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STUDY OF BACTERIAL COLONIES USING STEREOMICROSCOPE

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ABSTRACT
Introduction: Sometimes bacterial colonies may be missed by naked eye. Dissecting microscope helps view them better. It is an old but very simple instrument. It helps in 3-dimensional view of colonies. So, the routine usage of dissecting microscope seems justified. In this article we report use of dissecting microscope to view colonies, their morphotypes and also small colonies within zones of inhibition. *Materials and methods:* We used simple dissecting microscope to see the colonies, their shape, size, surface, margin and other parameters. Results: Different bacteria showed different types of colonies that were well appreciated by dissecting microscope. *Conclusion:* Dissecting microscope should be routinely used in routine microbiology laboratory so that colonies are not missed and identification and noting of antibiotic susceptibility is not hampered.

Keywords: *Dissecting microscope, stereomicroscope, colonies*

INTRODUCTION
Stereomicroscopes are also known as dissecting microscopes and are routinely used to study colony morphology and helps view colonies in 3 dimensions (1). They provide low magnification (typically 10x to 100x) with a large field of view and stereoscopic 3D imaging, ideal for observing opaque, three-dimensional specimens like bacterial colonies on agar plates (2).

This article explores the principles, techniques, applications, and advancements in using stereomicroscopes to study bacterial colony morphology, growth dynamics, and phenotypic traits, drawing from microbiological research and practical methodologies.

Principles of Stereomicroscopy for Colonies: Stereomicroscopes use two separate optical paths to deliver a true 3D image, allowing depth perception, with a working distance of several centimeters allowing observation of intact agar plates without coverslips (3).

Light paths converge at low angles, providing greater depth of field than compound microscopes, which is crucial for viewing colony elevations, textures, and edges—features often lost in high-magnification 2D imaging.

Episcopic (reflected) or transmitted illumination highlights surface irregularities, while darkfield or polarized light enhances contrast for translucent colonies (4).

Bacterial colonies, aggregates of millions of cells (diameters 0.5–10 mm), exhibit macroscopic traits like form (circular, irregular), elevation (flat, raised), margins (entire, undulate), and texture (smooth, rough), are all discernible under stereomicroscopy.

For instance, *Staphylococcus aureus* forms dome-shaped, golden colonies with steep slopes, while rod-shaped Escherichia coli yields flatter, irregular forms.

Historical Development: Early applications date to 1955, when Clifton developed a stereoscopic method for accurate colony counting on crowded plates, reducing errors from overlapping by exploiting 3D depth perception (5).

This predated automated counters, emphasizing manual stereoviewing for titers in bacteriology. By the 2010s, integration with digital imaging advanced the field. Arduino-based time-lapse stereomicroscopy

captured *Bacillus subtilis* biofilm structure and other details, revealing spatiotemporal dynamics at air-solid interfaces.

Instrumentation and Setup: A basic stereomicroscope features binocular eyepieces (10x–40x), zoom turret (0.67x–4.5x), and ring or fiber-optic illuminators. Modern units like Olympus SZX or Nikon SMZ add digital cameras for macro imaging.

For colonies, we place agar plates on a black/white stage, and then use oblique top lighting to minimize glare on reflective Petri dishes. Key accessories include mechanical stage for scanning plates, polarizers for birefringence in biofilms and confocal add-ons for 3D morphology maps.

Inexpensive DIY imaging boxes diffuse light, eliminating reflections for high-quality photos at ~$33, as validated against scanners and gel stations. Observing Colony Morphology provides phenotypic clues for identification before molecular tests. Under stereomicroscopy: Size and Form: Measure diameters (e.g., *Pseudomonas aeruginosa* proteolysis zones). Elevation and Profile: 3D views reveal aspect ratios (height/diameter); S. aureus averages 0.185 vs. 0.13 for *Salmonella* Typhimurium.

Margins and Texture: Undulate edges indicate motility; rough textures signal exopolysaccharides.

Color/pigment: Pigments (e.g., pyocyanin of *P. aeruginosa*) and translucency of bacterial colonies can be assessed live by dissecting microscope.

