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PetriJet Platform Technology: An Automated Platform for Culture Dish Handling and Monitoring of the Contents

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Abstract

Due to the size of the required equipment, automated laboratory systems are often unavailable or impractical for use in small- and mid-sized laboratories. However, recent developments in automation engineering provide endless possibilities for incorporating benchtop devices. Here, the authors describe the development of a platform technology to handle sealed culture dishes. The programming is based on the Petri net method and implemented via Codesys V3.5 pbF. The authors developed a system of three independent electrical driven axes capable of handling sealed culture dishes. The device performs two difference processes. First, it automatically obtains an image of every processed culture dish. Second, a server-based image analysis algorithm provides the user with several parameters of the cultivated sample on the culture dish. For demonstration purposes, the authors developed a continuous, systematic, nondestructive, and quantitative method for monitoring the growth of a hairy root culture. New results can be displayed with respect to the previous images. This system is highly accurate, and the results can be used to simulate the growth of biological cultures. The authors believe that the innovative features of this platform can be implemented, for example, in the food industry, clinical environments, and research laboratories.

Keywords

laboratory automation, culture dish handling, image analysis

Introduction

The culture dish was invented by Julius Petri in 1887 and is now commonly used in biological, clinical, food, and other laboratories. In addition, its dimensions are now defined to comply with several German Institute for Standardization (DIN) standards (e.g., DIN ISO 24998:2008).

After sampling their contents, culture dishes are usually left in an incubator to allow growth of an organism, followed by evaluation at specified time points. In small- and mid-scale laboratories, assistants evaluate the culture dishes manually; however, this can lead to incorrect results and data that are difficult to verify and document.

Due to limited financial resources and the complexity of biological systems, it can be beneficial to reduce the "human factor"[1] involved in analyzing cultures by introducing an automated culture dish analysis system.[2–4] With the development of high-throughput screening (HTS), a huge number of results are now generated, and it is necessary to record and store them in a safe location.[5] However, the current state of the art demands that laboratory staff must manually enter the results into various databases.

During the past 25 years, various types of robotic systems have been used in life sciences laboratories and other research facilities to make work flows more reliable, reproducible, and continuous.[6] These are important factors when certifying and validating processes and procedures used in the pharmaceutical and food industry.[7] In addition, a complete validation of every testing and production step is required to gain product approval from the European Food Safety Authority (EFSA) and/or the US Food and Drug Administration (FDA). While many laboratories are interested in implementing higher levels of automation for purposes of reducing production time and cost, an automated work flow must also remain flexible and accommodate

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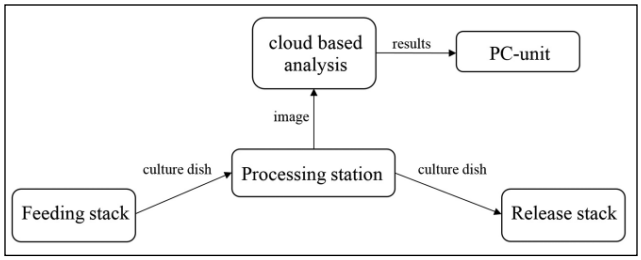

Figure 1. Schematic flowchart for culture dish handling and image analysis.

multitasking.[8] Hence, a single laboratory automation device should have a wide range of applications.[9]

The analysis methods used in large-scale microbiology laboratories and institutes are often rigidly configured,[6] making implementation of a totally automated system for HTS a reasonable goal.[10]

In contrast, small- and mid-scale laboratories have a growing demand for automated and adaptable laboratory platform technologies that can be applied in partially automated processes. This is because such laboratories lack the capacity and throughput needed to achieve efficient turnaround times when using totally automated laboratory devices.

The imaging and manipulation of deep-well plates and culture dishes are important functions provided by automated microbiology laboratories.[11] These capabilities provide new opportunities for quality control in both industrial and research laboratories (e.g., laboratories involved in studying physiological behavior and plant development).[12]

Currently, there are no technological solutions for small- and mid-scale microbiological laboratories, and most management of processing, throughput, and quality control is done by laboratory staff.

In this context, our aim was to develop a solely electrically powered device (no compressed air connection necessary) that could be carried by one person and would fit on a laboratory bench. This benchtop device would be capable of processing at least 100 culture dishes per hour and handling both sealed and unsealed culture dishes. A schematic drawing of the main components is shown in **Figure 1**.

