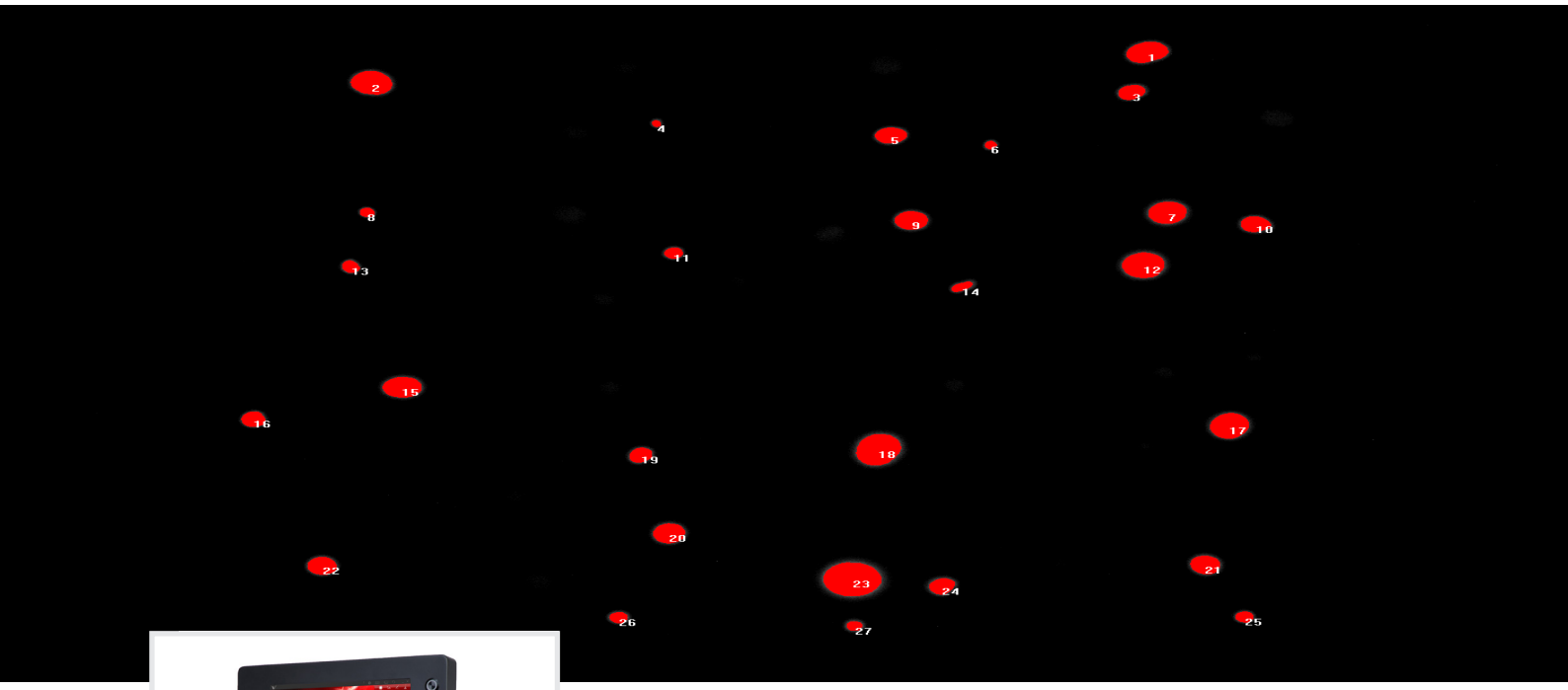

Task

Acquire colony images easily and without any need for training

Solution

Simple user interface and one touch automation enables researchers and students to acquire and analyze colony sample images without any need for training

Simplified Colony Counting

Introduction

Fluorescence imaging is a sensitive and quantitative method that has become integral to life sciences research and clinical applications. One form of fluorescence imaging involves visualization of bacterial colonies grown on agar plates. This technique can be applied to a variety of investigations including screening for gene insertions or mutations, studying protein-protein interactions or verifying fluorescent protein expression, in addition to the detection and evaluation of microbes in soil, water and food[1,2].

With current innovations in colony imaging and analysis technology, using vectors containing fluorescent reporter genes ligated with a gene of interest offers a more convenient and robust method to count and select transformed cells or screen for mutations. Liquid microbe cultures containing these vectors can be diluted and plated on agar plates. After incubating at the appropriate temperature and time depending on the microorganism used, the plated microbes form colonies, each growing from one progenitor cell. Colonies are then counted to determine the number of colony forming units (CFU) and the original concentration calculated. Conventional methods rely on manual counting, typically using grids drawn on an agar plate to ensure colonies are counted correctly. Fluorescence imaging introduces a more accurate and less time-consuming approach to assessing colony growth. Compact, stand-alone imaging systems like the UVP GelSolo provide high contrast image acquisition to ensure simple and effective identification of labeled colonies. The imager streamlines fluorescence imaging with live view capabilities and a touch screen with intuitive VisionWorks image acquisition and analysis software.

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This application note describes the colony counting process with specific emphasis on imaging of plates containing fluorescent colonies. Simulation of fluorescence imaging will be accomplished using a silicone colony plate marked with green and red fluorescent dye acting as fluorescing colonies. The plate is imaged using the UVP GelSolo imaging system to demonstrate the imaging process and how fluorescent colonies appear in a captured image. Colony count analysis is performed using VisionWorks imaging and analysis software. Non-research institutions can use this application note as educational material to supplement STEM curriculum. This same method to create and image a silicone plate can be applied as a training tool for those with access to imaging instruments.

