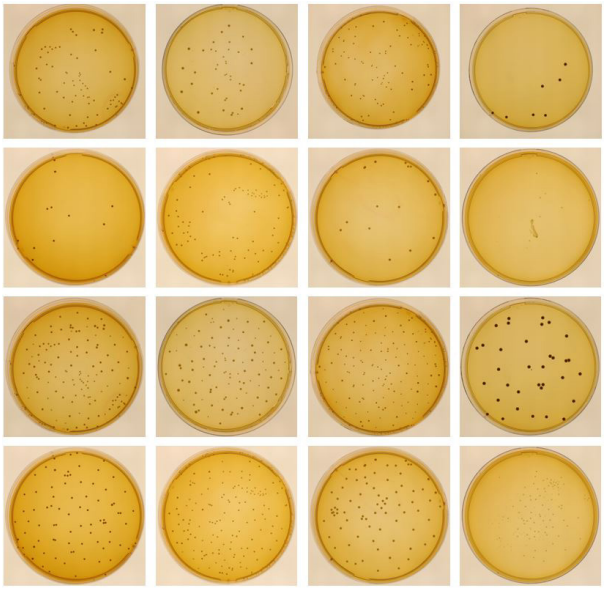
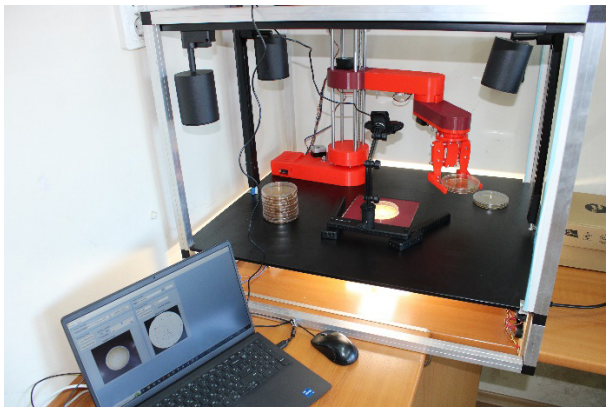


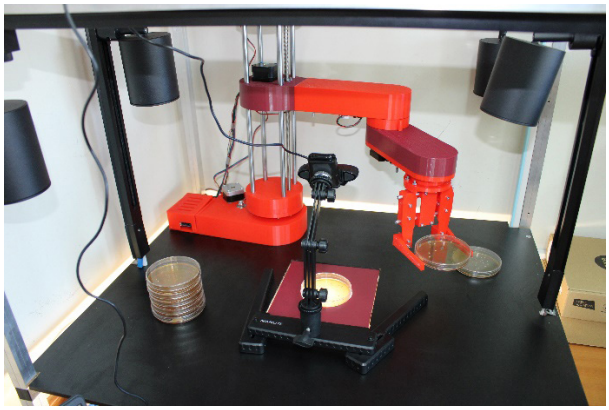
Figure 1. Agar plates images captured in a laboratory setting with controlled artificial lighting

2.2 Experimental setting

The developed system described in this research is shown in Figure 2. The experimental setting consists of several hardware components - the main corpus is constructed from aluminum profiles; the light source is a flat LED panel used for under illumination of the objects; 3D printed Selective Compliance Assembly Robot Arm (SCARA), used for automatically moving and positioning the Petri dishes; digital camera for obtaining the raw images, and a mobile personal computer with specially developed software installed for bacterial colonies detection and counting based on digital image processing techniques.



a)



b)

Figure 2. Robotic computer-based system for automatic counting of lactic acid bacterial colonies

The main corpus of the experimental setting is constructed from hollow aluminum profiles with a square cross-section with a side of 2 cm joined with special plastic connectors forming a square shape of the main corpus of the system.

A flat LED panel placed below the Petri dishes was used for the under-illumination of the objects. The panel has a built-in light diffuser to homogenize the emitting light. There is a thin glass plate permanently mounted directly below the hole for placing the Petri dishes. A light meter was used for measuring the luminosity directly below the Petri dishes.