In summary, while large laboratories with a high volume of samples can fully use Full Microbiology Lab Automation (FMLA),[13] small- and mid-scale laboratories can start using smart benchtop devices as an intermediary step in achieving a higher level of automation.

Materials and Methods

To address the issues described above, we are developing a benchtop device with the following features: (1) it is movable by one person, (2) the maximum weight is 30 kg, (3) it has combined hardware and software components that allow it to be placed under a laminar flow cabinet, (4) the surfaces can be disinfected, and (5) it has a wireless control mechanism.

Furthermore, the benchtop device can be developed and constructed using standardized industrial components, materials, and manufacturing techniques. The actuator linear axes, stepper motors, stepper motor controller, and control system used in the benchtop device are provided by FESTO (leading German industrial control and automation company, Esslingen, Germany) and are designed to be small in size. The sensors used to gain and transmit information are also FESTO components and mainly consist of inductive and optical proximity sensors.

The processing control system was developed based on the Petri net concept. A complete computer-aided design (CAD) for the system was developed prior to building the first prototype. Therefore, we used the CAD software package SolidWorks (Dassault Systèmes, SolidWorks Corporation, Waltham, MA, USA), and data were provided by FESTO. CAD was used to design all parts manufactured for the product prototype. We also used CAD software to implement a motion study designed to identify problems and solve them in the first iteration. A complete CAD design enabled us to scale the parameter of the actuators before they were assembled and installed in the prototype.

Programmable Logic Controller

Every system requires control mechanisms to gather and process data.[14] CODESYS (controller development system) is a programming environment development system used with a Programmable Logic Controller (PLC) and is widely used in industrial automation. In accordance with the IEC 61131-3 standard, we used language structured text for programming the PLC. Ethernet is the preferred option for connecting the new generation of miniaturized automated components. This is because it allows communication structures that can cross all levels of an automation system.[15]

Petri Net Concept

The Petri net concept, first described by Carl Adam Petri in 1962, is currently widely used as an established mathematical modeling language to describe distributed systems.[16,17] The concept is still being extended, developed, and applied in a variety of areas.[18–20] The Petri net concept is characterized by graphic symbols. By using a Petri net (PN), a process can be divided in several segments known as transitions ($T = \{t_1, t_2, \ldots, t_n\}$), places ($P = \{p_1, p_2, \ldots, p_m\}$), flow relations as arcs ($F \subseteq (P \times T) \cup (T \times P)$), an initial marking ($M_0: \rightarrow P \{1, 2, \ldots\}$), and a mapping to assign a weight to an arc ($W: F \rightarrow \{1, 2, \ldots\}$).[21] A place describes a state of the system; it is designed as a circle and shows the processes that proceed at that exact state. Transitions define the conditions needed for

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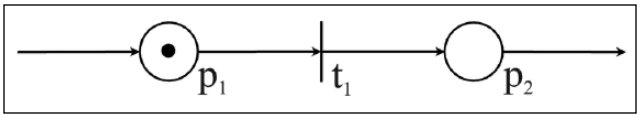

Figure 2. Scheme of a Petri net. A place (p_1 and p_2) describes a state of the system and is designed as a circle. Transitions (t_1) define the conditions needed for the system to reach another place. Arcs mark the link between two corresponding places and transitions. The marking symbolizes a distributed state of the system. After firing, the marking is placed in p_2.

the system to reach another place. Arcs mark the link between two corresponding places and transitions. Therefore, a Petri net can be summarized as a 5-tuple, PN = (P, T, F, W, M_0). When there is no specific initial marking provided, the Petri net structure is given by N = (P, T, F, W). A graphical description of these items is shown in **Figure 2**.

To change a state, the marking is moved from place p_1 to place p_2 (firing). Therefore, the transition (t_1) has to be enabled when the input place (the place before the transition) is marked with the corresponding weight (W) of the arc.

Results and Discussion

PetriJet Platform Technology

The state-of-the-art components for movement and control were made available through FESTO AG, in collaboration with ADIRO Automatisierungstechnik GmbH (Esslingen, Germany).

The fully functional benchtop device for automated culture dish handling and imaging (dimensions 800 × 400 × 650 mm) is shown in **Figure 3**.

