Microsc. Microanal. 21 (Suppl 3), 2015
doi:10.1017/S1431927615001117 Paper No. 0032

Microscopic Techniques for Sterility Assurance Support in the Medical Products Industry

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Assurance of sterility in intravenous solutions, pharmaceutical drug products, and medical devices is of paramount importance for ensuring patient safety. Microbiologists use several analytical techniques to design, validate, and assure sterility of finished products. As medical devices and pharmaceuticals grow more complex, so have the challenges related to the microbial control analyses, including complex part geometries and variable surface properties. In these cases, direct visualization of a microorganism's interaction with a material, the progression of biofilm formation and growth, or the organization of inoculated spores with microscopic techniques is invaluable.

Recent advances in highly sensitive camera technology have resulted in the creation of systems with large, light-tight chambers that can measure extremely low levels of luminescence or fluorescence emission. These systems, typically equipped with x-ray imaging and often used for live animal studies, are also extremely valuable for discerning the initial locations of microorganism attachment to surfaces and devices (Fig. 1). In some cases, a localization of mere tens of organisms can be detected, leading to a very accurate temporal assignment of biofilm colonization and growth events.

A wide range of fluorescent stains specifically designed for bacteria have been developed and can be exploited in fluorescence and confocal microscopy. These stains allow for the study of bacterial viability, sporulation, biofilm organization, morphology, and many other aspects of interest (Fig. 2).

Critical point drying of vegetative organisms and biofilms (and air drying of endospores, which are much more robust) allows for the use of scanning electron microscopy to extend the length scale available for study and view the details of individual microorganism and biofilm morphology (Fig. 3). Embedding in resins and sectioning for transmission electron microscopy yields the ultimate level of detail and resolution, providing information on the organization of biofilm extracellular matrix components, mechanisms of sterilization, and so on (Fig. 4).

We employed a range of correlative microscopy techniques to study vaporous hydrogen peroxide sterilization of spores inoculated on surfaces and compare biofilms grown using a modified standard CDC reactor method with colony biofilms grown on filters on the surface of agar plates [1,2]. In the first case, microscopy revealed problems with the inoculation technique and was critical in an optimization study to find the best procedure for the particular materials in use. In the second case, fluorescence and electron microscopy were used to characterize and compare the morphological details of the two methods of biofilm growth. This study serves as an example highlighting the utility of microscopy in establishing and verifying model systems.

[1] JH Merritt *et al*, Current Protocols in Microbiology **1B.1** (2005), p. 1-17.
[2] ASTM E2562-12: Standard Test Method for Quantification of *Pseudomonas aeruginosa* Biofilm Grown with High Shear and Continuous Flow using CDC Biofilm Reactor. 2012.

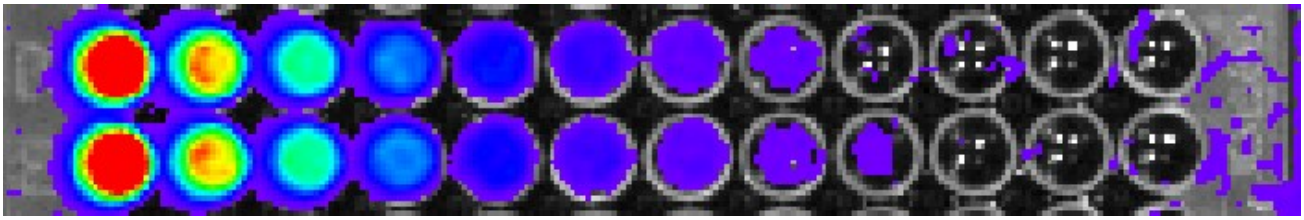

Figure 1. Two-fold dilution series of luminescent *Klebsiella pneuomoniae* in duplicate, demonstrating a detection limit of approximately 10-20 microorganisms emitting measurable photoluminescence within one well of a 96-well plate.

