**JOURNEY FROM LIGHT TO QUANTUM MICROSCOPY –**

**SPECIAL REFERENCE TO ADVANCES IN MICROSCOPY.**

Dr. Munaza Aman

M.B.B.S, M.D Microbiology

Sher-I-Kashmir Institute of Medical Sciences.

Soura. Kashmir. India

The journey from light to quantum microscopy has been an evolution of advancements in scientific understanding and technological innovation, paving the way for crescively precise imaging techniques. The microscope has considerably advanced human exploration in microbiology, emerging from light microscopes to quantum microscopes, with augmented resolution allowing research into microorganisms and even the nano-level. Microscopy has become pivotal in medical sciences, particularly microbiology, and has bestowed to the development of micro and nanotechnologies. Today, studying microorganisms involves various procedures, chemicals, and imaging tools to analyse their biological and biochemical properties. The future of microscopy in exploring the microbiology seems propitious (Breedlove & Partin, 2024; Khodavirdipour et al., 2019a). The earliest imaging technique, light microscopy, is extensively used in defiance of offering a low-resolution, 2D view of microorganisms. For more detailed imaging, electron microscopy—specifically scanning electron microscopy (SEM)—provides a clearer, more detailed view of microorganisms' morphology.(Jang et al., 2018; Khodavirdipour et al., 2019). However, electron microscopy necessitates sample fixation and a conductive cover for imaging, which makes it unsuitable for analyzing living cells.(Khodavirdipour et al., 2019) Furthermore, confocal microscopy accredits the distinction of organelles in microorganisms through fluorescent marker staining, providing exhaustive and specific imaging of cellular structures (Khodavirdipour et al., 2019). However, the high cost of confocal microscopy and the prepatent stumbling block of fluorescent molecules—such as photo-toxicity, photo-bleaching, and interference with cell mechanisms due to fluorescent marker aggregation—are notable disadvantages.(Allgeier et al., 2018; EC et al., 2018) New expansion in imaging techniques are vital in microbiology to obtain valuable information without the need for complex sample preparation or reagents that could potentially alter cell biology. (What Are The Three Main Types Of Microscopes? | Sciencing, n.d. ) Despite the adoption of various substitute methods for imaging live microorganisms, microbiologists still combat demur with modern light microscopy. Holotomographic Microscopy (HTM), also known as Optical Diffraction Tomography (ODT), is a state-of-the-art technique that uses the interaction of light with different media within microorganisms. HTM offers a versatile substitute for cell imaging, overcoming many of the shortcomingd of preceding techniques (Khodavirdipour et al., 2019).

**Journey from light microscopy to quantum microscopy:** The journey commences with the revelation of light's nature and the development of basic microscopy.

**Classical Optics and Light Microscopy:**

I. **Early Theories of Light:** The idea that light might consist of particles was first recommended by ancient Greek philosophers like Empedocles and Democritus. However, it wasn't until the 17th century that Isaac Newton's particle theory of light and Christiaan Huygens' wave theory formed the bedrock of our modern understanding of light.

II. **Invention of the Microscope:** In the late 16th and early 17th centuries, innovators like Hans and Zacharias Janssen, Galileo Galilei, and Robert Hooke refined and remodelled early microscopes. These amelioratons allowed scientists to observe microorganisms and begin understanding the micro-world.

III. **Optical Microscopy:** The 19th century saw the development of compound light microscopes, which used lenses to magnify and observe biological and material structures down to the micron scale. While light microscopes utilize visible light, their resolution is limited by light diffraction, typically to around 200 nm.

The evolution of magnification and microscopy:

* **Romans**: The concept of magnification began with the Romans, who established that objects appeared larger when viewed through certain types of glass, laying the groundwork for early microscopy.
* **12th Century AD**: Italian inventor **Salvino D'Armate** introduced the first magnifying eyepiece. (FIG:1)
* **1590**: **Zechariah and Hans Janssen** developed the first compound microscope by combining two lenses in a tube-like structure. (FIG:2)
* **1609**: **Galileo Galilei** improved the compound microscope by using a concave and a convex lens. (FIG:3)
* **1665**: **Robert Hooke** published *Micrographia*, where he documented his observations through microscopes and explored various instruments.
* **1674**: **Anton Van Leeuwenhoek** perfected the microscope after numerous attempts, grinding lenses to achieve better magnification. (FIG:4)
* **1826**: **Joseph Jackson Lister** introduced the **achromatic lens**, which removed chromatic aberration caused by different light wavelengths.
* **1860s**: **Ernst Abbe** made a significant breakthrough by discovering the **Abbe sine condition**, which outlined the necessary lens conditions for achieving sharp images.
* **1931**: **Ernst Ruska** introduced the **electron microscope**, marking a pivotal moment in the history of microscopy.

This timeline showcases the key milestones that led to modern microscopy, advancing our aptitude to explore the microscopic world.

**Classification of Microscopes:** Generally, microscopes can be categorized based on some factors like optical function, structure, or application. Basically, major classification is as follows:

***Optical microscope:***

1. Binocular stereoscopic microscope
2. Bright field microscope
3. Polarizing microscope
4. Phase contrast microscope
5. Differential interference contrast microscope
6. Fluorescence microscope
7. Total internal reflection fluorescence microscope
8. Laser microscope (laser scanning confocal microscope)
9. Multiphoton excitation microscope
10. Structured illumination microscope.

***Electron microscope:*** ( FIG: 5)

These microscopes instead of the light beams emit an electron beam toward an object in order to magnify them.

1. Transmission electron microscope (TEM)
2. Scanning electron microscope (SEM).

***Transmission Electron Microscope (TEM)***

A small source at the highest part of the microscope emits the electron which can travel via a vacuum column of the microscope. Instead of the lens which focuses the light in the light microscope, this microscope uses an electromagnetic lens that focuses the electrons into a beam. The electron beam then passes via a specimen. Depending on the density of the material and texture of the object, few electrons disappear from the beam. At the base part of microscope, electrons which remain unscattered collide to the screen, which produces a shadow-like image of the understudy specimen based on its density. The image can be analyzed and photographed for further study by the operator. (Allgeier et al., 2018)

***Scanning Electron Microscope (SEM)***

High-speed electrons in SEM have outstanding amounts of energy and above energy dispersed in very different signals produced by the electron–specimen interaction at the time the electrons speed decreases in solid objects. This signal may consist of secondary electrons, photons, visible lights, and heat. These electrons and reflexed electrons are commonly used in image production (Allgeier et al., 2018).

***Scanning Probe Microscope***

The scanning probe microscope scans the outer layer of an organism or cell by a probe and the intended interaction measures the area and identifies its properties (Jang et al., 2018).

1. Atomic force microscope (AFM);
2. Scanning near-field optical microscope (SNOM).

*Others*

1. X-ray microscope and Ultrasonic microscope.
2. Binocular Stereoscopic Microscope:

This is a type microscope which allows for easily observing the 3D at not very high magnification.