The device can be loaded with up to 20 culture dishes for a single-process flow of handling and imaging of each dish. This permits approximately 100 culture dishes to be handled per hour. The culture dishes are moved along electrically driven linear axles and lifted using a vacuum suction gripper. We are currently field testing a gripper for handling and separating unsealed culture dishes.

The process flow of culture dishes in the automated laboratory device can be divided into three segments. The first segment includes the feeding of 20 culture dishes, followed by the separation of one culture dish from the feeding stack. For this task, the device must detect both the correct position of the feeding stack and the number of dishes in the stack.

An automated image-capturing digital camera is positioned in the center of the benchtop device. After aquiring an image, the Petri dish is automatically transferred to the release segment.

The release segment includes the collection of 20 culture dishes and placing them in a fixed position until the user has removed the release stack from the automated benchtop device.

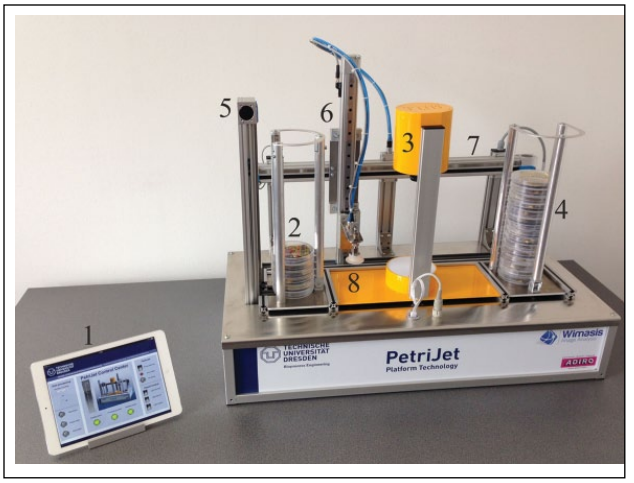

Figure 3. PetriJet platform device with the (1) wireless human interface, (2) feeding stack, (3) processing station PetriCam, (4) release stack, (5) toothed belt axis, (6) spindle axis, (7) toothed belt axis, and (8) vacuum suction gripper.

Handling

Once the PetriJet system is switched on, the device initializes itself automatically. Following a successful initialization and completion of the subsequent "homing process," the feeding and release stacks as well as the processing station are mounted into the device via the railing system.

As a result of its small frame size, and to optimize cleaning of its optical components, we designed the entire platform with only one on/off switch. To ensure user friendliness, we designed the device to be used with a wireless network. The device is controlled by an integrated webserver and can be accessed via any wireless local area network (LAN) smart device (**Fig. 3**, number 1).

The feeding stack can hold up to 20 culture dishes within a single batch (**Fig. 3**, number 2). We selected this number of dishes because it provides the total height of dishes most often stored in incubators. In addition, the device has a height of 650 cm, and thus 20 culture dishes is the maximum number it can hold.

When cultivation is continued for more than 2 weeks, culture dishes are sometimes sealed with Parafilm[22,23] to prevent media evaporation while allowing aeration. However, Parafilm can cause the plates to stick together, and this becomes a significant problem during long-term culture.

To prevent two culture dishes from sticking together, we installed T-Slot Nuts (Industrietechnik GmbH, Solingen, Germany) in every pole of the feeding stack. The spring plungers with the ball hold back the lower culture dish

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while the upper dish is being processed with the vacuum suction gripper. The electrical-driven linear axis next to the feeding stack positions the culture dishes directly below the separation unit (**Fig. 3**, number 5). This positioning guarantees separation between the upper culture dish and the culture dish immediately below and also prevents the lower culture dish from falling more than 2 mm onto the next culture dish.

Because a user might incorrectly place the feeding stack into the device, the bottom of the stack is designed with a rectangular shape rather than a quadratic shape, making it impossible to incorrectly insert the feeding stack.

Processing Station PetriCam

The first configuration of the PetriJet benchtop device has an imaging unit placed in the center of the platform (**Fig. 3**, number 3). The housing around the camera and the illumination unit under the culture dish tray are completely 3D printed using stereolithography.

