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Investigating Bio membrane Research's Structure, Composition, and New Analytical Methods

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ABSTRACT

Complex structures composed of adherent microbial populations contained in an extracellular polymeric substance (EPS) matrix are known as biofilms. These structures are ubiquitous in nature and are essential for the survival of many different kinds of microbes. Several steps are involved in the life cycle of biofilms: first, surface contact; second, persistent attachment; third, formation of tiny colonies; fourth, development to full maturity; and lastly, dispersion. Biofilm development is impacted by a multitude of elements, including hydrodynamics, substratum impacts, and environmental conditions. The growing resistance to antimicrobial treatments and contamination potential of biofilms make them a major problem in healthcare, food processing, and other sectors. The study of biofilms has been radically altered by the advent of new imaging tools, which have shed light on their physiology, structure, and composition. Some of the most popular methods for characterizing biofilms include light microscopy, atomic force microscopy (AFM), confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Biofilm formation, chemical composition, and gene expression can be explored using a variety of advanced techniques, including AFM-IR, FISH, AFM-based Raman spectroscopy, ATP bioluminescence, mass spectrometry, quantitative real-time PCR analysis, and Bio Finder. Research objectives and the level of geographical and temporal precision needed will determine the best approach. In order to create efficient techniques for controlling and eradicating biofilms, it is necessary to combine multiple methodologies in order to give a full knowledge of biofilm behavior.

Keywords: Advanced Analytical Techniques; Atomic Force Microscopy; Biofilms; Confocal Laser Scanning Microscopy; Extracellular Polymeric Substance (EPS); Imaging Techniques.

INTRODUCTION

Bacteria and other microbes that live in communities and reproduce create biofilms. The social structure of these biological systems provides safety and fosters development. To this day, scientists have not solved the mystery of this structure. 1. A biofilm is a "slimy adhesive" consisting of a very thin coating of microscopic organisms that adhere to a surface. This biofilm has a dual effect: it slows down bacterial development and speeds it up. Bacteria attach to surfaces and secrete structures composed of extracellular polymeric substances (EPS), resulting in its formation. 2 Dental plaque, water systems, air conditioners, food processing plants, and different healthcare surfaces are just a few examples of the many places you could find microbial colonies called biofilms. In biofilms, microbes may live in harmony, get food, and multiply. Bacteria inside biofilms have a resistance to antibiotics and cleaning chemicals that is about 200 times higher than the general population. They will keep endangering patients so long as they are alive. germs will continue to proliferate until something breaks the biofilm or the germs are discharged into the air, where they might possibly infect other surfaces via contact with unclean hands, gloves, or cleaning cloths. Bacillus, Staphylococcus, Shigella, Escherichia coli, and Enterobacter aerogenes are only a few genera of pathogenic bacteria. The capacity of these microbes to cause disease is what qualifies them as pathogenic. Their accumulation on hard surfaces gives rise to a biofilm, a thin, sticky covering. The development and spread of germs are facilitated by this protective layer. 3 Isolated, suspended cells do not constitute the majority of bacterial cells in nature. On the contrary, they are surface-attached groups of bacterial

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cells known as aggregates. Biofilms are collections of microorganisms that have attached themselves to a surface. In order to connect to solid surfaces, microorganisms produce an extracellular polysaccharide matrix. 4 As a vital survival strategy, this biofilm offers the bacteria substantial protection. In its barrier-forming role, the biofilm protects microbes from environmental hazards such as antibiotics and sanitizers. Bacteria, both benign and malicious, are capable of forming biofilms. Because biofilms provide dangerous bacteria a better chance of survival and raise contamination concerns, their formation in processing settings is a major cause for worry. As a result, getting rid of biofilms and preventing their growth in facilities is crucial. 5
Formation of Biofilms6

As shown in Figure 1 below, the usual manufacturing method for biofilms consists of five steps:
There is a multi-step process to biofilm formation:
First, there's primary adhesion, which starts when germs that are free to swim connect to something.
Second, the biofilm forms bonds that will last forever.