1. *Bright-Field Microscope:* This microscope utilizes lights which are transmitted to observe at high magnification.
2. *Polarizing Microscope:* The polarizing microscope has different types of lights which transmit the characteristics of materials, including crystalline structures in order to make a perfect image.
3. *Phase-Contrast Microscope:* This device brings to life even minute irregularities by utilizing the interference of the light. It is possible to observe living objects even without staining them.
4. *Differential Interference Contrast Microscope:* This is similar to the above-mentioned microscope but produces higher resolution pictures. However, it has its limitations due to the nature of the lights.
5. *Fluorescence Microscope:* This life science microscope captures fluorescence emitted from the sample by using a dedicated source of light like the mercury lamps. In combination with other equipment, a bright-field microscope also can produce fluorescence images (Jang et al., 2018).
6. *Total Internal Reflection Fluorescence Microscope:* It is a kind of fluorescent microscope that uses a passing wave only to illuminate the surface of the specimen. The viewed region is generally very thin in comparison to other regular microscopes. In addition, molecular unit observation is possible by using this microscope.
7. *Laser Microscope (Laser Scanning Confocal Microscope):* The laser beam in this microscope is used to clear the picture of samples with changes in focal distances (Khodavirdipour et al., 2019).
8. *Multiphoton Excitation Microscopy:* The multiphoton excitation microscope laser causes very little damage to living cells and interestingly allows higher resolution observation of obscure areas. Further, this microscope is usually used to observe the blood flow or nerve cells in the brain (Khodavirdipour et al., 2019).
9. *Structured Illumination Microscopy:* This very high-resolution microscope with highly sophisticated technology is utilized to handle the problem of the limited and poor resolution of optical microscopes that are only caused by light diffraction (EC et al., 2018).

**Advances in microscopy**

1. ***Atomic Force Microscope (AFM)*** (FIG: 6)

Almost all atomic force microscopes typically use a laser system in which the beam is reflected from behind the reflective atomic force microscope lever and into a detector. Furthermore, atomic force microscopes are mainly fabricated from Si3N4 and their radius is usually from couple to 10 seconds of a nanometer (Khodavirdipour et al., 2019).

***Atomic force microscopy principle and imaging modes***

AFM (Atomic Force Microscopy) has become one of the most accomplished techniques for analyzing samples with various surface characteristics. The primary principle behind AFM involves the deflection of a cantilever, which occurs due to the interacting forces between a sharp tip attached to the cantilever and the sample being analyzed. To detect these deflections and determine the position of the cantilever along the z-axis, a laser is typically reflected off the cantilever surface. This deflection measurement is key to obtaining high-resolution topographical and mechanical data of the sample's surface (Kreplak, 2016). FM offers multiple imaging modes, each tailored to suit the specific characteristics of the sample being studied. These different modes allow researchers to obtain a array of information reckoning on the proprium of the specimen and the AFM mode of operation chosen. Some common modes include **contact mode**, where the tip makes direct contact with the sample, **tapping mode**, which minimizes sample deformation, and **non-contact mode**, where the tip interacts with the sample without making physical contact. By selecting the appropriate imaging mode, AFM can provide acumen into topography, mechanical properties, and even nanoscale forces, making it a accomplished apparatus for evaluating various types of samples (Dufrêne et al., 2017). In its early stages, AFM was not universally adopted in biological research due to the intricacy of the technique. The setup, alignment, and adjustment of system parameters were ponderous, making it arduous to use in implicit applications. Additionally, the technology had limitations such as low spatio-temporal resolution, restricted operational modes, and small scan areas, making it demanding to analyse heterogeneous surfaces or soft samples. The first commercial AFM microscopes primarily operated in contact mode, which was not optimal for studying delicate or complex biological samples. However, momentous technological advances have propagated the purview of AFM applications. The enjoyment of various probe-sample interaction modes has fortified the technique's ambidexterity, permitting for more comprehensive analysis of different types of samples. In recent years, AFM has rapidly advanced with the integration of complementary techniques, including optical microscopy, spectroscopy, and artificial intelligence (AI), deep learning (DL), and machine learning (ML). These innovations have remarkably remodelled AFM’s capabilities, particularly in microbiological and biomedical research, making it an additionally robust appliance for studying the nanoscale properties and dynamics of living cells and biological systems (Miranda et al., 2021 ; Maver et al., 2016 ; Uchihashi & Ganser, 2020 ; V. D. dos Santos etal.,2023). AFM has become a thorough facility that accredit multiparametric scrutiny of living cells and microorganisms. One of its exclusive tenacities is its ability to execute label-free imaging of nanoscale biomolecular dynamics, which allows researchers to analyze molecular-scale mechanisms that other super-resolution imaging techniques cannot access. This competence has positioned AFM as an inestimable device in microbiology and biomedical research. The use of AFM in biomedical applications has grown significantly with the development of **tapping mode**. Tapping mode, which involves intermittent contact between the AFM tip and the sample, reduces the deformation of soft samples, making it especially useful for studying delicate biological specimens. This has led to numerous applications of AFM in tapping mode, as well as its variations, such as **HS-AFM (High-Speed AFM), AFM-IR (AFM-Infrared Spectroscopy),** and **AFM-SECM (Scanning Electrochemical Microscopy).** These advancements have enabled the study of live biological specimens, including microorganisms, tissues, and cells, providing insights into their mechanical properties, molecular interactions, and dynamics in real time.(Shi et al., 2020; Dufrêne, 2004 ;Martínez-Montelongo et al., 2020) Many analysts are engrossed in endowing AFM as a routine technique to study the bio-interactions of nanomaterials with different cell lines and microorganisms (Martínez-Montelongo et al., 2020). AFM is a highly adept and potent imaging technique that has endorsed valuable for uncovering baroque minutiae in biomedical research. It is label-free, fast, and provides abiding results, making it quintessential for evaluating biological systems at the nanoscale. One of the key potencies of AFM is its ability to render qualitative mechanical property mappings using intermittent contact mode, also known as phase imaging. AFM also comprise techniques like **force spectroscopy** and **force volume**, which allow for the measurement of tip-sample forces either at a specific point or across an area. However, both of these techniques tend to involve longer acquisition times, which can be a spur for high-throughput applications. Modern AFM systems are multifunctional, enabling the measurement of various mechanical properties of cells under physiological conditions. These measurements can include **force, pressure, tension, adhesion, friction, elasticity**, and **viscosity**, which are critical for evaluating the mechanical behavior and response of biological systems to controlled mechanical stimuli. This makes AFM a powerful tool for analysing cell/ microorganism/ tissue response to their mechanical environment, contributing acumen into areas such as cellular mechanics, drug delivery, and tissue engineering (Krieg et al., 2018). The combination of AFM with other integral techniques substantially strengthens its potential, endowing for a comprehensive investigation of mechanical, functional, and morphological properties. By assimilation of AFM with methods such as optical microscopy, spectroscopy, and fluorescence imaging, researchers can achieve a multi-dimensional view of microorganisms and their biological system. This combined approach not only measures the mechanical properties of cells, such as stiffness, elasticity, and adhesion, but also provides functional insights into biological processes, including molecular interactions and cellular dynamics. Additionally, AFM can be used alongside imaging techniques to observe the morphology of cells and tissues in high resolution.