Materials and methods

Silicone Colony Plate and Imaging

The silicone colony plate is made from semi-transparent silicone rubber poured into a standard sized petri dish, typical of agar plates. Red and green fluorescent dyes that signify expressed fluorescence, were dotted on the surface of the solid silicone rubber to demonstrate how red and green fluorescent colonies would appear. The silicone plate was imaged using the UVP GelSolo imaging system equipped with a 5.0-megapixel camera and 8-48mm f/1.2 manual zoom lens. To detect the wavelengths of red fluorescent and green fluorescent colonies, 575-640nm and 513-557nm emission filters were placed in the filter slot located on top of the system. Table 1 details the image capture data for each color dye.

	Exposure Time	Filter
Red Fluorescent (GelRed)	258ms	575nm-640nm
Green Fluorescent (GelGreen)	170ms	513nm-557nm

Table 1. Capture Parameters

Discussion

Utilizing an imager with single, dual or triple wavelength detection can expand imaging to multiple fluorescent markers using associated wavelengths. As shown in Figure 1A-B, the UVP GelSolo distinctively captured both green and red fluorescent markers for larger colonies and those that might not be caught by the human eye. The composite image in Figure 1C allows further comparison of the two fluorescent markers. By translating this process to a research environment, one can better understand how fluorescence imaging is applied to and simplifies studies that involve colony counting.

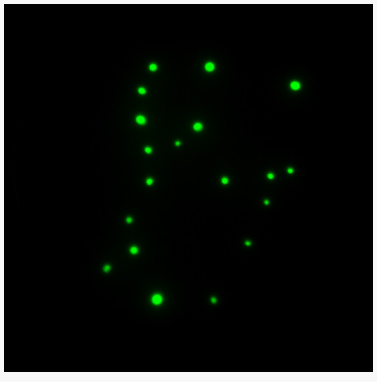

Figure 1. a) Green fluorescent colonies

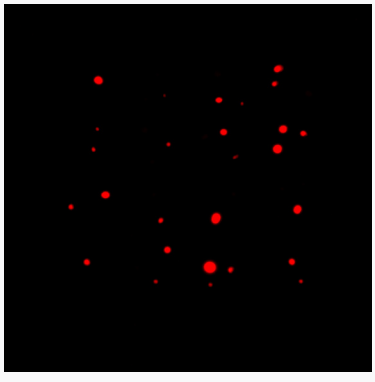

Figure 1. b) Red fluorescent colonies

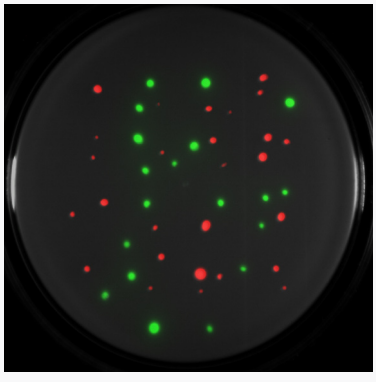

Figure 1. c) Composited green and red fluorescent colonies on a white light image

These results confirm the ability of imaging systems like the UVP GelSolo to precisely and accurately detect various colony sizes, increase visibility to all colonies and provide highly sensitive image acquisition and analysis. After imaging, automated colony count analysis was performed on the green and red fluorescent colony images using VisionWorks software. According to the results displayed in Figure 2A-B, the green fluorescent plate contains a total of 19 colonies and the red fluorescent plate contains a total of 27 colonies. VisionWorks imaging and analysis software provides a more intuitive and accurate colony counting approach compared to manual conventional methods. For more in-depth analysis, colonies can be filtered by circularity and total pixel size after automatic colony counting. Researchers can also add, delete, split or merge colonies using editing tools included in the software.

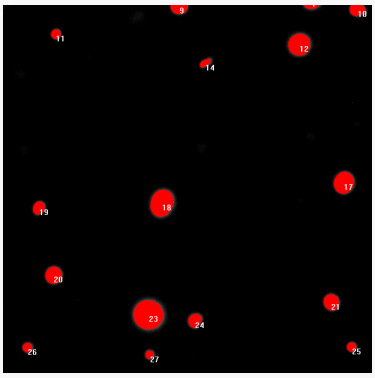

Figure 1. a) Green fluorescent colony count analysis

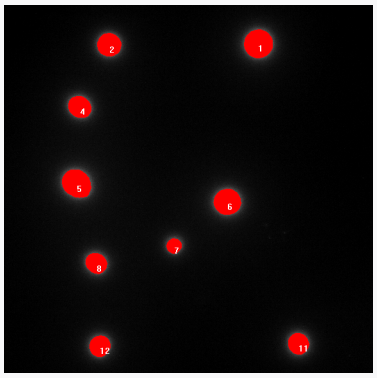

Figure 1. b) Red fluorescent colony count analysis

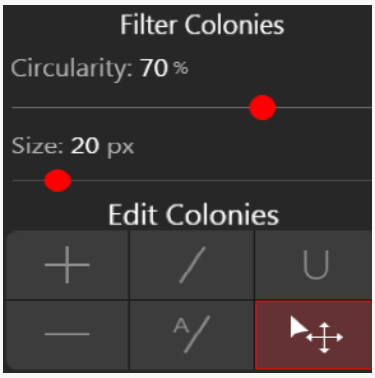

Figure 1. c) VisionWorks colony count editing and filter options

Conclusion

As an easy-to-use system for colony documentation, the UVP GelSolo is designed for imaging and analysis without the need for any additional training. The user-friendly automation and straightforward features make the UVP GelSolo an ideal entry level imager for school laboratories, multi-user laboratories and practical trainings.

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2.Mahboob Nemati, Aliasghar Hamidi, Solmaz Maleki Dizaj, Vahid Javaherzadeh, and Farzaneh Lotfipour. An overview on novel microbial determination methods in pharmaceutical and food quality control. Adv Pharm Bull. 2016 Sep; 6(3): 301–308.

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