the cells' ability to adhere to surfaces and one another is enhanced by the extracellular polymeric substance (EPS) matrix that is generated during cell division. The EPS makes the bacterial cell-substrate bond stronger. As time passes, these relationships strengthen, and eventually, the attachment is permanent. 3. Development: Over time, the biofilm changes into a more organized and tough structure that can withstand more damaging chemicals and biocides.
3. Bacteria colonize preconditioned surfaces, forming microcolonies. After this still-reversible phase, the biofilm may enter its last, permanent stage of development, which is followed by its real establishment.
4. Maturation and dispersal: As a biofilm develops, its cells multiply, their density rises, and they begin to feel and interact with one another via the synthesis and release of signaling chemicals.
Phase 5, sometimes called the dispersal or detachment phase, occurs when a considerable number of cells leave the cell body and return to the planktonic state. The environment is contaminated with bacterial cells that have been unleashed.
Because of their importance in analyzing and comprehending complicated things and processes, imaging techniques serve a pivotal role in several scientific fields, including biology and medicine. The use of these methods enables the non-invasive, multi-scale, three-dimensional examination of specimens. 8 Macromolecules are studied at nanometer scales, cellular structures, microbial clusters, and biofilms at micrometer scales, and microbial mats, tissues, organs, and the human body at centimeter scales. Magnifying glasses and primitive optical microscopes were the first instruments to go into this tiny world. In 1665, Robert Hooke used the first light microscope. Antonie van Leeuwenhoek, who became a pioneer in microbiology by studying oral biofilms, examined samples from his mouth soon after. The principal techniques for investigating and viewing biological materials for more than a century have been versions of conventional light microscopy. Light microscopy was thought to have hit a plateau in its evolution at one time. 9
A plethora of state-of-the-art approaches have been developed or refined in recent times.

with the purpose of studying them in order to learn more about their composition, structural aspects, and physiological traits. Biofilm viability, structure, composition, and physiological characteristics, as well as their biomass, are examined in this article using both classic and cutting-edge methodologies. 10 Here are some tactics:
Detailed examinations under the microscope Optical microscope
An essential imaging tool for visually detecting biofilms and providing helpful predictive data is light microscopy (LM). It is an appropriate method for estimating biofilm biomass due to its ease of use and low cost. Unfortunately,

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LM can't capture the shape of biofilm cells or the finer features of extracellular polymeric substance (EPS) structure due to its magnification and resolution limitations. With these caveats in mind, it is possible to conduct correlative research using LM in conjunction with SEM and TEM. 11
A TEM, or transmission electron microscope,
The utilization of electrons allows for the creation of very enlarged and intricate pictures of cells and their constituents, such as nucleic acids and proteins. Transmission electron microscopy (TEM) uses negative staining methods to directly see cellular components. Due to the restricted penetration of photons and electrons into cells, agents such osmic acid, permanganate, uranium, lanthanum, or lead salts are used to stabilize and stain tiny portions of cellular material. The large atomic weight of the elements used to make these staining compounds increases electron scattering from the material, which in turn increases contrast. 12 Advanced imaging technology using a scanning electron microscope
Scientists use precious metals like gold to make three-dimensional models of cell samples. These samples are coated with metal, and scanning electron microscopy (SEM) uses electrons released by the materials to create pictures. Contrary to TEM, which requires infiltration, resin embedding, polymerization, and staining with lead citrate and uranyl acetate, SEM does not need any of these steps. 13 Nevertheless, SEM necessitates the incorporation of supplementary materials, such as gold. SEM gathers data regarding surface topography by monitoring changes in energy signals, which are caused by accelerated electrons acting as a light source.

Secondary electrons provide the most useful signal for scanning electron microscopy (SEM), allowing for the visualization of very small objects with a resolution of 0.5 nm. The reason for the impressive amount of detail is that electrons have wavelengths that are up to 100,000 times shorter than visible light photons. 14 Although scanning electron microscopy (SEM) pictures do not have very high vertical resolution, their large depth of field makes them seem quite three-dimensional. The remarkable spatial order inside biofilms may be clearly seen using SEM's great depth of focus, making it an ideal tool for studying biofilm structures.

Confocal laser scanning microscopy (CLSM)

Researchers may examine biofilms that develop on the see-through surfaces of flow cells using confocal laser scanning microscopy (CLSM), a cutting-edge imaging technology. It makes it easier to examine biofilms in three dimensions (3D) in terms of their anatomy and physiology. Biofilms and other thick samples, as well as the microorganisms entrenched deep inside them, are excellent examples of situations where CLSM shines. This technique has great promise for real-time imaging of completely hydrated, live specimens and is essential for studying biofilm architecture. 15 Optical microscopy's spatial resolution is enhanced using a fluorescent technique, allowing for imaging capabilities at the nanoscale. Aside from quantitative information, this "super- resolution optical microscopy" also gives qualitative details on biofilms. As biofilms grow and create high-diffusion zones in real time, CLSM provides a unique way to assess biofilm growth rates and cell behaviors including attachment, separation, and re-attachment. 16