Together, these tools enable researchers to investigate the biological response of complex systems to various stimuli, such as drug treatments, environmental changes, or mechanical forces, providing a more complete understanding of the underlying mechanisms driving cellular behavior and function. This makes AFM a powerful tool for advancing research in fields like nanomedicine, tissue engineering, and microbiology (Kasas et al., 2018 ; Guillaume-Gentil et al., 2014). Late reports remark on the recent discoveries that help AFM expand its reach. Penedo and co-workers discuss the implementation of Nanoendoscopy-AFM (3D-AFM), a new mode of AFM operation that allows the visualization of intracellular structures (Kasas et al., 2018). In this technique, inserting a needle-like nanoprobe into a living cell allows real-time (2D and 3D) imaging of intracellular nano dynamics without decreasing cell viability. This method surpasses the AFM limitation to 2D surface imaging, imaging unsupported 3D structures. The following studies comment on some technical considerations and applications (to observe organelles in living cells) for nano endoscopic imaging (Ichikawa et al., 2023). A recent review summarizes the main applications of 3D-AFM to study 3D self-organizing systems (3D-SOSs) with particular emphasis on intracellular components inside live cells(Fukuma et al., 2024). Despite the numerous advantages of AFM, acquisition time had historically been a challenge for studying real-time biochemical or conformational changes of biomolecules. However, **HS-AFM** has significantly addressed this issue by increasing image acquisition speed, making it suitable for observing biological processes in real-time and analyzing dynamic cellular activities. However, one limitation of HS-AFM is its relatively small scanning area, but recent advances in electronics, cantilever design, and piezoelectric scanners have expanded the scan range, both in air and in liquids. These innovations in modern AFM equipment, including improved stability in liquids, larger fields of view, and high spatial and temporal resolution, have enabled the study of biological systems under physiological conditions with exceptional precision. With these advancements, HS-AFM is now capable of imaging at high speeds, allowing researchers to observe and record the dynamics of biomolecules (such as mitochondrial DNA replication) or live cells in real time. This makes it possible to study biological processes at the **single-cell** or organism level, providing deeper insights into molecular mechanisms and cellular activities that were previously difficult to observe with conventional techniques (Ando, 2018). The possibility of AFM imaging in liquids allows the study of living cellsunder different conditions. The capability of AFM to image and interactwith the surfaces of living cells or tissues under physiological conditions gives researchers endless possibilities to develop new methodologies for more realistic and accurate biomedical applications (Dufrêne et al., 2021). Initially, AFM imaging in biological contexts was limited by the use of liquid cells, which provided a confined physiological environment for the samples. However, a major drawback of this approach was that the liquid volume in the cell often caused biomolecules to become diluted, limiting the accuracy and sensitivity of the analysis. To address this challenge, **microfluidics technology** was integrated with AFM, and the design of cantilevers and liquid chambers was re-engineered, leading to significant improvements in **Fluid AFM** imaging. One key innovation was the development of **hollow cantilevers** that are application-specific and enable **Fluidic Force Microscopy (Fluid-FM).** This technology allows for force-controlled manipulation of single cells in fluid environments. Fluid-FM has proven to be a powerful tool for investigating various biological processes at the single-cell level, including cell adhesion. Cell adhesion plays a crucial role in many biological functions, such as microbial biofilm formation, survival, and pathogenesis, as well as mammalian processes like embryonic development, tissue morphogenesis, and inflammation. Fluid-FM enables precise measurement of living cell adhesion forces in physiological conditions, providing valuable insights into how cells interact with their environments. Recent reviews have highlighted the growing number of biological applications for Fluid-FM, particularly in studying cell mechanics, interactions, and responses under realistic, in vivo-like conditions (Schoenwald et al., 2010; Efremov et al., 2019). Traditional AFM has limitations when it comes to discriminating the material composition of the sampled surface unless various properties like elasticity, electronic, and magnetic characteristics are studied. However, by coupling **infrared (IR) spectroscopy** with AFM, **AFM-IR nano spectroscopy** was developed, which opened up new possibilities for molecular analysis along the surface. When a sample is irradiated with infrared light, it experiences thermal expansion, which the AFM tip can measure. This allows the tip's response to be correlated with the excitation wavelength, thus providing **IR patterns** that offer chemical and structural information about the surface of an organism. AFM-IR is a highly sensitive technique that enables direct visualization of specific molecular changes, such as the drug loading of biomolecule carriers at the nanoscale. This technique has been used to optimize drug loading protocols for single biomolecule carriers. For instance, **Hanke et al.** successfully applied AFM-IR to study the loading of DNA origami nanostructures with the photosensitizer **methylene blue,** demonstrating its potential for characterizing and optimizing drug delivery systems. AFM-IR is also applied in studying molecular changes in living bacteria, offering insights into their biochemistry and interactions with various compounds. Recent reviews have highlighted the use of AFM-IR in the characterization of various **drug delivery systems,** including polymer-based, lipid-contained, and metal-based carriers, emphasizing its versatility and precision in molecular analysis for pharmaceutical and biomedical applications (Dazzi & Prater, 2017; Mathurin et al., 2022). AFM has evolved into a robust and multifunctional tool for manipulating and detecting bio-interactions with nanometer resolution. However, one limitation of traditional AFM is that it cannot provide chemical composition or detailed internal structure information. To overcome this, **AFM** is increasingly being combined with other microscopy techniques to provide more comprehensive insights. For example, **super-resolution optical microscopy** can be paired with AFM to simultaneously identify cellular components or exogenous agents and visualize their behaviour inside the cell. Modern **correlative microscopy**—which integrates AFM with other imaging modalities—has become essential in life sciences. By combining structural, compositional, and functional data, researchers can gain a deeper understanding of the complex relationships within biological systems. This synergy helps paint a more complete picture by associating structural and compositional details with functional information, which is critical for advancing biological research. Many types of AFM equipment are now commercially available, and these tools are designed to be **user-friendly** and operate automatically, minimizing the need for manual adjustments. These systems can quickly set up, align, and readjust parameters, which makes them especially appealing for biomedical applications. Moreover, these advanced AFM systems offer **high spatio-temporal resolution**, large scan areas, and fast scanning capabilities, all of which are essential for studying complex biological samples. Beyond imaging, AFM is capable of measuring a variety of **nano-mechanical properties** such as stiffness, elasticity, dissipation, viscoelasticity, and hardness, which are important for understanding cell mechanics and behaviour. The latest-generation AFM systems can also operate under a range of conditions (such as varying temperature, in air, or in fluid), making them adaptable for diverse research needs. Additionally, **data analysis** from these AFM systems is simplified, accurate, and versatile. It’s even possible to overlay AFM data with data generated from other advanced optical microscopy techniques, enhancing the analysis further. For instance, the combination of **confocal microscopy** and AFM **indentation** has been used to study cell mechanics in 3D environments, revealing changes in the mechanical properties of metastatic cancer cells as they invade collagen I matrices. This ability to integrate multiple techniques provides powerful new insights into cellular behaviours and disease processes at the nanoscale (Geisse, 2009; Staunton et al., 2016).

1. ***Holotomographic microscopy (HTM)*** (FIG: 7, 8, 9)

HTM principles are grounded in advancements from quantitative phase microscopy, combining techniques like microscopy, holography, and light scattering. Recently, optical microscopy has surpassed the diffraction limit, leading to super-resolution microscopy, or "nanoscopy." Fluorescence nanoscopy (FN) has been key in uncovering fine structural details of subdiffraction-level cellular architectures (Meyer, 1979). Additionally, multi-batch labelling allows researchers to track dynamic changes in cells and their interactions with exogenous agents. Recently, HTM has gained prominence as a powerful label-free 3D imaging technique, enabling the observation of microorganisms and cell components without the need for staining or label-free nanoparticles inside cells (Liu et al., 2016). Modern HT microscopes offer several advantages, including the absence of label requirements, low photo-toxicity, and quick analysis. They can also acquire quantitative data, such as refractive index (RI), dry mass, and protein content. In many samples, protein concentration is linearly proportional to RI values (Moreno et al., 2021 ;Sandoz et al., 2018, 2019). Measuring refractive index (RI) differences in cell components to create a 3D image of living organisms is complex due to light refraction in media with varying RIs, which causes multiple light scattering effects. HTM overcomes this by reconstructing phase difference images, providing 3D RI and volume information for both living and fixed cells. Early HTM applications focused on classifying organisms based on cell and organelle morphology, specifically looking at species-related properties (Choi et al., 2021; Y. K. Park et al., 2018). HTM is a valuable tool for studying pathological characteristics of various cells, tracking changes after exposure to different agents (such as chemicals, microorganisms, or nanomaterials), and monitoring dynamic processes in cells and their organelles. However, HTM has limitations in providing molecular-level information and in the structural reliability of organelles, which are only expressed through RI. To address these limitations, it's essential to correlate HTM with complementary imaging techniques. Today, HTM microscopes are commercially available, and while their designs may vary, modern models generally use low-energy wavelengths for measurements to reduce phototoxicity (Moreno et al., 2021; Sandoz et al., 2018). A recent study explored the efficiency of levofloxacin dry powder aerosols for tuberculosis treatment, using both confocal laser scanning microscopy (CLSM) and HTM to examine how the levofloxacin formulations were taken up by microorganisms (cells). Both techniques revealed that the formulations were internalized by NR8383 cells. HTM further showed changes in the volume and concentration of lipid droplets, as well as alterations in cell volume of the treated cells. Another study highlighted HTM's ability to distinguish between healthy and apoptotic cells, while also monitoring changes both on the outer surface and within the cells (Salucci et al., 2020; Srichana et al., 2023). Camptothecin-treated cells show both morphological changes, such as membrane blebbing and chromatin condensation, as well as biochemical changes like an increase in lipid droplets. In a separate study, Koutsogiannis et al. used HTM to investigate the effects of Toxoplasma gondii on host cells. They reported noticeable changes in the cell volume, dry mass, and surface area of infected cells (Koutsogiannis et al., 2023). Recent HTM equipment integrates Artificial Intelligence to assist with automated single-cell segmentation and quantification. Additionally, HTM microscopes can operate under various conditions, making them suitable for analyzing living cells (both adherent and suspended) or living cells interacting with exogenous agents. HTM offers several advantages for live cell imaging, including:

(a) high resolution (nanoscale),

(b) label-free imaging,

(c) real-time observation,

(d) quantitative phase imaging, and

(e) fluorescence capabilities.

The versatility of HTM allows it to be combined with other analytical techniques to enhance its functionality. Ryu et al. also contribute to exploring these advanced applications, reports in a research on combining a microfluidic device and HTM for red blood cell histopathological analysis. The microfluidic device has been integrated with the HT microscope to obtain both biochemical (such as hemoglobin content) and morphological properties (like corpuscular volume) of cells. HTM can also be coupled with mass spectrometry for more precise localization and identification of organelle composition. However, as an emerging technique, HTM still faces challenges related to data reproducibility across different research groups. To address this, researchers need to establish standardized protocols for sample preparation and create libraries with RI values for organelles across various cell lines. Additionally, recent studies have pointed out that cell fixation can alter the refractive index of cells and cellular compartments, which may impact the accuracy and reproducibility of HTM's morphological analysis. The latest HT microscopes are suitable for analyzing organoids or tick specimens. HTM is a versatile technique to image and analyze dynamics of biological events because of its label-free and quantitative imaging capabilities. Among optical cell nanoscopy techniques, the advantages of HTM are its non-invasive nature, simplicity, and fast acquisition times. (Baczewska et al., 2021; D. Park et al., 2023). Accurate measurement of the mechanical properties of live cells is of utmost importance in today´s biomedical research. While much of the HTM research has focused on bacteria and fungi, parasites, including protozoans, are also suitable for identification using HT microscopy. Larrazabal et al. investigated the antiparasitic effects of ezetimibe on human and veterinary parasites (Toxoplasma gondii, Neospora caninum, and Besnoitia besnoiti), which cause significant diseases in both humans and animals. The study revealed that during the asexual reproduction phase of these parasites, they affect host cells. Using HTM, the researchers observed that ezetimibe reduced the size of meronts in all three parasites without altering the morphology of non-infected cells. Investigations on sporogony are limited in the literature, as commonly used fluorescent dyes cannot penetrate the resistant bi-layered walls of oocysts. However, HT microscopy proves to be a suitable technique for studying sporogonial oocysts and observing any alterations, whether they are time-dependent changes or effects induced by anti-coccidial drugs (Larrazabal et al., 2021; Lopez-Osorio et al., 2022). The versatility of HT microscopy, particularly its ability to study living cells, makes it an excellent tool for examining the life cycles of parasites. For instance, using 3D HT microscopy, researchers were able to analyze the differing processes of sporozoite egress, providing detailed insights into this crucial stage of the parasite life cycle (López-Osorio et al., 2020). HTM has recently been used to study the Toxoplasma lytic cycle, revealing alterations in the volume, surface area, and dry mass of genetically engineered Toxoplasma mutant cells. This application highlights HTM's capability to track dynamic changes in cellular properties during the course of infection or mutation (Koutsogiannis et al., 2023). Recent studies have highlighted the involvement of lipid droplets in the cellular stress response, particularly during detoxification processes or in response to various diseases across multiple cell lines. These lipid droplets play a key role in managing cellular stress by storing lipids and helping regulate metabolic and inflammatory responses (Hammoudeh et al., 2020). A recent study highlights the unique advantage of HTM for real-time monitoring of bacterial cells, allowing for the observation of both morphological and biochemical changes when in contact with antimicrobial films. The authors note that current methods for investigating the antibacterial activity of 2D nanomaterials (such as colony counting, SEM, and live/dead fluorescent staining) have limitations, particularly in tracking the changes in bacterial cells over time and observing continuous alterations in the bacterial membrane. HTM provides a more dynamic and detailed view, helping to decipher the antibacterial mechanisms of these 2D nanomaterials (Kim et al., 2017). Another study demonstrates the suitability of HTM for observing virus-induced cytopathic effects in live cells. The results revealed noticeable differences in the refractive index gradient of infected cells, providing valuable insights into the structural and functional changes that occur during viral infection, without the need for labels or staining (Yakimovich et al., 2018). Furthermore, the study highlights the relevance of using HTM to investigate the severity of COVID-19, particularly due to the presence of microclots even after the recovery period. This capability allows researchers to track and analyze lingering effects of the disease on blood cells and vascular health, offering a deeper understanding of the long-term consequences of COVID-19 (Bergaglio et al., 2024).

***Atomic force and holotomography microscopy in microbiology:***

The growing resistance to antimicrobials is a major concern, as the number of resistant species and cases of multidrug resistance continues to rise. Recent advances in microscopy techniques, such as AFM, are playing a key role in the search for new antimicrobial agents. AFM analysis, for instance, allows researchers to observe morphological and mechanical changes in microorganisms after exposure to antimicrobial agents, offering valuable insights into how these agents affect microbial structures and their mechanisms of action (Formosa-Dague et al., 2018). AFM has numerous applications in biomedical studies due to its ability to provide both nanoscale imaging and nanomechanical characterization of biological materials in physiological environments. Recent studies have used AFM to examine the effects of antibiotics on the mechanical properties of bacteria. For example, researchers observed that only the virulent strain of Bordetella pertussis experienced a decrease in height and elasticity when exposed to antimicrobial agents. Another study investigated the impact of different antibiotics (nitrofurantoin, furazolidone, and nitrofurazone) on probiotic bacteria, noting changes in bacterial cell morphology, topography, and adhesion properties—alterations that could potentially impair biofilm formation (Villalba et al., 2022; Zdarta & Kaczorek, 2023). A recent review emphasizes the use of AFM to understand the mechanisms of antimicrobial drugs at the nanoscale level on microbial interfaces. The study explores how AFM can reveal alterations in bacterial cell morphology, nanomechanical properties, and the adhesive abilities of microbes. Additionally, it highlights the potential of AFM as NanoMechanical Sensors (NEMS), which can quickly and accurately detect microbial resistance, offering a valuable tool for monitoring and diagnosing antimicrobial resistance in real-time (Formosa-Dague et al., 2018). A recent review highlights the diverse applications of AFM in cellular and molecular microbiology. Moreover, focusing on its relevance in studying non-pathogenic microorganisms in environmental contexts. Cyanobacteria and microalgae, as key primary organisms in aquatic environments, were examined in a recent AFM study using force-distance curves. The study revealed the mechanical heterogeneity of the external layers of these microorganisms, providing insights into their structural properties and how they interact with their environment (Dufrêne et al., 2021b; Xiao et al., 2021). This study gives a better understanding of the biophysical mechanism that helps these microorganisms to adapt to turbulency. HS-AFM has proven invaluable in studying the dynamic behaviour of biomolecules and structures on or beneath the cell membrane. For example, Kobayashi et al. used HS-AFM to investigate the sliding mechanisms of the Mycoplasma mobile parasite. Their results revealed spatial and temporal characteristics of particles on the cell surface, showing that particles moved up to 9 nm in just 330 milliseconds. These movements, driven by ATP hydrolysis reactions, are crucial for cell movement, providing a deeper understanding of the parasite's mobility at a molecular level (Kobayashi et al., 2021). The use of AFM to study microalgae has been steadily increasing, though it still represents only about 0.17% of the total research on microalgae. Similar to studies on bacteria, AFM research on microalgae has mainly focused on their morphological characteristics, topography, and mechanical and adhesion properties. However, AFM has also enabled the study of substances produced by microalgae, such as exopolysaccharides. Additionally, techniques like Fluid-AFM are being used to evaluate parameters such as lipid profiles, which can help optimize the efficiency of microalgae cultivation processes (Demir-Yilmaz et al., 2021).