Sometimes manual picking of the bacterial colonies for subculturing is preferable. For this, a dissecting microscope placed within a microbiological safety cabinet is ideal (6).

Multi-channel stereosystems combine brightfield, 3D confocal topography, and optical density (OD) maps; *S. aureus* shows steeper profiles and higher OD due to spherical cells.

Techniques for Detailed Analysis and Colony Counting: Stereoviewing resolves overlaps; so we should scan at 20x for 100–500 colonies/plate.

Time-Lapse Imaging: Arduino-controlled stereomicroscopy tracks growth every 20 min, quantifying expansion rates (e.g., B. subtilis biofilms).

Elastic Light Scattering (ELS): Laser interrogation via stereomicroscope yields forward-scatter patterns correlating to morphology; steeper profiles produce wider patterns. Environmental Effects: Cold stress alters OD; E. coli smoothens, increasing transparency and ring-dominant scatters. Fluorescence: GFP-labeled strains reveal subpopulations in 3D. For quantification, software like ImageJ analyzes profiles from stereophotos.

Applications in Microbiology, phenotyping and others:

In teaching, stereomicroscopy is able to distinguish bacterial colonies like *S. aureus* (yellow, opaque, low convex, domed colonies) from contaminants and also helps see the colony edges better (7). Research screens transposon libraries; e.g., *P. aeruginosa* NAG utilization mutants identified via morphology.

Biofilm Studies: 3D mapping reveals spatial OD gradients, linking to virulence.

Antimicrobial Susceptibility: Zones of inhibition can be measured precisely; stereoviewing detects subtle halos and also heaping at margin of zones of inhibition.

Quality Control: Food/pathology labs inspect *Listeria* colonies on agar. High-Throughput: ePetri on-chip stereo counts microcolonies (20 μm) in 6h. In India, relevant for TB/leprosy diagnostics via colony traits on Lowenstein-Jensen media. Advantages Over Other Methods Versus compound microscopes: Larger depth of field, no thin-section prep, native 3D. Versus scanners: Top-view captures surface texture; no agar distortion. Versus macro cameras: Glare-free, magnified detail.

Limitations: Lower resolution for cells (<10 μm); not for intracellular views. Recent Advances (2021–2026) Multi-Modal Instruments: 2021 Purdue system integrates stereo with confocal/ELS for 5 channels: morphology, OD, scatter—improves genus-level ID (e.g., E. coli vs. S. aureus). DIY High-Res Imaging: 2021 Frontiers box for phenotypic diversity (*P. aeruginosa* proteolysis). AI-Enhanced: IEEE stereo-AI for explainable leprosy diagnosis; 2025 3D MSI for biofilms. Portable Systems: Wide-FOV on-chip for real-time microcolonies.

Future directions: Integration with hyperspectral imaging can also be used for metabolic profiling.

(a) Practical Protocols: Streak/isolate on agar; incubate 24–48h at 37°C.

(b) Position plate under stereo (10–40x);
(c) Adjust oblique light.
(d)Record traits: Use grid for counts, calipers for size. Image: DSLR/ phone through eyepiece or trinocular. Analyze: ImageJ for profiles; correlate to scatter if equipped.
Safety: Biosafety level 2 for pathogens; UV for sterilization.
Challenges and Future Directions Challenges: Glare on plastic plates (mitigated by diffusers), subjectivity in morphology (AI aids objectivity).
Future: Portable stereo-AI for field diagnostics (e.g., Kolkata labs for leprosy); VR 3D colony modeling; CRISPR screens via automated stereo-phenotyping. In conclusion, stereomicroscopy remains indispensable for holistic colony studies, bridging macro-phenotypes to micro-mechanisms in an era of genomics.
Keeping all these things in mind, we planned use of dissecting microscope for observation of colony size, surface, edge and margin.

MATERIALS AND METHODS

Type:- Observational study
Time;- August 2025 to March 2026
Place of study:- Departmental laboratory.
Sample size:- We studied the colony morphology of a total of 50 isolates by dissecting microscope.
Methodology:- The plates were placed under dissecting microscope and colonies observed directly. Images were also clicked with the help of smartphones.