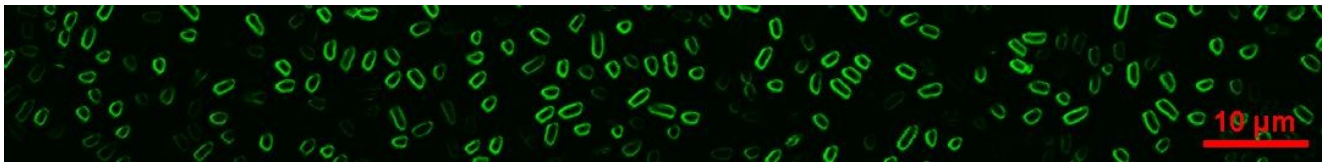

Figure 2. Membrane staining of individual bacteria in laser-scanning confocal microscopy. A wide variety of dyes allow for the study of viability, biofilm morphology, etc.

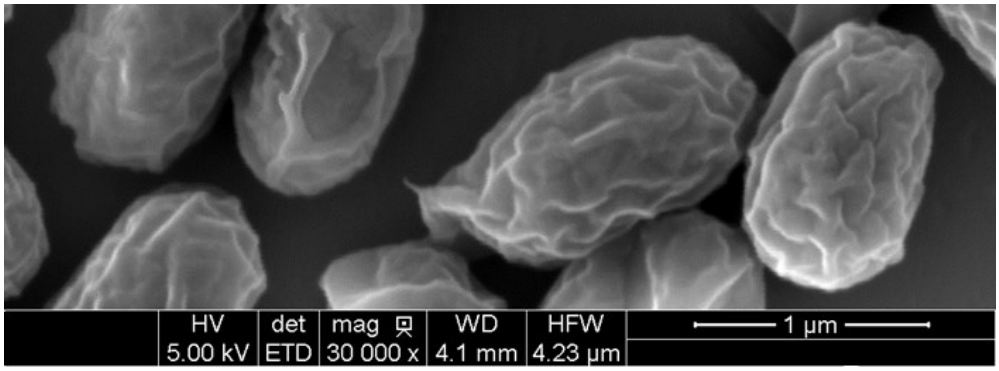

Figure 3. Scanning electron microscopy of bacterial endospores, a useful model organism in testing worst-case scenario sterilization conditions.

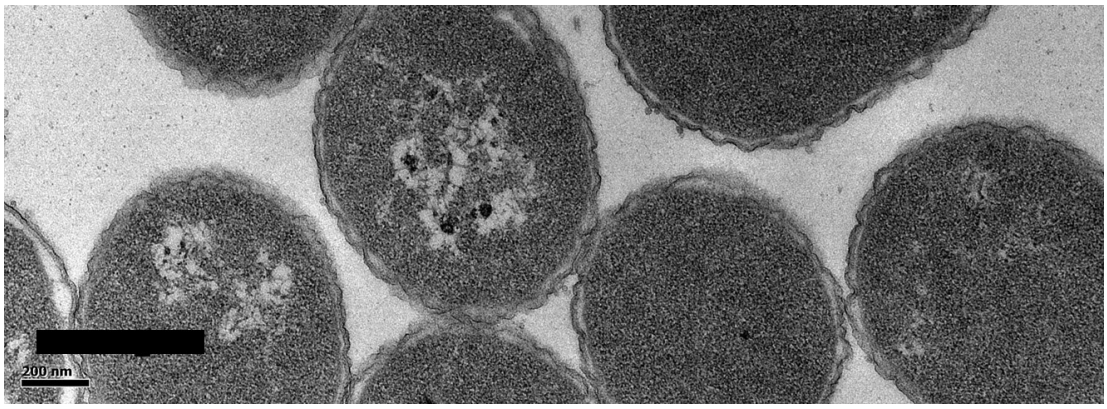

Figure 4. Transmission electron microscopy of bacteria, revealing high-resolution details of the layers of their membranes and inner organelles.