An open-source SCARA robot with 4 degrees of freedom was used for moving and positioning the tested objects into the working space of the system. The corpus of the robot was designed by Dejan Nedelkovski [10] and it was 3D printed using the Flashforge Adventurer 4 3D FDM printer, located at the University of Food Technologies - Plovdiv, Bulgaria. The robot is driven by 4 NEMA 17 stepper motors and a small servo motor is used for controlling the gripper of the robot. Figure 3 shows the complete list of all parts used to assemble the robot.

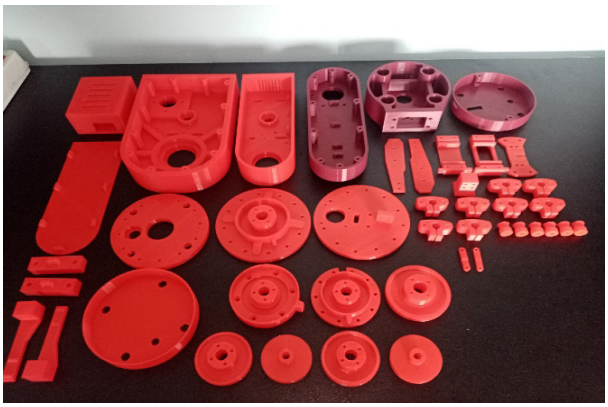


Figure 3. 3D printed construction elements used for assembling the SCARA robot

The brain of the robot is an Arduino UNO board and a CNC shield is used for connecting four A4988 stepper drivers for controlling the stepper motors. Using the Arduino platform software with the graphical user interface is adapted. The program is designed to perform Inverse Kinematics for positioning the gripper of the robot and automatically calculating the angles for each joint for the robot to get to the desired positions.

A Logitech HD USB web camera model C920 is used for obtaining the initial raw images. The camera has the following technical specifications:

- Max Resolution: 1080 p/30 fps - 720p/ 30 fps;
- Camera megapixel: 3;
- Focus type: Autofocus;
- Lens type: Glass
- Diagonal field of view (dFoV): 78°.

To prepare the system for work, the robot arm must be trained using Forward and Inverse Kinematic techniques to perform all necessary movements - grabbing a Petri dish from a stack; positioning the Petri dish right on the Petri dish stand; removing the lid; closing the lid after initial image acquisition, than grabbing the Petri dish again and put it in another stack where the processed samples are stored. The process of training the robot arm was performed using specially adapted software with a graphical user interface shown in Figure 4.

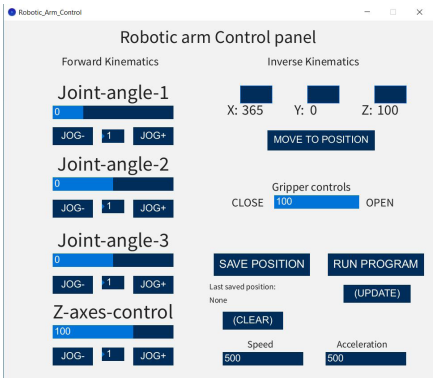
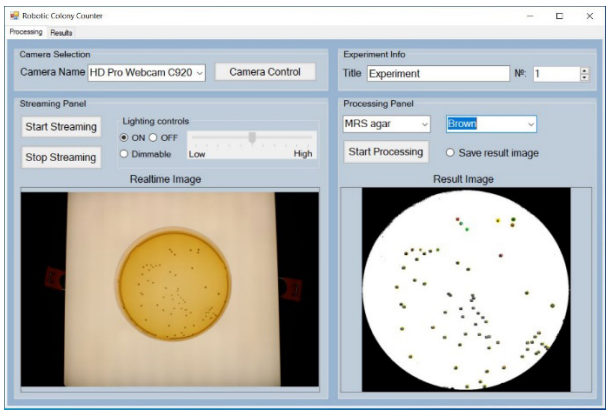


