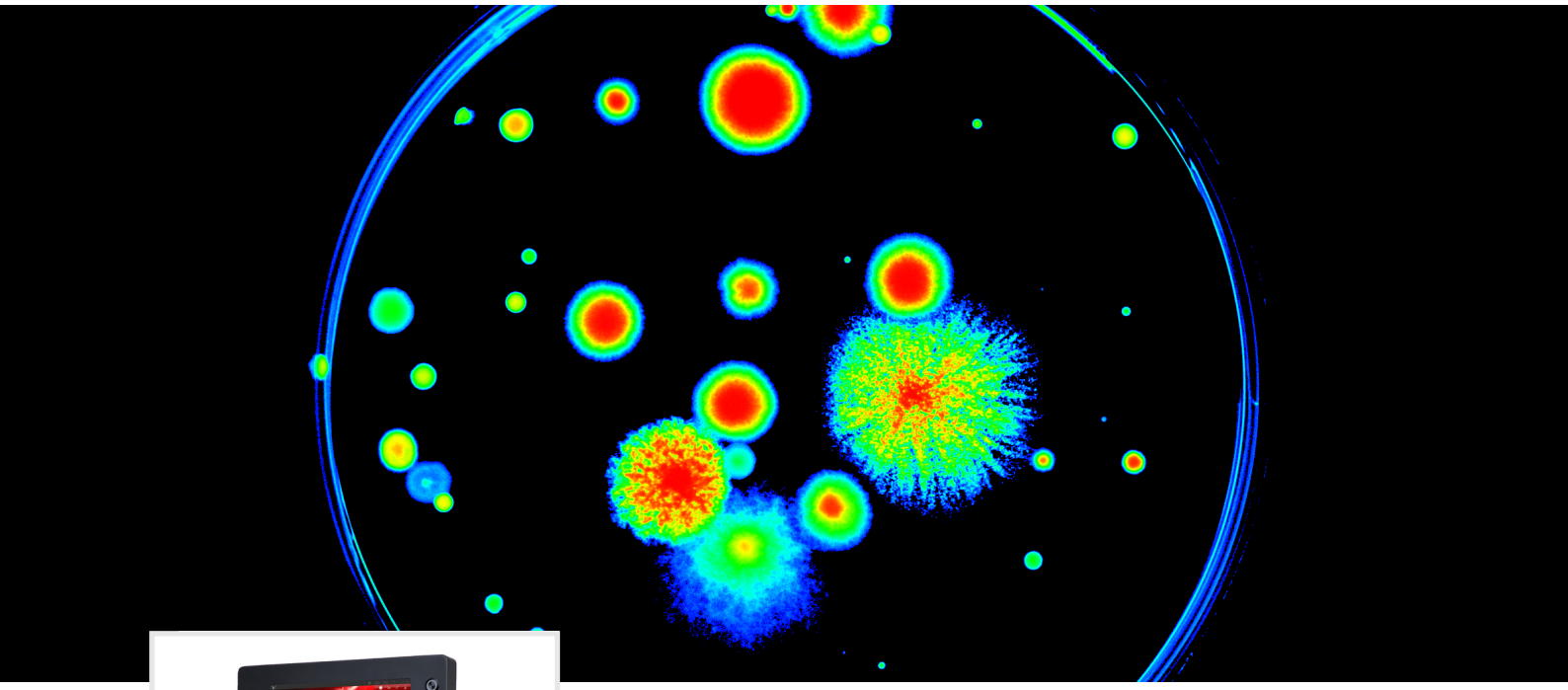

Task

Precision colony counting

Solution

Analytik Jena's UVP GelSolo with a high resolution 5MP camera, one touch automation, and artificial intelligence driven colony counting software

Theory and Practice: Counting *Escherichia coli* colonies on Analytik Jena's UVP GelSolo

Introduction

In 1887, Richard Julius Petri published his rather dull prescription for cultivating microorganisms—homogenize a microbial laden sample in a molten gelatin medium, distribute into a round glass dish, and cover with a slightly larger glass dish. The petri dish was born. About 130 years later, and the petri dish is arguably the most popular method to cultivate and quantify bacteria. Plastic may have replaced glass, but Petri's publication can still serve as a guide to researchers new to cultivating microbes, thus cementing his discovery as one of the most important contributions in the annals of microbiology.