Images are acquired using a camera (IDS imaging 5481VSE-C-SD32, IDS Imaging Development Systems GmbH, Obersulm, Germany) equipped with a SV-0814H f8 objective mounted on the image-processing station. While the camera has manual capture settings that can be adjusted for the environment, images can also be captured using fixed settings. The optical axis of the camera is aligned to face the illuminated base of the processing station. Six high-power light-emitting diodes (LEDs) provide illumination from beneath the culture dishes, and 12 LEDs are positioned next to the lens to eliminate possible shadows and reflections from the top of the culture dish. Due to the device's compact design, the distance between the lens and the processed culture dish has been made as small as possible (~180 mm). Following image acquisition, the culture dish is moved to the release stack. The images are saved in JPEG format without additional compression.

The processing station is connected to the platform via two interfaces (Ethernet and wire for electricity), which allows the user to switch processing stations within 60 s.

Release Stack

The release stack is designed as simply as possible, as its only functions are to receive and hold the processed culture dishes (**Fig. 3**, number 4). To provide a maximum amount of free space for the gripper to move into the release stack, the space between poles is as wide as possible but not wide enough to let the culture dishes slip through.

Similar to the feeding stack, the release stack has a rectangular base to prevent system errors due to user action.

Electrically Driven Linear Axes

We developed a system of three independent electrical-driven linear axes for use in moving culture dishes. Two toothed belt axes (EGC-TB-KF) with a recirculating ball bearing guide (frame size 50) are used to move and separate culture dishes (**Fig. 3**, numbers 5 and 7). We chose a spindle axes DGE-SP (frame size 18; **Fig. 3**, number 6) to implement the "take up" of the culture dishes.

These axes are connected with a CMMO-ST (Festo AG & Co., Esslingen, Germany) motor controller and the data recorder.

Gripper Technology

Vacuum suction grippers are often used for handling operations,[24] and for the PetriJet platform, a customary 20-mm diameter vacuum suction gripper was found to be sufficient (**Fig. 3**, number 8). To detect the grip on culture dishes, we inserted two sensors into the device. One inductive sensor is situated immediately above the gripper to detect any upright movement when touching a culture dish. The other pressure sensor is set into the base of the PetriJet to detect a positive grip of the vacuum suction gripper upon the culture dish. The axis with the gripper attached moves only when a signal is received from the pressure sensor.

An electrically driven parallel gripper for handling both unsealed and sealed culture dishes is being developed. We are currently field testing the parallel gripper, and the results will be published soon.

Petri Net Structure

A complete Petri net structure was developed to represent the overall process and possible error states. This is an established method when developing a software for an automated device. A summarized Petri net for nominal operation is shown in **Figure 4** and represents the main structure of the developed program using CODESYS. After switching the device on (t_1), reference runs and homing processes for all electrical linear axes are executed (p_1). If this initializing process is executed successfully (t_2), the feeding stack, release stack, and processing station can be mounted into the PetriJet (p_2). If the initializing process is not successful, place p_{11} is obtained, after which the homing of the axes stops, a signal is given, and the device must be switched off and on again. When the control lamps flash green and the settings are properly set, the process can start (t_3). Place p_3 represents the separation of two culture dishes, as well as the movement of one culture dish from the feeding stack to the processing station. t_4 marks the transition from movement to image acquisition. It contains the successful positioning of the culture dish in the processing station, the positioning of the vacuum suction gripper aside the processing station, and the signal given to the camera for image acquisition. When all of these conditions are met, an image is obtained and automatically saved in cloud storage and on a microSD card (Secure Digital, Global Inventures, Inc., San Ramon, CA, USA) (p_4). After obtaining a saved image (t_5), the vacuum suction gripper