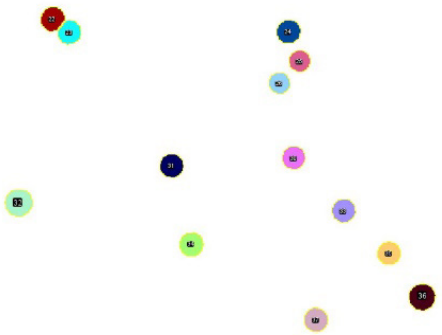
Figure 4. Software for training the robotic arm

2.3 Software for performing the analyses

For this research, a stand-alone program (called Robotic Colony Counter) with a graphical user interface (GUI) for desktop PCs is developed using C# language. The main window of the program is shown in Figure 5 a).



a) Main windows of the program



b) Detected and marked colonies

Figure 5. The developed software for automatic detection and counting bacterial colonies

The program consists of two tab controls - "Processing" and "Results". In the "Processing" tab there are several individual control panels with different control objects. The "Camera selection" panel provides functionalities for selecting a specific camera device that is connected to the working station via a USB port. The combo box (1) contains a list of all connected and active camera devices. The idea of the "Streaming panel" is to visualize the image in real-time (panel 3) and to provide the user functions for controlling the lighting of the experimental setting and some basic camera controls. Once a streaming process is started camera control settings can be managed using the button "Camera control" (2). The lighting in the experimental setting has three operating states - On, Off, and Dimmable. In the Dimmable state, the user can adjust the brightness of the lighting using a track bar control (4). Panel "Experiment Info" (5) provides a function for typing the name and the number of the current experiment. The "Processing Panel" provides functions for agar type and agar color selection and a "Start Processing" button for conducting digital processing of the original image and the automatic detecting and counting of the bacterial colonies. The resulting image with all detected and counted colonies is visualized in panel (7) and a sample is shown in Figure 5 b). If the detection and counting is successfully made the program marks each colony with a number and unique color. The results obtained after successful image processing are saved in a local database and can be listed in a table view in the Result window of the program shown in Figure 6. The result table (panel 8) contains several columns: experiment information; sample number; name of the processed image file; number of all detected and counted colonies; total area of all colonies in mm^2; mean size in millimeters and total area in percentages. The user can choose one of two options for saving the results data - saving to a text file or saving to a local Database (panel 9).

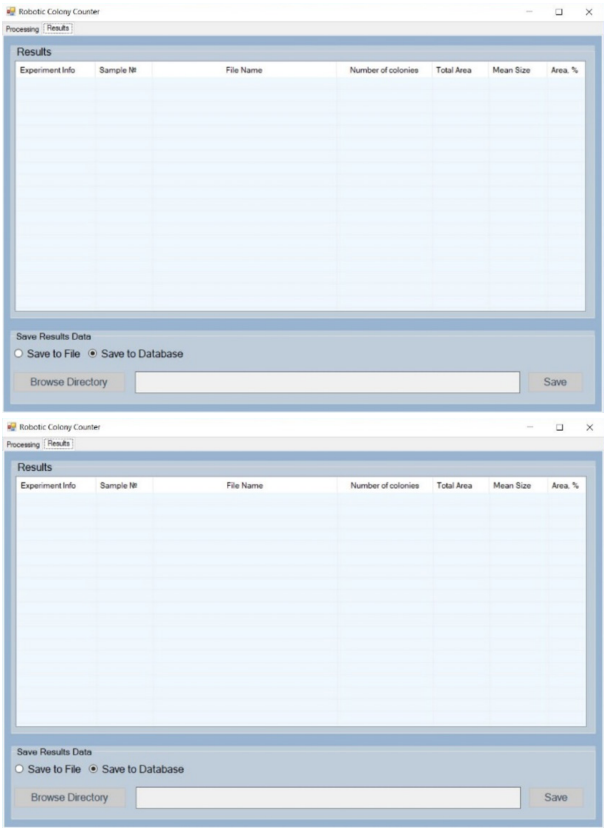


Figure 6. Result window

The workflow of the developed program is shown in Figure 7. It consists of 10 main steps from starting the program to finishing the analysis and observing the results. First, the desired camera should be selected and a streaming mode must be started to observe the live picture in real-time and to check if the camera settings have to be adjusted (step 2, step 3, and step