1. ***Scanning Near-field Optical Microscope (SNOM/ NSOM)***

A near-field scanning optical microscope (NSOM), also known as scanning near-field optical microscopy (SNOM), is a technique used to study nanostructures. In SNOM, a laser beam is focused through a tiny aperture, smaller than the wavelength of light. This results in the laser beam being confined to the near field, allowing it to interact with the specimen at distances smaller than the aperture. The specimen is then scanned at this very close distance, enabling the study of fine details at the nanoscale that are beyond the diffraction limit of conventional optical microscopes (EC et al., 2018).

1. ***X-ray Microscope***

An X-ray microscope uses soft X-ray radiation to create enlarged images of specimens. Since X-rays can penetrate almost all materials, there is no need for specific staining techniques, unlike in traditional light or compound microscopes. X-ray microscopes don't rely on refraction or reflection and are not visible to the human eye. Instead, they use a film or charge-coupled device to detect the X-rays that pass through the object. This detection is based on contrast, utilizing the difference in absorption of soft X-rays in the water window region, with wavelengths between 2.34–4.4 nm and energy levels ranging from 280 to 530 eV. In microorganisms and water, carbon atoms and oxygen cells are the primary elements interacting with the X-rays, respectively, allowing for detailed imaging of biological samples (Khodavirdipour et al., 2019).

1. ***Ultrasonic Microscope***

Acoustic microscopy is a microscopy technique that uses very high or ultra-high ultrasound frequencies to generate images. These microscopes can penetrate almost all solid objects, allowing for the visualization of internal properties that are not visible through conventional methods. Acoustic microscopy is particularly useful for detecting issues such as cracks, defects, or other internal problems within materials, providing valuable insights into the structural integrity of various specimens (Khodavirdipour et al., 2019).

1. ***Biological quantum light microscope***

Researchers have developed quantum light microscopes based on [squeezed states of light](https://en.wikipedia.org/wiki/Squeezed_states_of_light) (Casacio et al., 2021; Taylor et al., 2013, 2014). Squeezed states of light have noise characteristics that are reduced beneath the shot noise level in one quadrature (such as amplitude or phase) at the expense of increased noise in the orthogonal quadrature. This reduced noise can be used to improve signal-to-noise ratio. Squeezed states have been shown to allow a signal-to-noise ratio improvement of as much as a factor of thirty (Casacio et al., 2021). The first biological quantum light microscope used squeezed light in an [optical tweezer](https://en.wikipedia.org/wiki/Optical_tweezer) to probe the interior of a living yeast cell (Taylor et al., 2013). In experiments it was shown that squeezed light allowed more precise tracking of lipid granules that naturally occur within the cell, and that this provided a more accurate measurement of the local viscosity of the cell. Viscosity is an important property of cells that is connected to their health, structural properties and local function. Later, the same microscope was employed as a photonic force microscope, tracking a granule as it diffused spatially (Taylor et al., 2014). This allowed quantum enhanced resolution to be demonstrated, and for this to be achieved in a far-sub-diffraction limited microscope. Squeezed light has also been used to improve nonlinear microscopy (Casacio et al., 2021). Nonlinear microscopes use intense laser illumination, close to the levels at which biological damage can occur. This damage is a key barrier to improving their performance, preventing the intensity from being increased and therefore putting a hard limit on SNR. By using squeezed light in such a microscope, researchers have shown that this limit can be broken - that SNR beyond that achievable beneath photo-damage limits of regular microscopy can be achieved (Casacio et al., 2021).

1. ***Quantum enhanced fluorescence super-resolution***

In a [fluorescence microscope](https://en.wikipedia.org/wiki/Fluorescence_microscope), images of objects that contain fluorescent particles are recorded. Each such particle can emit not more than one [photon](https://en.wikipedia.org/wiki/Photon) at a time, a quantum-mechanical effect known as [photon antibunching](https://en.wikipedia.org/wiki/Photon_antibunching). Recording anti-bunching in a fluorescence image provides additional information that can be used to enhance the microscope's resolution beyond the [diffraction limit](https://en.wikipedia.org/wiki/Diffraction_limit), and was demonstrated for several types of fluorescent particles. However, improved detector technology enabled demonstrations of quantum enhanced super-resolution using fast detector arrays, such as [single-photon avalanche diode](https://en.wikipedia.org/wiki/Single-photon_avalanche_diode) arrays (Israel et al., 2017).

1. ***Quantum enhanced Raman microscopy***

[Quantum correlations](https://en.wikipedia.org/wiki/Quantum_correlation) offer an SNR beyond the photo-damage limit (the amount of energy that can be delivered without damage to the sample) of conventional microscopy. A coherent [Raman microscope](https://en.wikipedia.org/wiki/Raman_microscope) offers sub-wavelength resolution and incorporates bright quantum correlated illumination. Molecular bonds within a cell can be imaged with a 35 per cent improved SNR compared with conventional microscopy, corresponding to a 14% concentration sensitivity improvement (Casacio et al., 2021). More precisely, these microscopes are further utilized in clinical microbiology, virology, and mycology and specifically become the gold standard in molecular biology.

**References:**

Allgeier, S., Bartschat, A., Bohn, S., Peschel, S., Reichert, K. M., Sperlich, K., Walckling, M., Hagenmeyer, V., Mikut, R., Stachs, O., & Köhler, B. (2018). 3D confocal laser-scanning microscopy for large-area imaging of the corneal subbasal nerve plexus. *Scientific Reports 2018 8:1*, *8*(1), 1–10. https://doi.org/10.1038/s41598-018-25915-6

Ando, T. (2018). High-speed atomic force microscopy and its future prospects. *Biophysical Reviews*, *10*(2), 285–292. https://doi.org/10.1007/S12551-017-0356-5/METRICS

Baczewska, M., Eder, K., Ketelhut, S., Kemper, B., & Kujawińska, M. (2021). Refractive Index Changes of Cells and Cellular Compartments Upon Paraformaldehyde Fixation Acquired by Tomographic Phase Microscopy. *Cytometry. Part A : The Journal of the International Society for Analytical Cytology*, *99*(4), 388–398. https://doi.org/10.1002/CYTO.A.24229

Bergaglio, T., Synhaivska, O., & Nirmalraj, P. N. (2024). 3D Holo-tomographic Mapping of COVID-19 Microclots in Blood to Assess Disease Severity. *Chemical & Biomedical Imaging*, *2*(3), 194–204. https://doi.org/10.1021/CBMI.3C00126

Breedlove, B., & Partin, C. (2024). From Observing Little Animalcules to Detecting Fastidious Bacteria. *Emerging Infectious Diseases*, *30*(1), 208. https://doi.org/10.3201/EID3001.AC3001