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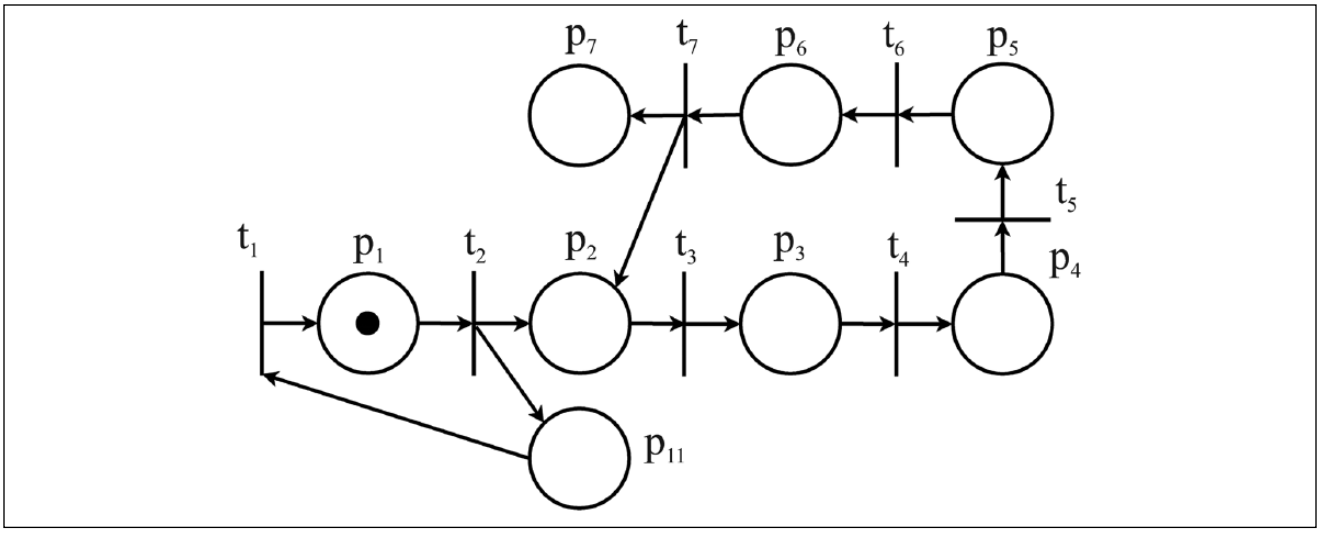

Figure 4. Simplified Petri net of the platform device for nominal function. t_n represents a transition and p_n a place. A detailed explanation is given in the subtitle Petri net structure in the Results and Discussion section: t_1, switching the device on; p_1, homing process for the linear axes; t_2, successful initializing process; p_{11}, rerun of the homing process; p_2, mounting the stacks and processing station; t_3, settings properly set; p_3, separation of two culture dishes; t_4, transition from movement to image acquisition; p_4, acquiring an image; t_5, image safely stored; p_5, processing the culture dish into the release stack; t_6, release of the culture dish into the release stack; p_6, movement of the vacuum suction gripper next to the feeding stack; t_7, distinguish between two different possibilities according to the program and p_7 end position.

moves back over the processing station, picks up the culture dish, and processes it into the release stack (p_5). The release of the culture dish into the release stack marks transition t_6. The processing of one dish is completed with the movement of the vacuum suction gripper next to the release stack (p_6). At transition t_7, the Petri net distinguishes between two possible places. p_7 is selected when the chosen number of culture dishes has been processed. In this case, the gripper stays next to the feeding stack, and the stacks can be removed. The other place (p_2) is selected if the chosen number of culture dishes is not yet obtained. In this case, another culture dish is processed until the place p_7 is reached.

Innovative Control via Ethernet/Codesys V3.5

The phrase "Internet of things" is commonly used to describe a new approach toward the shift to an Internet used more for interconnecting physical objects that communicate with each other and/or with humans rather than for interconnecting end-user devices.[25]

By using the new Codesys V3.5 pbF, we were able to implement a web-based control center that made the PetriJet platform a part of the "Internet of things."

Due to the constant evolution of process control software,[9,26] one can access the control center via the platform-independent browser interface through a wireless LAN connection to the PetriJet. In addition to the control center, the stepper motor controller CMMO-ST as well as the PLC can also be addressed directly over an IP address. The user-friendly interface is shown in **Figure 5**.

After connecting the handheld device to the wireless LAN network of the PetriJet platform, the human-machine interface (**Fig. 5**) will appear. Methods for human interaction with the device were developed based on standards of the German Institute for Standardization (DIN EN ISO 9241-110, DIN EN ISO 9241-210) for human-machine interfaces.

In the dish-processing area, a user may either choose the number of dishes to be processed or simply mark the "all" box. After pressing the "Start batch" button, the device will start processing the culture dishes. Buttons marked "Pause batch" and "Stop batch" can be used to halt the process. An indicator located in the left middle of the interface shows the number of processed culture dishes.