5). Step 4 provides possibilities for controlling the light source placed under the Petri dish stand. The user has three options for light control - off, on, and dimmable. If it is necessary the light brightness can be adjusted using the track-bar control (Figure 5 - (4)) in the panel. For more than 1 experiment the user must type relevant information for the current experiment in the appropriate fields in panel (5). In step 7 of the workflow, the user is prompted to select the type of agar used in the current batch of Petri dishes. The common agar type used in such microbiological analyses is a semi-solid agar medium called MRS agar, so the program is developed for using this type of agar. The program has control over selecting a base color for the agar medium, but the current version of the program is developed only to use one color. In addition, different base colors will be added for future optimization.

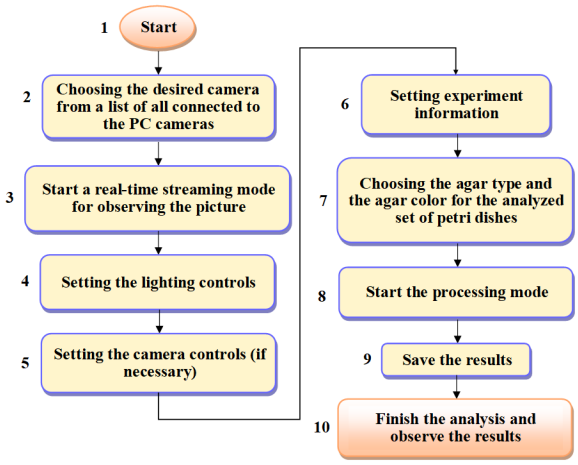


Figure 7. Workflow of the program

The next step from the workflow of the program (Step 8 - Processing mode) is most important for this research. Starting the processing mode immediately triggers the robotic arm and the analysis begins. The whole process is presented in a sample timeline diagram shown in Figure 8. It consists of seven stages. Stage 1 represents the time needed for the robotic arm to move from its initial position to the position of the unprocessed stack of Petri dishes and grab one Petri dish. This stage lasts about 3 seconds. In Stage 2 the arm moves to the next position and places the Petri dish in the middle of the experimental setting where a Petri dish stand is located (this stage lasts about 8 seconds). Stage 3 represents the time needed for the arm to open the Petri dish lid. The next stage (Stage 4) shows the time needed for obtaining the initial image and processing the image to detect and count the bacterial colonies and save the results. The time measured in this stage is approximately 3 seconds and it depends on the size of the initial images and the technical characteristics of the working station i.e. the PC. Next, comes Stage 5 where the robotic arm closes the lid, and in Stage 6 the arm grabs the analyzed Petri dish and places it on another stack with already analyzed samples. The two stacks with Petri dishes are equidistant from the center of the experimental setting. This stage lasts about 8 seconds. The last stage is Stage 7 where the arm moves again to the stack with unanalyzed Petri dishes and this stage takes about 16 seconds. During the experiments, small differences (tens of a second) in the measured time at some stages are observed, but they do not lead to significant changes in the total time needed for the robotic arm to complete one working cycle. Therefore, for the convenience of subsequence analyses, the small differences in the time are ignored and the time required for performing one cycle is assumed to be 42 seconds, as shown in Figure 8. The robotic arm movements can be accelerated programmatically by controlling the stepper motors, but due to the rigidity and the durability of the materials that have been used to construct the robot, no studies have been done with the highest possible acceleration. It is assumed that if stronger construction materials are used, a higher acceleration of the arm's movements and a correspondingly shorter time required for one working cycle can be achieved.