Atomic force microscopy (AFM)

Atomic Force Microscopy has unique characteristics that make it an effective tool for obtaining authentic 3-D surface topography. This method presents specific challenges and techniques associated with imaging biofilm formation. Researchers have successfully gathered high- resolution qualitative and quantitative data on the structure of biofilms.[17] Unlike Scanning Electron Microscopy (SEM), AFM enables the evaluation of biomass height and surface corrosion. In recent years, AFM has rapidly gained popularity for studying biofilms on various substrate surfaces and bacterial species, as evidenced by numerous examples in review articles. Its high sensitivity and resolution in topography analysis have been extensively used to examine individual cells and biofilms on surfaces that come into contact with food.[18]

AFM-based infrared spectroscopy (AFM-IR)

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Infrared spectroscopy is often used to measure molecular species in both laboratory and industrial settings. By using this method, data on organic molecules inside biofilms may be collected while the materials are still in their natural forms, such as solids or liquids. In order to better understand how bacteria behave during biofilm development, the AFM-infrared (AFM-IR) technique may be used in conjunction with atomic force microscopy (AFM) to examine the molecular makeup of specific bacterial cells. 19
By interacting the tip of an AFM with pulsed infrared light, AFM-IR is able to determine the atomic-scale thermal expansion of the material. An infrared-transmissive zinc selenide (ZnSe) prism is used to mount the sample, and an internal reflection laser is used to illuminate it. The cantilever oscillates because the sample expands thermally in response to light absorption at certain wavelengths. In order to extract the infrared spectrum, researchers analyze the cantilever's reaction and apply a fast Fourier transform (FFT) to the original time-domain oscillation signal. 20
Using fluorescent in situ hybridization (FISH)
One method using fluorescent in situ hybridization (FISH) in the lab involves combining histology, fluorescence microscopy, and molecular biology to investigate microscopic cellular features. You can see the microbes in the tissue samples and find out how many of them there are, where they're located, and what their metabolic status is. This technique allows for the identification of fungus and bacteria down to the species level by use of fluorescently tagged probes that bind to microbial ribosomal RNA (rRNA). One example is the ability to tailor probes to detect specific germs, such as Staphylococcus aureus, or to detect all microbes. Fluorescently tagged bacteria in biofilms may also be studied using FISH. 21 The probes are prepared to hybridize with the 16S rRNA of microbes by being linked with enzymes like horseradish peroxidase or fluorescent dyes like FITC or Rhodamine. The fact that horseradish peroxidase-conjugated probes are not toxic to bacteria found in biofilms is a benefit of their use. It is possible to determine the growth rates of bacteria inside biofilms using FISH since there is a correlation between the ribosome count and growth activity. It is necessary to create probes in order to identify conserved areas of certain species. 22
To find out what kinds of microbes are active, FISH looks at the ribosome content of certain cells. When biofilms are effectively treated with antimicrobials, the fluorescence signals are lower than when the biofilms are active. Digital image analysis quantifies the efficacy of antimicrobial drugs by observing the reduction in the FISH-positive percentage compared to the overall biofilm mass. Biofilms, antimicrobial susceptibility, and other such topics are well-suited to FISH.

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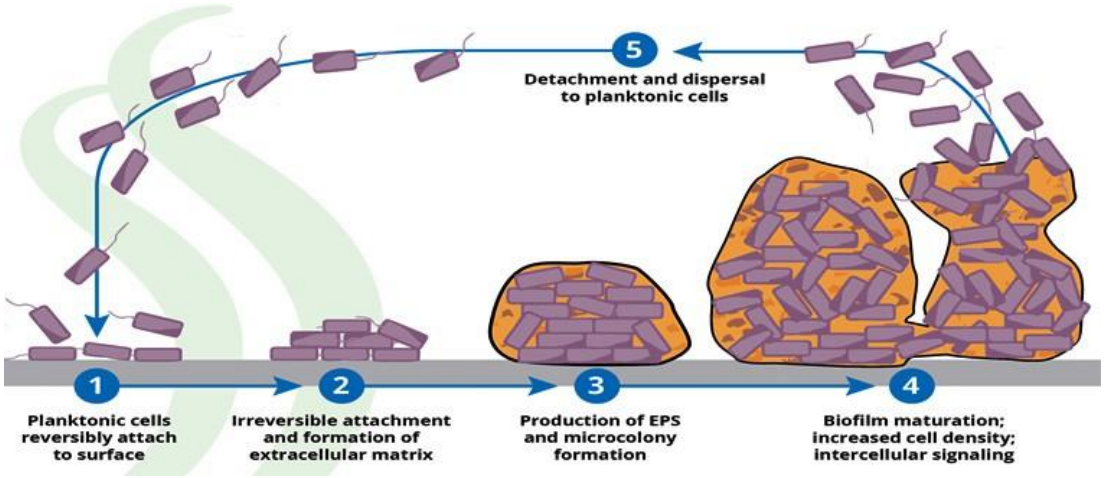