RESULTS

Routine specimens like urine, sputum and others were inoculated on microbiological culture media and incubated aerobically. Next day, colonies were observed. The shape, size and margins of the colonies were better appreciated and observed by using the dissecting microscope. It was also useful for the observation of small colonies seen within the zone of inhibition in disk diffusion test.
Enterococcus spp.:- mucoid, small, glossy, well defined colonies
Klebsiella spp.:- dome-shaped mucoid colonies.
Staphylococcus aureus:- small, low convex, opaque colonies.
Escherichia coli:- mucoid, translucent colonies with good differentiation of morphotypes.
Pseudomonas aeruginosa:- mucoid, translucent, shiny, well defined colonies.
Lactobacillus spp.:- small, white, pearly, shiny mucoid colonies.
Rhodotorula spp (basidiomycetous yeast).:- pink and mucoid colonies
The figures below show the images of colonies observed after use of dissecting microscope.

Figure 1: Translucent, well defined colonies of *Pseudomonas aeruginosa*

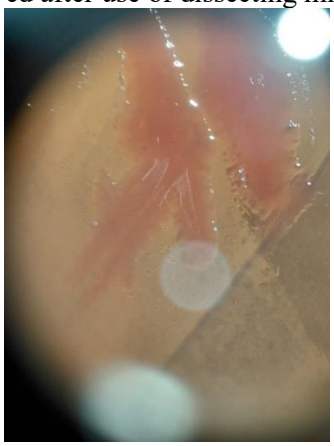

Figure 2: Translucent, shiny, well defined colonies of *Pseudomonas aeruginosa*

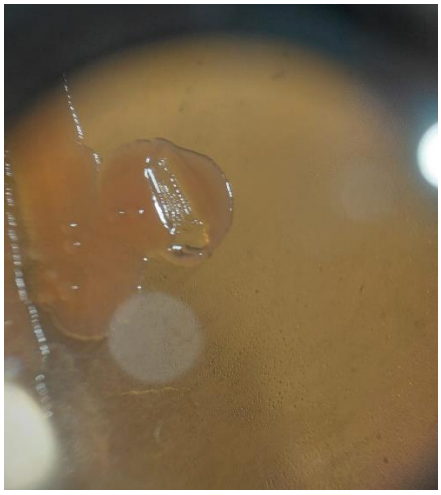

Figure 3: LF colony of *Escherichia coli* under dissecting microscope

Figure 4: Corrugated, rugose colonies of *Bacillus* spp.

Figure 5: Colony morphogenesis of LF *E. coli* on MacConkey agar

Figure 6: A simple Dissecting microscope (Image:- author)

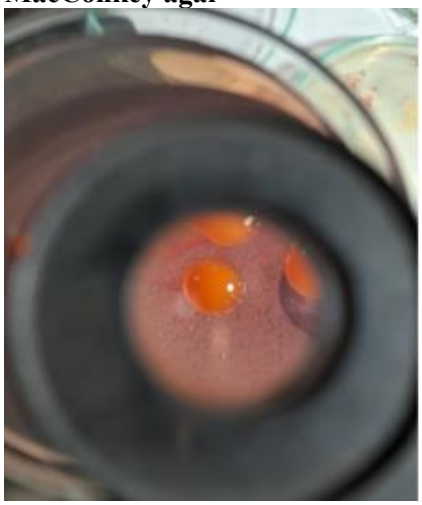

Figure 7: Mucoid, dome-shaped colonies of *Klebsiella* spp. as seen under dissecting mnicroscope

The heaping of colonies at margin of zones of inhibition were also noted well by dissecting microscope. So it helped appreciate and demarcate the zones of inhibition also very well.

CONCLUSION

Use of dissecting microscope facilitates better study of the colony morphology of bacteria and thus helps in more accurate identification. Also, the existence of 2 or 3 morphotypes of colonies is better demonstrated by dissecting microscope. Colonies will not be missed when dissecting microscope is used. So the routine use of dissecting microscope may open up a new vista of diagnostic microbiology.

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