Casacio, C. A., Madsen, L. S., Terrasson, A., Waleed, M., Barnscheidt, K., Hage, B., Taylor, M. A., & Bowen, W. P. (2021). Quantum-enhanced nonlinear microscopy. *Nature 2021 594:7862*, *594*(7862), 201–206. https://doi.org/10.1038/s41586-021-03528-w

Choi, J., Kim, H.-J., Sim, G., Lee, S., Park, W. S., Park, J. H., Kang, H.-Y., Lee, M., Heo, W. Do, Choo, J., Min, H., & Park, Y. (2021). Label-free three-dimensional analyses of live cells with deep-learning-based segmentation exploiting refractive index distributions. *BioRxiv*, 2021.05.23.445351. https://doi.org/10.1101/2021.05.23.445351

Dazzi, A., & Prater, C. B. (2017). AFM-IR: Technology and Applications in Nanoscale Infrared Spectroscopy and Chemical Imaging. *Chemical Reviews*, *117*(7), 5146–5173. https://doi.org/10.1021/ACS.CHEMREV.6B00448

Demir-Yilmaz, I., Guiraud, P., & Formosa-Dague, C. (2021). The contribution of Atomic Force Microscopy (AFM) in microalgae studies: A review. *Algal Research*, *60*, 102506. https://doi.org/10.1016/J.ALGAL.2021.102506

Dufrêne, Y. F. (2004). Using nanotechniques to explore microbial surfaces. *Nature Reviews. Microbiology*, *2*(6), 451–460. https://doi.org/10.1038/NRMICRO905

Dufrêne, Y. F., Ando, T., Garcia, R., Alsteens, D., Martinez-Martin, D., Engel, A., Gerber, C., & Müller, D. J. (2017). Imaging modes of atomic force microscopy for application in molecular and cell biology. *Nature Nanotechnology*, *12*(4), 295–307. https://doi.org/10.1038/NNANO.2017.45

Dufrêne, Y. F., Viljoen, A., Mignolet, J., & Mathelié-Guinlet, M. (2021a). AFM in cellular and molecular microbiology. *Cellular Microbiology*, *23*(7). https://doi.org/10.1111/CMI.13324

Dufrêne, Y. F., Viljoen, A., Mignolet, J., & Mathelié-Guinlet, M. (2021b). AFM in cellular and molecular microbiology. *Cellular Microbiology*, *23*(7). https://doi.org/10.1111/CMI.13324

EC, C., AF, M., D, de M.-D., & IJ, C. (2018). Polyethylene glycol molecular weight influences the ClearT2 optical clearing method for spheroids imaging by confocal laser scanning microscopy. *Journal of Biomedical Optics*, *23*(5), 1. https://doi.org/10.1117/1.JBO.23.5.055003

Efremov, Y. M., Okajima, T., & Raman, A. (2019). Measuring viscoelasticity of soft biological samples using atomic force microscopy. *Soft Matter*, *16*(1), 64–81. https://doi.org/10.1039/C9SM01020C

Formosa-Dague, C., Duval, R. E., & Dague, E. (2018). Cell biology of microbes and pharmacology of antimicrobial drugs explored by Atomic Force Microscopy. *Seminars in Cell & Developmental Biology*, *73*, 165–176. https://doi.org/10.1016/J.SEMCDB.2017.06.022

Fukuma, T., Fukuma, & Takeshi. (2024). Visualizing the inside of three-dimensional self-organizing systems by three-dimensional atomic force microscopy. *JaJAP*, *63*(1), 010801. https://doi.org/10.35848/1347-4065/ACF721

Geisse, N. A. (2009). AFM and combined optical techniques. *Materials Today*, *12*(7–8), 40–45. https://doi.org/10.1016/S1369-7021(09)70201-9

Guillaume-Gentil, O., Potthoff, E., Ossola, D., Franz, C. M., Zambelli, T., & Vorholt, J. A. (2014). Force-controlled manipulation of single cells: from AFM to FluidFM. *Trends in Biotechnology*, *32*(7), 381–388. https://doi.org/10.1016/J.TIBTECH.2014.04.008

Hammoudeh, N., Soukkarieh, C., Murphy, D. J., & Hanano, A. (2020). Involvement of hepatic lipid droplets and their associated proteins in the detoxification of aflatoxin B1 in aflatoxin-resistance BALB/C mouse. *Toxicology Reports*, *7*, 795–804. https://doi.org/10.1016/J.TOXREP.2020.06.005

Ichikawa, T., Alam, M. S., Penedo, M., Matsumoto, K., Fujita, S., Miyazawa, K., Furusho, H., Miyata, K., Nakamura, C., & Fukuma, T. (2023). Protocol for live imaging of intracellular nanoscale structures using atomic force microscopy with nanoneedle probes. *STAR Protocols*, *4*(3), 102468. https://doi.org/10.1016/J.XPRO.2023.102468

Israel, Y., Tenne, R., Oron, D., & Silberberg, Y. (2017). Quantum correlation enhanced super-resolution localization microscopy enabled by a fibre bundle camera. *Nature Communications 2017 8:1*, *8*(1), 1–5. https://doi.org/10.1038/ncomms14786

Jang, W. H., Kwon, S., Shim, S., Jang, W. S., Myung, J. K., Yang, S., Park, S., & Kim, K. H. (2018). Comparison between reflectance confocal microscopy and 2-photon microscopy in early detection of cutaneous radiation injury in a mouse model in vivo. *Journal of Biophotonics*, *11*(10). https://doi.org/10.1002/JBIO.201700337

Kasas, S., Stupar, P., & Dietler, G. (2018). AFM contribution to unveil pro- and eukaryotic cell mechanical properties. *Seminars in Cell & Developmental Biology*, *73*, 177–187. https://doi.org/10.1016/J.SEMCDB.2017.08.032

Khodavirdipour, A., Mehregan, M., Rajabi, A., & Shiri, Y. (2019a). Microscopy and its Application in Microbiology and Medicine From Light to Quantum Microscopy: A Mini Review. *Avicenna Journal of Clinical Microbiology and Infection*, *6*(4), 133–137. https://doi.org/10.34172/AJCMI.2019.24

Khodavirdipour, A., Mehregan, M., Rajabi, A., & Shiri, Y. (2019b). Microscopy and its Application in Microbiology and Medicine From Light to Quantum Microscopy: A Mini Review. *Avicenna Journal of Clinical Microbiology and Infection*, *6*(4), 133–137. https://doi.org/10.34172/AJCMI.2019.24

Kim, T. I., Kwon, B., Yoon, J., Park, I. J., Bang, G. S., Park, Y. K., Seo, Y. S., & Choi, S. Y. (2017). Antibacterial Activities of Graphene Oxide-Molybdenum Disulfide Nanocomposite Films. *ACS Applied Materials & Interfaces*, *9*(9), 7908–7917. https://doi.org/10.1021/ACSAMI.6B12464

Kobayashi, K., Kodera, N., Kasai, T., Tahara, Y. O., Toyonaga, T., Mizutani, M., Fujiwara, I., Ando, T., & Miyata, M. (2021). Movements of mycoplasma mobile gliding machinery detected by high-speed atomic force microscopy. *MBio*, *12*(3). https://doi.org/10.1128/MBIO.00040-21/SUPPL\_FILE/MBIO.00040-21-SM003.AVI

Koutsogiannis, Z., Mina, J. G. M., Suman, R., & Denny, P. W. (2023). Assessment of Toxoplasma gondii lytic cycle and the impact of a gene deletion using 3D label-free optical diffraction holotomography. *Frontiers in Cellular and Infection Microbiology*, *13*, 1237594. https://doi.org/10.3389/FCIMB.2023.1237594/BIBTEX

Kreplak, L. (2016). Introduction to Atomic Force Microscopy (AFM) in Biology. *Current Protocols in Protein Science*, *85*, 17.7.1-17.7.21. https://doi.org/10.1002/CPPS.14