The lower middle section of the interface includes three signal indicators. They flash green if the feeding stack, processing station, and release stack are properly mounted. If one of these items is improperly mounted, the system will not start until all components are correctly installed. Additional features such as the "Pump stand-by" and "Init device" buttons allow the operator to correct an error state.

In addition, in its current state of development, it is possible to adjust light intensity at the imaging station.

Cloud-Based Image Analysis

When using PetriJet platform technology, the obtained images can be processed in several ways, ranging from basic actions such as saving them in cloud-based storage to more advanced applications. An example of a more

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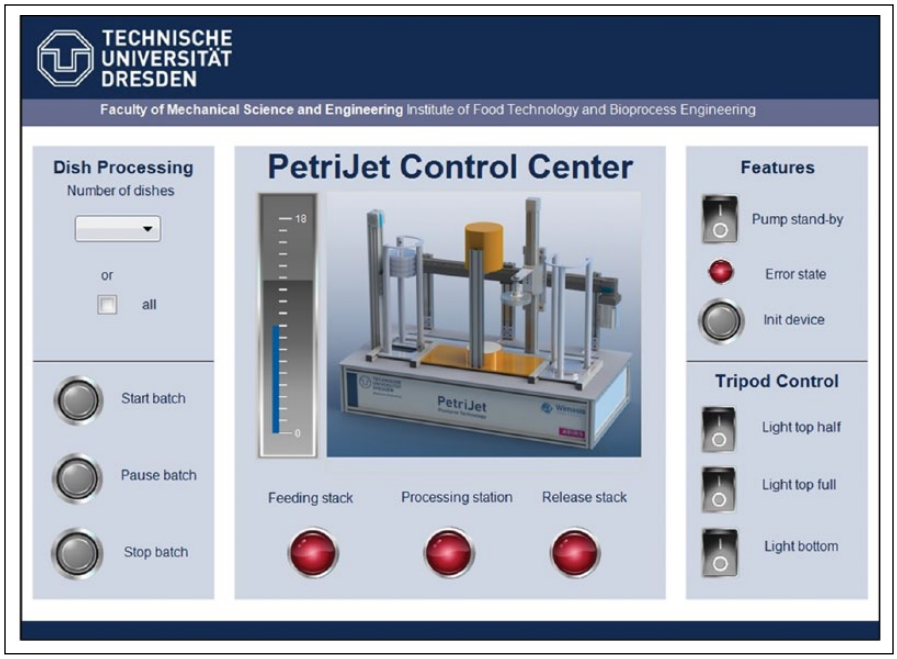

Figure 5. The PetriJet user interface, which can be addressed from any wireless local area network device. The control center is subdivided in the three segments: dish processing, visual supervision, and features/tripod control.

complex application is the hairy root analysis we described earlier. This application might be commercially available when using the Wimasis Image Analysis system (Wimasis Image Analysis GmbH, Munich, Germany).

Roots form highly complex 3D systems within a plant. Root system architecture (RSA) differs widely among different species and is also highly specific for each species.[27–29]

Most current software does not automatically analyze images without human intervention.[30,31] Therefore, we partnered with Wimasis Image Analysis GmbH to develop a cloud-based automatic image analysis solution for evaluating the growth of hairy roots. Hairy root cultures are an innovative biotechnology-based alternative method for producing important nutritionally, physiologically, and pharmaceutically active secondary metabolites.[32]

When an image is obtained (as shown in **Fig. 6**), it is automatically uploaded to the Wimasis Image Analysis system. The software automatically recognizes the orientation of the acquired image and allows the creation of a time series of every culture dish. Therefore, the software is able to recognize subsequent images of one culture dish by the ID on the culture dish. The image analysis procedure can be broken down into several independent steps[22]:

- Image preprocessing
- Creation of a digital image showing the skeleton of the hairy root
- Measuring and implementing the results into a comma-separated value-file (*.csv)
- Creation of a new image file for visual confirmation

Several articles have already been published that prove the functionality of the image analysis algorithm by showing an automated cloud-based root analysis.[22,33]

In addition, the image analysis interface can be commercially adapted for customized applications (e.g., the detection and counting of cell colonies and obtaining measurements from complex patterns).