Microbiology has experienced a renaissance several times over since Petri, but go into any laboratory today, and you are bound to find someone who in recent times has hunched over a bench with marker in hand and painstakingly counted colonies—no different than Petri reported in his 1887 publication. At Analytik Jena, we want our customers spending less time with mundane tasks like colony counting, and more time making discoveries. This is why we have embedded robust colony counting software into the UVP GelSolo.

The UVP GelSolo is an entry-level imaging system well-suited for any laboratory, and provides exceptional documentation of nucleic acid and protein gels, colony counting, and beyond (contact our Applications Scientists for additional applications). Equipped with a 5 megapixel CMOS camera, the UVP GelSolo can capture the finest details in your samples. Below we provide a brief overview of bacteria enumeration using our colony counting software to ensure you get precise counts for your research needs.

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Theory

Sampling

The plate count method is the best approximation of number of cells present in a sample. It is why it is routinely carried out by labs interested in colony forming units/mL (or CFUs/mL) with samples from an infected animal, soil, fomites—the list goes on. When harvesting samples for bacterial counts, it is best practice to harvest the sample, and store on ice until the samples are diluted and plated. This will ensure that bacteria do not continue dividing, which would result in misleading counts.

Serial Dilutions

When carrying out serial dilutions of the samples, use fresh pre-chilled media (nutrient limiting if possible), and place samples back on ice until you are ready for plating. There is no proper serial dilution range since samples can vary, so this is often determined empirically. Alternatively, researchers can use optical density, which is determined by using a spectrophotometer, or comparing the turbidity of their samples to McFarland Standards, to guide their plating dilutions. Nonetheless, a good place to start is a 1/10 dilution series carried out to 10^6. If you suspect your samples are very concentrated, you can carry dilutions out further. Once you have an idea what range your samples typically fall in, you can select a subset of the dilution series to plate—saving you time and money.

Plating, Incubation and Counting

There are several plating techniques, but here we discuss spread plating. Spread plating is more of an art than a science. Researchers need a plate spinner, a spreader, an agar plate, and a Bunsen burner. With a flame on, an aliquot of a dilution is dispensed onto the center of the agar plate. With a sterile spreader, the researcher gently spreads the aliquot on the plate while simultaneously rotating the plate spinner, taking care to avoid gouging the agar and spreading the aliquot to the sides of the plate—both which make counting colonies manually or automatically difficult, if not impossible. Spreading is continued until there is no visible moisture left on the surface of the agar plate which helps keep colonies isolated for straightforward counting. Each sample should be plated in technical triplicates in order to account for any variation in pipetting and plating technique. Depending on the type of the bacteria, samples are incubated for some length of time until visible colonies are present on the plate. For statistical and regulatory purposes, plates with colonies ranging between 25-250 are considered within countable range. To determine the amount of bacteria in your original samples, simply multiply the dilution factor, by the volume plated in milliliters, and then divide your CFU count by this value (Equation 1).

Equation 1: Colony forming units / [Dilution Factor x Volume Plated (mL)]

Practice

Culturing and Plating Bacteria

Escherichia coli was grown overnight at 37°C in a 5mL culture of LB under ampicillin selection on a rotary incubator. Samples were diluted 1/10 out to 10^7. 100µl of each dilution was plated. Plates were incubated at 37°C overnight.

Imaging and Enumerating Bacteria

The 10^4 dilution plate was used for enumeration. The plate was placed inside the GelSolo with the lid off and plate facing upward. A UV transilluminator with the white-light converter plate was used to illuminate the plate. A 20 ms exposure time used. After image capture, the self-guided colony count analysis was performed. The software determined that there were 127 colonies (Figure 1A). Upon closer inspection we noticed there were two aberrations in the agar plate that were being picked up by the software—this was due to gouging during plating which can cause issue as mentioned above. Using the Filter Colonies option from the Edit Menu we were able to remove the aberration from the analysis since they did not fit the size and shape of the colonies on the plate (Figure 1B, 1E, 1F). In addition, we noticed that some colonies were too close together and were picked up as one colony (Figure 1C). Using the Edit Colonies feature from the Edit Menu (Figure 1E, 1G), the researcher can use a manual or auto-split feature to make the count more accurate (Figure C, D, before and after). The final

count was 128 colony forming units. Using equation 1, we determined there were 12.8 million bacteria/mL in our sample. With precision and throughput top of mind during the design of the colony counting software and all of Analytik Jena's imaging products, we hope this feature helps you accelerate discovery.