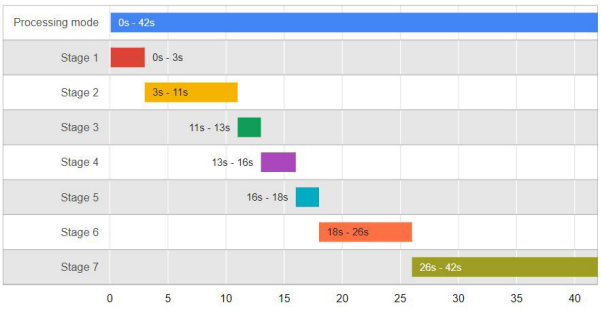


Figure 8. A timeline diagram for processing mode

3. Results and Discussion

A batch of sixteen Petri dishes with fully grown lactic acid bacteria colonies was analyzed. All the Petri dishes were automatically moved by the robotic arm and processed by specially adapted software for detecting and counting bacterial colonies through digital image processing techniques. The initial images were captured using a USB HD digital camera with 3MP resolution.

Two parameters of the proposed system are analyzed - the time needed for processing and the accuracy in detecting and counting bacterial colonies.

To evaluate the overall performance of the system three different approaches are used in detecting and counting the bacterial colonies. Manual and semi-automatic approaches are used as a reference method and a fully-automatic computer-based approach is used.

The total number of counting iterations for each sample (Petri dish) was six. The results obtained after manual counting are shown in Table 1.

Table 1. Colony count results were obtained using manual counting as a reference method

Sample №	Iter. 1	Iter. 2	Iter. 3	Iter. 4	Iter. 5	Iter. 6	Average Count
Sample 0	73	72	78	78	71	71	74
Sample 1	39	39	39	39	39	39	39
Sample 2	70	70	70	72	65	70	70
Sample 3	7	7	7	7	7	7	7
Sample 4	11	11	11	11	11	11	11
Sample 5	90	91	91	91	86	88	90
Sample 6	15	15	15	15	15	15	15
Sample 7	8	8	8	8	8	8	8
Sample 8	120	120	129	128	121	121	123
Sample 9	103	103	102	102	103	103	103
Sample 10	131	130	135	135	137	133	134
Sample 11	33	33	33	33	33	33	33
Sample 12	81	81	81	81	82	81	81
Sample 13	140	140	152	153	144	143	145
Sample 14	80	80	80	80	89	85	82
Sample 15	128	128	128	128	131	129	129
SUM:	1129,00	1128,00	1159,00	1161,00	1142,00	1137,00	1142,67

The results show that when using a manual counting approach, it is possible to make errors and have differences in the total number of colonies for each sample. After six iterations of counting, the average count of colonies for each sample is calculated and these results are used as reference data. For each sample, the time required for colony counting is measured and the results are shown in Table 2.

Table 2. Results for the time measured after performing manual counting

Sample №	T1, s	T2, s	T3, s	T4, s	T5, s	T6, s	Average Time, for each sample
Sample 0	42,00	38,30	47,23	48,54	45,78	44,00	44
Sample 1	28,15	28,64	33,65	32,45	32,27	31,78	31
Sample 2	54,78	51,21	55,28	57,21	56,74	55,24	55
Sample 3	3,56	4,88	3,21	3,12	4,35	4,12	4
Sample 4	12,00	10,80	12,78	13,11	11,68	10,05	12
Sample 5	48,48	54,05	58,26	59.23	59,48	56,15	55
Sample 6	11,20	11,15	10,20	13,89	11,26	10,27	11
Sample 7	5,15	5,49	6,58	7,18	7,98	7,45	7
Sample 8	116,42	102,00	137,34	132,50	125,63	120,72	122
Sample 9	65,47	61,43	64,72	58,70	117,35	107,97	79
Sample 10	98,17	87,24	132,25	103,42	102,34	96,87	103
Sample 11	20,51	21,06	18,43	19,76	20,15	21,46	20
Sample 12	62,67	59,54	69,57	67,23	65,23	61,37	64
Sample 13	165,82	86,51	118,69	115,34	124,73	98,71	118
Sample 14	51,49	50,76	54,32	52,65	52,46	59,23	53
Sample 15	97,24	91,65	95,14	98,25	92,19	98,57	96
AVG T, for the whole batch:	55,19	47,79	57,35	54,89	58,10	55,25	54,77
SUM T, sec. :	883,11	764,71	917,65	823,35	929,62	883,96	867,07
T, minutes:	14,72	12,75	15,29	13,72	15,49	14,73	14,45

The average time for the six iterations of counting was calculated together with the average time for all sixteen Petri dishes. The calculations showed that the average time required for processing one Petri dish was less than 1 minute - about 54.77 seconds, and the average time for processing the whole batch of sixteen dishes was about 14.45 minutes.