As previously shown,[22,32,33] the cultivation and analysis of hairy root networks in culture dishes has produced some interesting results. However, other plants and bacteria that can be grown in culture dishes are also suitable targets for analysis with PetriJet platform technology.

To demonstrate applicability of the system, several images of different cultivated organisms were acquired. The original images are shown in **Figure 7**. It can be seen that the images were almost totally free of reflections or other parameters that might influence their analysis. These results indicate that the imaging unit functioned as planned and that only minor improvements are needed.

Laboratory Automation in the Context of Culture Dish Handling

As mentioned previously, the need for an automated system for handling culture dishes is a given fact. To analyze the benefits provided when using the PetriJet platform, we compared the average working time required by a laboratory assistant with the time required when using the PetriJet

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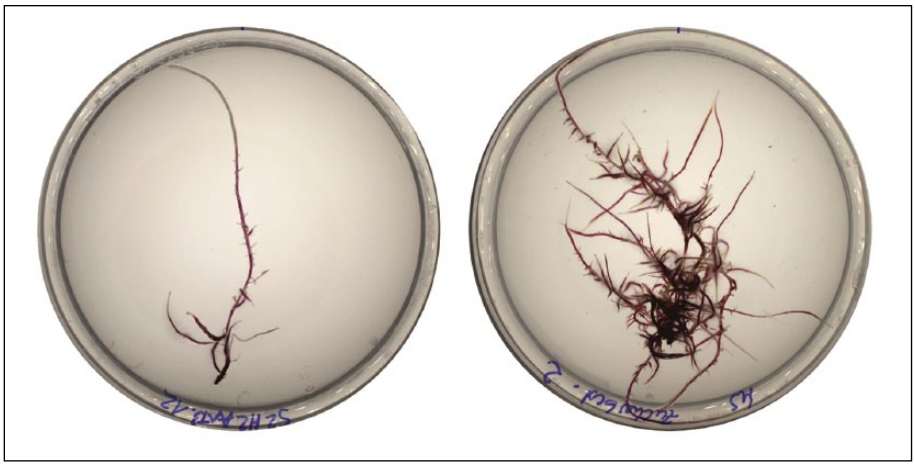

Figure 6. Images of two hairy root cultures. The amount of branching points makes it difficult to analyze the root structure.

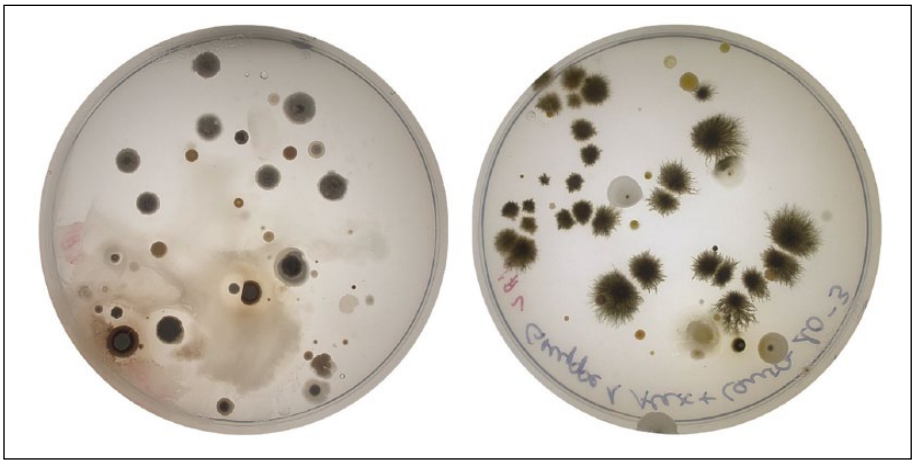

Figure 7. Representative images (bacteria and fungi cultures) acquired with the PetriJet platform to demonstrate utility of the device.

for handling 20 culture dishes. The elapsed times are itemized in **Table 1**.

It was noted that approximately 60 s more preparation time was required when using the PetriJet device. This included time required for initializing the PetriJet and starting the computer, as well as mounting the processing station into the device. Preparation for using PetriJet was defined as walking to the incubator to load culture dishes into the feeding stack, as well as handling the culture dishes manually.

After completing the preparation phase, both operations (PetriJet and manual) were started. We recorded an average handling time of 700 s when using PetriJet to handle 20 culture dishes and 220 s when a laboratory assistant handled the same number of dishes. Thus, the laboratory assistant appeared to be faster.