Fig. 1. Representation of biofilm formation process in five steps[7]

AFM-based Raman spectroscopy

Raman spectroscopy is an additional form of label-free spectroscopic analysis. In fact, Raman and IR spectroscopy complement each other in chemical analysis, both utilizing different approaches to examine molecular vibrations. This allows Raman spectroscopy to overcome certain difficulties associated with IR spectrum identification. Raman spectroscopy is a powerful tool for examining biological systems, showcasing its versatility in analyzing various states of matter, including solids, liquids, gels, and various mixtures in different environments, such as aqueous settings. This analytical method can be used without causing harm or making changes to the specimen, making it especially valuable for non-invasive investigations.[24]

The technique employs the measurement of Raman scattering to determine chemical composition. A major challenge in Raman spectroscopy is the weak Raman signal, which is often obscured by strong background noise. The integration of Raman spectroscopy with atomic force microscopy (AFM) provides more than just an extra chemical analysis tool to complement high-resolution AFM images. By coating AFM tips with conductive metals, such as gold or silver nanoparticles, it is possible to enhance Raman signals by up to 108 times in specific regions between the tip and the sample surface. This combination merges AFM's nanoscale topographic resolution with Raman spectroscopy's chemical fingerprint identification capabilities. The integration of Raman spectroscopy and AFM is facilitated by the stable and robust nature of Raman spectral detection equipment, which consists of a limited number of standard and modular components. Consequently, TERS instruments are easily commercialized.[25]

ATP bioluminescence

The enzyme luciferase plays a crucial role in ATP bioluminescence by producing measurable light in the presence of ATP, which can be detected using a luminometer. This method is advantageous

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Table 1. Different advanced and Imaginary techniques and their applications

Sr. No.	Name of techniques	Applications
1.	Light microscope	Imaging and quantitative evaluation of biofilm growth and mass.
2.	Transmission electron microscope (TEM)-	For generating highly magnified views of a sample's interior structure.
3.	Scanning electron microscope (SEM)-	Analyzing biofilms using highly magnified and detailed imagery.
4.	Confocal laser scanning microscopy (CLSM)-	Evaluation of biofilm structural characteristics, three-dimensional arrangement, and identification and localization of both viable and non-viable cells.
5.	Atomic force microscopy (AFM)	Analysis of biofilm quantity, measurement of adhesion forces, examination of biofilm surface features, and real-time visualization techniques.
6.	AFM-based infrared spectroscopy (AFM-IR)	Tasked with measuring the adhesive forces between goethite and bacteria in water.
7.	Fluorescent in situ hybridization (FISH)	An effective method for observing and measuring the distribution of various microbial species within biofilm structures is proposed.
8.	AFM-based Raman spectroscopy	For identifying biofilm forming bacterial strains
9.	ATP bioluminescence	Indirect measurement of the amount of organic/ food residue on a surface.
10.	Mass spectrometry	Characterized new functional metabolites that regulate biofilm formation.
11.	Quantitative real time PCR analysis	Detection of biofilm genes.
12.	Bio Finder	For the detection of biofilms and surface contamination.

since you should expect to get results in around 20 seconds at most. Nevertheless, it encounters difficulties when used on biofilms, particularly older ones. The major problem is that these biofilms prevent ATP from moving around much, leading to low ATP levels. For this reason, it is possible to underestimate the true microbial population in a mature biofilm with a high microbial density due to the low ATP levels. 26