Krieg, M., Fläschner, G., Alsteens, D., Gaub, B. M., Roos, W. H., Wuite, G. J. L., Gaub, H. E., Gerber, C., Dufrêne, Y. F., & Müller, D. J. (2018). Atomic force microscopy-based mechanobiology. *Nature Reviews Physics 2018 1:1*, *1*(1), 41–57. https://doi.org/10.1038/s42254-018-0001-7

Larrazabal, C., Silva, L. M. R., Hermosilla, C., & Taubert, A. (2021). Ezetimibe blocks Toxoplasma gondii-, Neospora caninum- and Besnoitia besnoiti-tachyzoite infectivity and replication in primary bovine endothelial host cells. *Parasitology*, *148*(9), 1107–1115. https://doi.org/10.1017/S0031182021000822

Liu, P. Y., Chin, L. K., Ser, W., Chen, H. F., Hsieh, C. M., Lee, C. H., Sung, K. B., Ayi, T. C., Yap, P. H., Liedberg, B., Wang, K., Bourouina, T., & Leprince-Wang, Y. (2016). Cell refractive index for cell biology and disease diagnosis: past, present and future. *Lab on a Chip*, *16*(4), 634–644. https://doi.org/10.1039/C5LC01445J

López-Osorio, S., Silva, L. M. R., Chaparro-Gutierréz, J. J., Velásquez, Z. D., Taubert, A., & Hermosilla, C. (2020). Optimized excystation protocol for ruminant Eimeria bovis- and Eimeria arloingi-sporulated oocysts and first 3D holotomographic microscopy analysis of differing sporozoite egress. *Parasitology International*, *76*. https://doi.org/10.1016/J.PARINT.2020.102068

Lopez-Osorio, S., Velasquez, Z. D., Conejeros, I., Taubert, A., & Hermosilla, C. (2022). Morphometric analysis of aerobic Eimeria bovis sporogony using live cell 3D holotomographic microscopy imaging. *Parasitology Research*, *121*(4), 1179–1189. https://doi.org/10.1007/S00436-021-07338-X

Martínez-Montelongo, J. H., Medina-Ramírez, I. E., Romo-Lozano, Y., & Zapien, J. A. (2020). Development of a sustainable photocatalytic process for air purification. *Chemosphere*, *257*. https://doi.org/10.1016/J.CHEMOSPHERE.2020.127236

Mathurin, J., Deniset-Besseau, A., Bazin, D., Dartois, E., Wagner, M., & Dazzi, A. (2022). Photothermal AFM-IR spectroscopy and imaging: Status, challenges, and trends. *Journal of Applied Physics*, *131*(1). https://doi.org/10.1063/5.0063902

Maver, U., Velnar, T., Gaberšček, M., Planinšek, O., & Finšgar, M. (2016). Recent progressive use of atomic force microscopy in biomedical applications. *TrAC Trends in Analytical Chemistry*, *80*, 96–111. https://doi.org/10.1016/J.TRAC.2016.03.014

Meyer, R. A. (1979). Light scattering from biological cells: dependence of backscatter radiation on membrane thickness and refractive index. *Applied Optics*, *18*(5), 585. https://doi.org/10.1364/AO.18.000585

Miranda, A., Gómez-Varela, A. I., Stylianou, A., Hirvonen, L. M., Sánchez, H., & De Beule, P. A. A. (2021). How did correlative atomic force microscopy and super-resolution microscopy evolve in the quest for unravelling enigmas in biology? *Nanoscale*, *13*(4), 2082–2099. https://doi.org/10.1039/D0NR07203F

Moreno, H., Archetti, L., Gibbin, E., Grandchamp, A. E., & Fréchin, M. (2021). Artificial Intelligence-Powered Automated Holotomographic Microscopy Enables Label-Free Quantitative Biology. *Microscopy Today*, *29*(5), 24–32. https://doi.org/10.1017/S1551929521001139

Park, D., Lee, D., Kim, Y., Park, Y., Lee, Y. J., Lee, J. E., Yeo, M. K., Kang, M. W., Chong, Y., Han, S. J., Choi, J., Park, J. E., Koh, Y., Lee, J., Park, Y. K., Kim, R., Lee, J. S., Choi, J., Lee, S. H., … Chung, C. (2023). Cryobiopsy: A Breakthrough Strategy for Clinical Utilization of Lung Cancer Organoids. *Cells*, *12*(14), 1854. https://doi.org/10.3390/CELLS12141854/S1

Park, Y. K., Depeursinge, C., & Popescu, G. (2018). Quantitative phase imaging in biomedicine. *Nature Photonics 2018 12:10*, *12*(10), 578–589. https://doi.org/10.1038/s41566-018-0253-x

Salucci, S., Battistelli, M., Burattini, S., Sbrana, F., & Falcieri, E. (2020). Holotomographic microscopy: A new approach to detect apoptotic cell features. *Microscopy Research and Technique*, *83*(12), 1464–1470. https://doi.org/10.1002/JEMT.23539

Sandoz, P. A., Tremblay, C., Equis, S., Pop, S., Pollaro, L., Cotte, Y., van der Goot, F. G., & Frechin, M. (2018). *Label free 3D analysis of organelles in living cells by refractive index shows pre-mitotic organelle spinning in mammalian stem cells*. https://doi.org/10.1101/407239

Schoenwald, K., Peng, Z. C., Noga, D., Qiu, S. R., & Sulchek, T. (2010). Integration of atomic force microscopy and a microfluidic liquid cell for aqueous imaging and force spectroscopy. *The Review of Scientific Instruments*, *81*(5). https://doi.org/10.1063/1.3395879

Shi, X., Qing, W., Marhaba, T., & Zhang, W. (2020). Atomic force microscopy - Scanning electrochemical microscopy (AFM-SECM) for nanoscale topographical and electrochemical characterization: Principles, applications and perspectives. *Electrochimica Acta*, *332*, 135472. https://doi.org/10.1016/J.ELECTACTA.2019.135472

Srichana, T., Thawithong, E., Nakpheng, T., & Paul, P. K. (2023). Flow cytometric analysis, confocal laser scanning microscopic, and holotomographic imaging demonstrate potentials of levofloxacin dry powder aerosols for TB treatment. *Journal of Drug Delivery Science and Technology*, *84*. https://doi.org/10.1016/J.JDDST.2023.104464

Staunton, J. R., Doss, B. L., Lindsay, S., & Ros, R. (2016). Correlating confocal microscopy and atomic force indentation reveals metastatic cancer cells stiffen during invasion into collagen I matrices. *Scientific Reports*, *6*. https://doi.org/10.1038/SREP19686

Taylor, M. A., Janousek, J., Daria, V., Knittel, J., Hage, B., Bachor, H. A., & Bowen, W. P. (2013). Biological measurement beyond the quantum limit. *Nature Photonics*, *7*(3), 229–233. https://doi.org/10.1038/nphoton.2012.346

Taylor, M. A., Janousek, J., Daria, V., Knittel, J., Hage, B., Bachor, H. A., & Bowen, W. P. (2014). Subdiffraction-limited quantum imaging within a living cell. *Physical Review X*, *4*(1). https://doi.org/10.1103/PHYSREVX.4.011017/FIGURES/3/THUMBNAIL

Uchihashi, T., & Ganser, C. (2020). Recent advances in bioimaging with high-speed atomic force microscopy. *Biophysical Reviews*, *12*(2), 363. https://doi.org/10.1007/S12551-020-00670-Z

V. D. dos Santos, A. C., Hondl, N., Ramos-Garcia, V., Kuligowski, J., Lendl, B., & Ramer, G. (2023). AFM-IR for Nanoscale Chemical Characterization in Life Sciences: Recent Developments and Future Directions. *ACS Measurement Science Au*, *3*(5), 301–314. https://doi.org/10.1021/ACSMEASURESCIAU.3C00010