A semi-automatic approach is used as a second reference method for colony counting. This approach is based on using an open-source computer program (ImageJ) and its features for manually marking and labeling each colony in the currently analyzed Petri dish using the pointer of the mouse. The advantage of this approach is that the program (ImageJ) automatically counts each marked colony and the user does not have to remember the current number of colonies. Another advantage is the possibility of magnifying different sections of the processing image to recognize and count smaller-sized colonies and achieve better precision. The semi-automatic approach is more useful when there are more than 100 colonies in a Petri dish and they are too small for accurate marking with a felt tip pen. The obtained results are shown in Table 3 and Table 4.

Table 3. Colony count results were obtained using semi-automatic counting as a second reference method

Sample №	Iter. 1	Iter. 2	Iter. 3	Iter. 4	Iter. 5	Iter. 6	Average Count
Sample 0	70	70	71	71	70	70	70
Sample 1	39	39	39	39	40	39	39
Sample 2	79	79	79	79	78	79	79
Sample 3	7	7	7	7	7	7	7
Sample 4	11	11	11	11	11	11	11
Sample 5	84	84	84	85	84	84	84
Sample 6	16	15	15	15	15	15	15
Sample 7	9	8	8	8	8	8	8
Sample 8	126	125	125	127	125	125	126
Sample 9	105	104	104	104	104	103	104
Sample 10	134	131	131	131	131	132	132
Sample 11	33	33	33	33	33	33	33
Sample 12	82	82	81	82	82	82	82
Sample 13	144	143	143	143	144	143	143
Sample 14	80	80	80	80	80	80	80
Sample 15	134	134	133	134	134	134	134
SUM:	1153,00	1145,00	1144,00	1149,00	1146,00	1145,00	1147,00

Table 4. Results for the time measured after performing semi-automatic counting

Sample №	T1, s	T2, s	T3, s	T4, s	T5, s	T6, s	Average Time, for each sample
Sample 0	86,00	85,65	78,54	76,32	88,54	104,27	87
Sample 1	42,64	43,26	55,23	54,30	47,21	54,21	49
Sample 2	70,30	71,25	68,21	69,74	74,72	65,48	70
Sample 3	13,23	15,22	11,30	12,43	15,47	14,78	14
Sample 4	19,08	20,12	21,35	22,12	20,45	23,83	21
Sample 5	68,63	71.24	66,87	64,39	72,62	75,34	70
Sample 6	18,10	20.32	17,25	18,95	19,37	18,27	18
Sample 7	13,01	16.78	12,00	17,32	15,83	16,94	15
Sample 8	96,00	102,25	102,34	98,23	123,42	134,17	109
Sample 9	77,60	82,65	98,45	92,65	72,19	73,98	83
Sample 10	102,07	119,45	115,26	112,32	108,48	106,34	111
Sample 11	27,77	30,21	33,76	31,63	31,84	45,82	34
Sample 12	64,76	66,45	67,21	64,85	72,18	77,41	69
Sample 13	105,05	110.32	118,12	116,31	121,42	147,29	122
Sample 14	60,79	66.63	64,23	63,74	64,72	71,45	65
Sample 15	82,69	88.32	87,45	85,35	81,39	85,63	85
AVG T, for the whole batch:	59,23	63,65	63,60	62,54	64,37	69,70	63,77
SUM T, sec. :	947,72	636,51	1017,57	1000,65	1029,85	1115,21	957,92
T, minutes:	15,80	10,61	16,96	16,68	17,16	18,59	15,97

The results show that the average time required for counting colonies in one Petri dish was about 64 seconds and the average time for processing the whole batch was about 16 minutes. Despite the slower

execution of this approach, it gives more accurate results in comparison to the manual one.