Gas chromatography

Extracellular polymeric substances (EPS) and other big macromolecules present in complex biological formations, such as biofilms, may be identified and analyzed using mass spectrometry (MS), a powerful tool. The chemical components involved in biofilm growth may be studied in depth using this approach. Either matrix-assisted laser desorption ionization (MALDI) or electrospray ionization (ESI) are the two most used forms of mass spectrometry. By studying ions that have been desorbed in a controlled environment, a time-of-flight (TOF) mass spectrometer can determine mass. 27 The acronym MALDI-TOF stands for the combination of these methods. The procedure involves ionizing and vaporizing the sample using a laser. The produced ions are then guided towards the TOF detector by means of an electric field that runs the length of the MALDI-TOF device's column. Ions move more quickly when the mass-to-charge ratio is less, which is the basis for the TOF measurements. Among the many uses for MALDI methods are the following: bacterial identification; measuring bacterial growth; and monitoring bacterial protein expression in response to antimicrobials, including surface proteins and exoenzymes like â-lactamase. 28 Analyzing quantitative real-time PCR results

Gene expression patterns may be very different between planktonic and biofilm-dwelling bacteria. For accurate gene expression measurements in bacteria that form biofilms, one trustworthy tool is quantitative reverse transcriptase real-time polymerase chain reaction (qRT-PCR). Gene expression data from microarray research may be validated using this approach, thanks to its large dynamic range. 29 In addition, the sensitivity of qRT-PCR allows for the determination of gene expression in biofilm samples that contain little biological material, such those acquired using LCMM. One

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of the most used qRT-PCR methods

methodologies, which include SYBR Green and the dual-labeled probe (Taqman) approach. Both methods use reverse transcription to transform messenger RNA (mRNA) into complementary DNA (cDNA), which is then amplified using polymerase chain reaction (PCR). Here are the main points to remember while doing quantitative real-time polymerase chain reaction (qRT-PCR): (1) primer design; (2) primer and probe effectiveness evaluation; (3) qRT-PCR execution utilizing the Corbett Rotor-Gene platform; and (4) data extraction and analysis. 30 Find a Bio Finding biofilms on surfaces that come into touch with food using traditional microbiological culture methods might take a long time. The food sector, on the other hand, needs ways to verify microbiological contamination, evaluate environmental cleanliness, and put fixes in place right away. In a matter of seconds or minutes after application, BioFinder can identify biofilms, offering a remedy. Catalase is an enzyme found in almost all live cells and biofilms; this material interacts with it. A noticeable shift in hue and the appearance of tiny bubbles are telltale signs of biofilms. It is also possible to use BioFinder to check the efficacy of manufacturing facility sanitation procedures and to evaluate key inspection locations just before disinfection. 31

CONCLUSION

The complex and pervasive nature of biofilms has been revealed via research into these structures, which have implications in many fields, including as medicine and food processing. Biofilms are infamously difficult to eradicate because they are the product of a multi-stage process that produces tenacious microbial colonies. Modern imaging and analytical tools have substantially improved our understanding of biofilm structure, composition, and behavior. Microscopic analysis, spectroscopy, and molecular biology techniques are some of the technologies that give light on biofilm characteristics and point the way toward better management measures. More comprehensive knowledge and innovative strategies for dealing with biofilms in many contexts are expected to result from the combination of multiple analytical methods as this field of study progresses. Studies using biomembranes have used cutting-edge analytical methods to make considerable progress in elucidating the structure and composition of membranes. The high-resolution imaging of biomembranes made possible by atomic force microscopy (AFM) allows for the nanoscale assessment of their physical characteristics and dynamics. 32 Similarly, cryo-electron microscopy (Cryo-EM) has allowed us to better understand biomembrane architecture by determining the structures of membrane proteins. 33 Intrigued by the interactions and dynamics of membrane proteins and lipids, Single- Molecule Localization Microscopy (SMLM) offers unmatched resolution for examining these components. 34 In addition, Neutron Scattering is an effective method for studying the structure of membranes and the interactions between proteins and lipids, which provides important details on the molecular level behavior of membranes. 35 Biomembranes play an important part in many different cellular processes, therefore understanding how membrane proteins work is key to understanding their role in general. 36 When used together, these methods provide a thorough structure for investigating biomembrane studies.

REFERENCES

1. The development of biofilms and their regulation by pathogenic bacteria in food (Liu X., Yao H., Zhao X., & Ge C.). Chemicals, 2023, 28, 6, 2432. doi: 10.3390/molecules28062432.
Contribution of bacterial extracellular polymeric substances (EPS) in surface water filtration. In: Li W., Liu M., Siddique M.S., Graham N., and Yu W. Public Health and Environmental Research. 2021; 280: 116998. DOI: 10.1016/j. envpol.2021.116998.
3. Zhao A., Sun J., & Liu Y. Bacterial biofilms: A comprehensive guide to their description and treatment options. This article was published in the journal Frontiers in Cellular and Infection Microbiology in 2023 and has the DOI: 1137947. Doi: 10.3389/fcimb.2023.1137947.