However, for processing 20 culture dishes, the laboratory assistant has a hands-on device time of 285 s when using the PetriJet versus 425 s when done manually.

Hence, the laboratory assistant gained approximately 140 s of idle working time compared with manual handling. Other tasks can be executed during that additional time. The postprocessing portion of the operation required approximately the same amount of time when using PetriJet or a laboratory assistant.

When analyzing the turnaround time of the device, one might calculate only the number of culture dishes processed per day and the increased idle work time of the laboratory assistants. However, another positive effect is the decrease in incorrect analytical methodology achieved when using the automated PetriJet platform. For example, errors that can be eliminated include those that typically occur during the manual validation of a single culture dish and transfer of visual results to the PC together with the correct culture dish identification number. Another positive aspect of PetriJet platform technology is that it provides instant availability of the acquired images.

In conclusion, the acquisition of automated but still modular laboratory devices is of significant importance to small- and mid-scale laboratories. This is due to the minimal space required for such devices and their large number of applications, ranging from single-vessel handling to advanced image recognition. While attending several

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Table 1. Times Required to Process 20 Culture Dishes: PetriJet vs. Manual Processing.

Parameter	PetriJet Time, s	Manual Processing Time, s
Preparation		
Initializing	30	5
Mounting PetriCam	15	
Walking to incubator	45	45
Filling in-stack with 20 dishes	15	5
Walking to PetriJet	45	45
Mounting in-stack	5	5
Mounting out-stack	5	
Performing controls	10	
	170	105
Processing 20 culture dishes		
Loading one dish	15	3
Imaging	5	5
Unloading one dish	15	3
	700*	220*
Postprocessing		
Dismounting out-stack	5	5
Dismounting in-stack	5	
Walking to incubator	45	45
Unloading out-stack	15	5
Walking to PetriJet	45	45
	115	100
Results		
Total processing time required for 20 culture dishes	985	425
Hands-on time for user processing 20 culture dishes	285	425
Idle hands-on time for user	140	0

*The sums 700 and 220 represent the total time per 20 dishes (one batch).

conferences and symposia, we asked potential customers about their requirements for an automated culture dish handling device and found that most of their concerns centered on frame size and modularity.

Our newly developed device meets all of the above-mentioned requirements: (1) it can be moved by one person; (2) its weight is limited to 30 kg; (3) its dimensions are limited and the hardware and software components are combined, allowing its use under a laminar flow cabinet; (4) its surfaces can be disinfected; and (5) it has a wireless control system.

Furthermore, it can handle both sealed and unsealed culture dishes, with the separation of sealed culture dishes being accomplished via T-Slot Nuts.

A camera system provides the capability for acquiring images of processed culture dishes. The images are automatically uploaded and analyzed by the development partner, Wimasis GmbH. The results are stored as .csv-files and sent to an email account of choice.

In exemplary experiments, we obtained images of several culture dishes and analyzed them for shades and reflections. We found only minor occurrences of such problems, and these drawbacks will be totally eliminated in the near future due to further developments in image acquisition technology.

In the context of the PLC, we were successful in establishing an innovative wiring system via Ethernet TCP/IP when using Codesys V3.5 pbF. Hence, we reduced the cost and effort associated with electromechanical installation and simplified the implementation of smaller frame sizes.

Furthermore, we created a user-friendly interface for controlling the device via any wireless LAN device that works with any HTML-5 web browser.

We are currently field testing the electric parallel gripper, and the results will be published soon. As a next step, we want to use magnets to hold the stacks of plates and processing station on the device when replacing the aluminum profiles on top of the platform. Furthermore, we will enclose the section on the platform where the vertically mounted electrical linear axes go through the platform. We believe these last two enhancements will permit the PetriJet to be used without any possibility of damaging its electrical components (according to the International Protection Marking [IP] 41). To achieve IP standard 53, we are going to enclose the entire platform. We also have other processing stations in various stages of development. For example, stations capable of filling culture dishes with nutrient medium, stations equipped with stereo cameras

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for capturing 3D images of processed culture dishes, and stations capable of transferring materials from one dish to another and evaluating the growth characteristics or the productivity of a culture based on visual inspection are all under development.

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