The present research proposes a fully automatic approach to detecting and counting lactic acid bacterial colonies based on the robotic arm and specially developed software. After setting up the system and conducting several experiments, the final results were obtained. The results are shown in Table 5. An absolute error ΔA (in number of the colonies) was calculated. After processing the data relative errors between fully automatic, manual, and semi-automatic approaches were calculated too. The relative errors (in percentages) do not exceed 2.5%.

Table 5. Results obtained after using the fully automatic approach for counting

Sample №	Fully-automatic	Manual counting		Semi-automatic	
	Count	ΔA	δ, [%]	ΔA	δ, [%]
Sample 0	71	3	3,99	1	0,94
Sample 1	40	1	2,50	1	2,08
Sample 2	72	3	3,47	7	9,49
Sample 3	7	0	0,00	0	0,00
Sample 4	11	0	0,00	0	0,00
Sample 5	89	1	0,56	5	5,43
Sample 6	15	0	0,00	0	1,11
Sample 7	9	1	11,11	1	9,26
Sample 8	123	0	0,14	3	2,03
Sample 9	102	1	0,65	2	1,96
Sample 10	128	6	4,30	4	2,86
Sample 11	33	0	0,00	0	0,00
Sample 12	82	1	1,02	0	0,20
Sample 13	142	3	2,35	1	0,94
Sample 14	81	1	1,65	1	1,23
Sample 15	132	3	2,53	2	1,39
SUM:	**1137**	**23**		**27**	
Average:			**2,14**		**2,43**

In addition, a time analysis was made. The time needed for the robotic arm to perform one cycle of operation is experimentally measured and it is rounded to 42 seconds for processing one Petri dish. It was calculated that the time required for processing the whole batch of sixteen Petri dishes is 672 seconds (about 11.2 minutes). The calculations for the time of the other approaches (manual and semi-automatic) showed that the process of counting bacterial colonies grown in semi-solid MRS agar conducted by the proposed open-source robotic computer-based system for automatic counting was faster than the manual and semi-automatic methods of counting.

4. Conclusions

The current research aims to develop a compact automated system for objective counting of lactic acid bacterial colonies grown in a semi-solid medium - MRS agar, by using modern computer methods for digital image processing and an appropriate approach for automatization of the process. The system consists of a hardware and a software part. To achieve the goal of this study the following tasks are completed - 1) a hardware module is developed and a prototype of a computer-controlled robotic arm with 4 degrees of freedom is built using 3D printing technology; 2) a digital camera for obtaining the primary images is selected, together with appropriate lighting necessary to provide illumination of the tested objects; 3) a computer program with graphical user interface is developed for objective and automatic detection and enumeration of bacterial colonies. The program is developed in Visual Studio IDE and C# programming language is used. The software was developed for desktop personal computers with Windows OS. Modern methods of digital image processing were integrated into the program. The software was based on a working algorithm that has proven its effectiveness in previous scientific studies related to the subject of the current research; 4) to evaluate the overall effectiveness of the proposed system two reference methods for counting bacterial colonies were used and during the conduction of the reference methods two parameters are examined - time and accuracy; 5) the obtained results with the proposed system were analyzed and compared with the results obtained with the reference methods.

- Based on the obtained results the following conclusions can be made:

A. The proposed system is accurate enough in the detection and counting of lactic acid bacterial colonies grown in semi-solid medium - MRS agar. The calculated relative errors between the fully automated system and the two reference methods are less than 2.5%;

B. The time required for processing the whole batch of sixteen Petri dishes is about 3 to 4 minutes less than the time needed for manual and semi-automatic counting;

C. The better performance (in time) of the system can be achieved using stronger construction materials for building the robotic arm.

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