Chapter 4: Dinh D., Awad T.S., Baker P., Howell P.L., and Hatton B.D. Surface-bound enzymes that do not elute interfere with bacterial adhesion by degrading continuous biofilm polysaccharide. The article is published in Biomaterials in 2018 and spans pages 168 to 176. You may get the article at this link: https://doi.org/10.1016/j. biomaterials.2018.03.016.
Authors: Mgomi F.C., Yang Y.R., Cheng G., and Yang Z.Q. The biofilms formed

ISSN:0976-0172
Journal of Bioscience And Technology
https://jbstonline.in/

by lactic acid bacteria and the antibacterial properties they possess in relation to harmful microbes. Published in 2023 in the journal Biofilm, volume 5, pages 100118. Doi: 10.1016/j.bioflm.2023.100118. Biofilm production in bacteria (Kahraman H., 2006). Physiology and Biochemistry: An International Journal. 2022; 7(1). The link to the article is https://doi.org/10.23880/sjbp-16000200. 7. QualiTru. (n.d.) outlines the stages of biofilm development. Systems for QualiTru Sampling. The five primary stages of biofilm growth are described in https://qualitru. com/. 8. Lu X., Hu Z., Liu J., and Ren S. Light and

Analysis of the taxonomic relevance of the morphological variety of Rosaceae stigma using scanning electron microscopy. Volume 85, Issue 8, Pages 2866–2879, Microscopy Research and Technique, 2022. The publication's DOI is 10.1002/jemt.24143. Biofilm research: cutting-edge tools for understanding microbial ecology and antibiotic resistance (Almatroudi A., 2009). Volume 29, Issue 4, Page 133 of the 2024 edition of Frontiers in Bioscience (Landmark Edition). The publication may be accessed at this URL: https://doi.org/10.31083/j.fbl201504133. "Implications for visualizing EPS architecture, mineral nucleation, and microfossil generation: Advanced biofilm staining techniques for TEM and SEM in geomicrobiology" (McCutcheon J. & Southam G., 2010). Published in 2018 in volume 498, pages 115–127, Chemical Geology. Doi: 10.1016/j.chemgeo.2018.09.016. Transmission electron microscope (TEM) v1. Zappylab. 2022. Choi M.-L. 11. 12. Seifert P. Enhanced Hiraoka TEM grid staining system and methodology using 3D printed components and gadolinium triacetate tetrahydrate, an alternative to radioactive uranyl acetate. The article may be found at https://doi. org/10.17504/protocols.io.ewov1n3zpgr2 /v1. The link to the article is in the 2017 Journal of Histotechnology, volume 40, issue 4, pages 130–135. At the following URL: https://doi.org/10.1080/01478885.2017.1 3 61117. 13. Huang A. Zappylab. 2022. Sample preconditioning before scanning electron microscopy (SEM) v1. The authors of the article are Relucenti, Familiari, Donfrancesco, Taurino, Li, Chen, Artini, Papa, and Selan et al. We will examine the advantages and disadvantages of scanning electron microscopy (SEM) and VP-SEM as they pertain to biofilm imaging. Scientific Reports in Biology, Volume 10, Issue 1, 2021, Pages 51. Agarwal, Jain, Pippal, and Gurnani (2015) published a paper in the journal Biology with the DOI 10.3390/biology10010051. Bacterial biofilm architecture and biogenesis: capturing micro-communities. Journal of Biological Chemistry and Biotechnology. 2025; 7: 100133. 16. K.H.S. Main, J.I. Provan, P.J. Haynes, G. Wells, J.A. Hartley, and A.L.B. Pyne. https://doi. org/10.1016/j.bbadva.2024.100133. The use of atomic force microscopy has greatly aided studies of DNA structure and translation. Publication: APL Bioengineering. Volume 5, Issue 3, Page 31504, 2021. Cattò and Cappitelli (2017) published a work with the DOI 10.1063/5.0054294. Testing polymeric surfaces for anti-biofilm potential: where to begin? Molecular and Cellular Biology, Volume 20, Issue 15, 2019, Pages 3794–3799. The citation is: 18. Mathurin et al., Wagner et al., Dazzi et al., Dartois et al., Bazin et al., and Deniset-Besseau. Current state, future directions, and obstacles in photothermal AFM-IR imaging and spectroscopy. “Journal of Applied Physics” published in 2022 with the DOI: 010901. The URL is https://doi