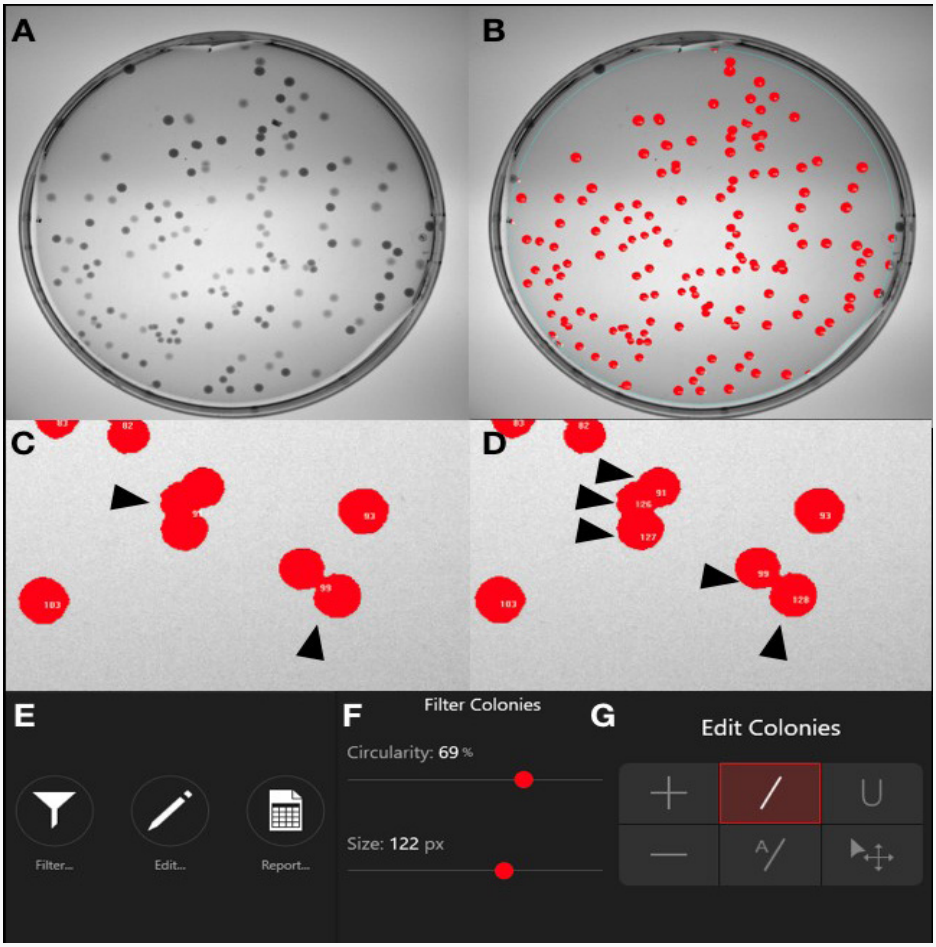

Figure 1: Colony counting using the UVP Gel Solo. Colonies are detected using a self-guided algorithm. Users can make post-detection modifications to improve analysis using the simple user interface such as manual and auto colony splitting.

Expert Tips

- Prior to plating, desiccate agar plates for 30 minutes with lid off in an incubator. Inoculum will dry quicker, speeding up the plating process.
- When available, use blood agar to improve the contrast of colonies on the agar.
- Avoid using large print on the bottom of the agar plate to prevent errors in colony detection.
- When spreading, avoid making contact with the edge of the plate. This makes colony counting more accurate as colonies will not be obfuscated by the plate walls.

References

1. Petri, RJ (1887) Eine kleine modification des Koch'schen plattenverfahrens. Untersuchungsmethoden, Istrumente etc. pp 279-281.

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