Villalba, M. I., Venturelli, L., Arnal, L., Masson, C., Dietler, G., Vela, M. E., Yantorno, O., & Kasas, S. (2022). Effect of antibiotics on mechanical properties of Bordetella pertussis examined by atomic force microscopy. *Micron*, *155*, 103229. https://doi.org/10.1016/J.MICRON.2022.103229

*What Are The Three Main Types Of Microscopes? | Sciencing*. (n.d.). Retrieved January 27, 2025, from https://www.sciencing.com/three-main-types-microscopes-12507/

Xiao, Y., Cheng, Y., He, P., Wu, X., & Li, Z. (2021). New insights into external layers of cyanobacteria and microalgae based on multiscale analysis of AFM force-distance curves. *The Science of the Total Environment*, *774*. https://doi.org/10.1016/J.SCITOTENV.2021.145680

Yakimovich, A., Witte, R., Andriasyan, V., Georgi, F., & Greber, U. F. (2018). Label-Free Digital Holo-tomographic Microscopy Reveals Virus-Induced Cytopathic Effects in Live Cells. *MSphere*, *3*(6). https://doi.org/10.1128/MSPHEREDIRECT.00599-18

Zdarta, A., & Kaczorek, E. (2023). Nanomechanical changes in probiotic bacteria under antibiotics exposure: Implications on Lactobacillus biofilm formation. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1870*(7), 119533. https://doi.org/10.1016/J.BBAMCR.2023.119533



FIG 1: Salvino D’Armates’s Magnifying Eyepiece



FIG 2: Zechariah and Hans’s First Compound Microscope



FIG 3: Galilei’s Compound Microscope



FIG 4: Anton Van Leeuwenhoek’s Lens Grinding Device

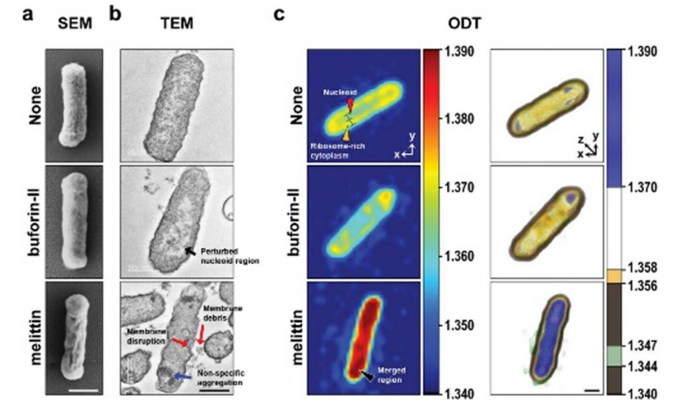


FIG 5: Comparison of Electronic Microscopy (SEM, TEM) imaging of E coli with HTM

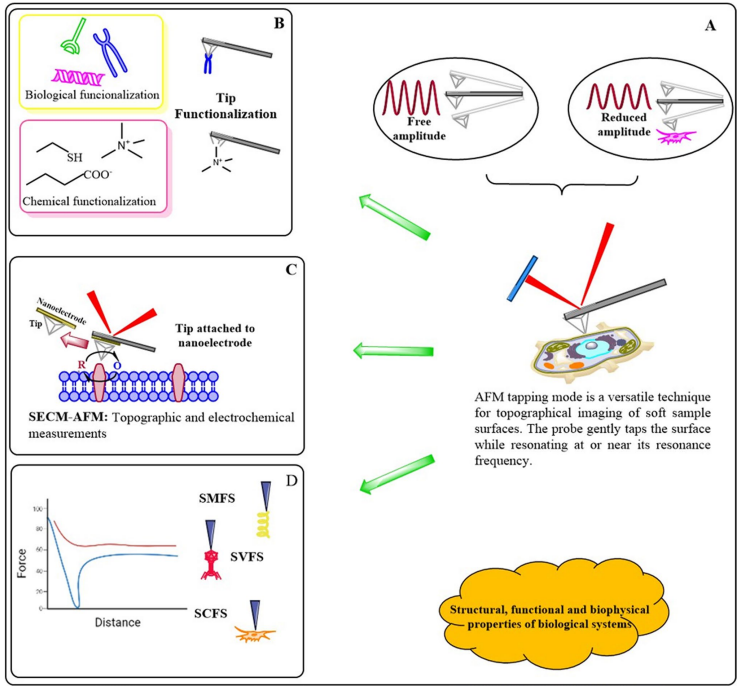


FIG 6: AFM - examine (BIO)Chemical properties of biological systems (cells, cellular components, living tissues).

A Dynamic Atomic Force Micorscopy is a suitable technique for biomedical research.

B Biological functionalization of AFM tips can be used to examine topographic and biomechanical properties (binding –unnfolding proteins- and rupture –to pull apart single receptor-ligand complexesforces). Chemically functionalized tips can be used to probe hydrophobic, hydrophilic domains or electrostatic interactions.

C, D SFMS (Single molecule Force Spectroscopy) uses tips derivatized with biomolecules, drugs, viruses (SVFS; Single Virus Force Spectroscopy) or cells (SCFS; Single Cell Force Spectroscopy) to study ligand receptor interactions.

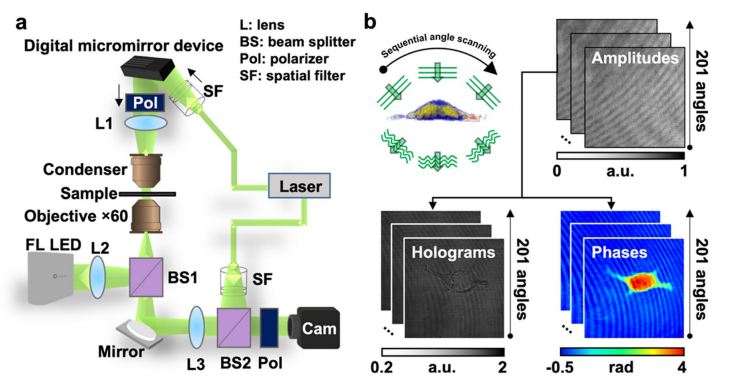


FIG 7: Holotomography microscopy (HTM) principle.

a Structure of HTM. (The sample and the reference beam combine to generate a 2D hologram).

b Holotomography mechanisms for 3D image construction.

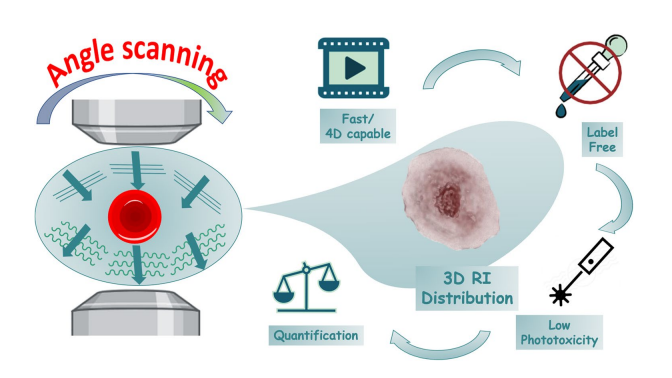


FIG 8: HTM: fast 3D visualization of living cells and tissues. Imaging of living cells.

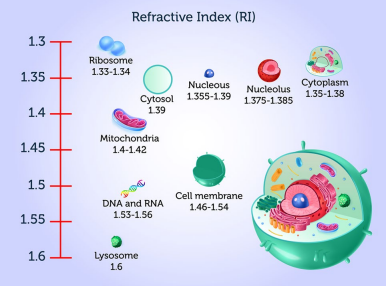


FIG 9: Refractive index value for different parts of cell.