# Benefits of Low Voltage Transmission Electron Microscopy in Biopolymer Research

## Introduction

Low-voltage transmission electron microscopy (LVEM) is a powerful technique for imaging biological samples, including biopolymers. Biopolymers play a key role in materials and life sciences because they are sustainable and widely used in eco-friendly materials and biomedical applications., but imaging them using conventional electron microscopy is challenging. These challenges are caused by the composition of light elements and, thus, the low natural contrast of the image. One important class of biopolymers is polyhydroxyalkanoates (PHAs), which are found inside some microorganisms in the form of intracellular granules. These microorganisms produce them as energy storage, similar to how humans store fat for "rainy days." Visualizing these structures often requires staining agents like uranyl acetate or osmium tetroxide, which can be toxic and may cover fine details of the sample.

LVEM solves this problem by increasing image contrast without the need for staining, thus preserving the natural structure of biopolymers. Unlike high-voltage TEM, which operates at voltages 60–300 kV, LVEM operates at significantly lower voltages (5–25 kV). This lower energy leads to increased electron scattering, which naturally increases contrast. Electron microscope LVEM 25E (see Fig. 1) integrates multiple imaging and analytical modes – TEM, SEM, STEM, ED, and EDS – within a single device. These capabilities allow researchers to perform multimodal microscopy for more comprehensive studies of biopolymer structures.

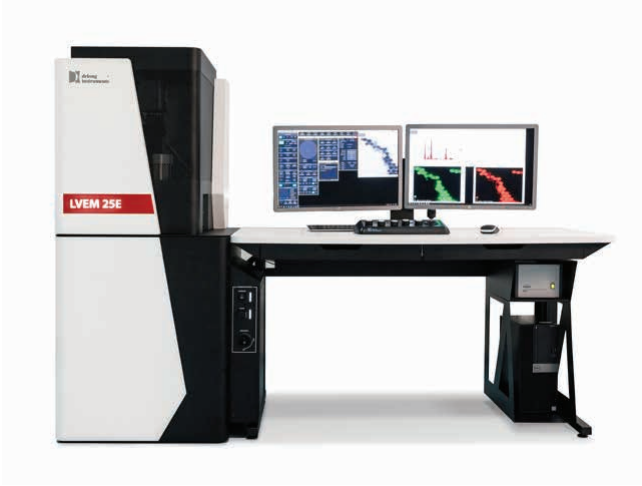


*Figure 1: Low voltage electron microscope (LVEM 25E) from Delong Instruments.*

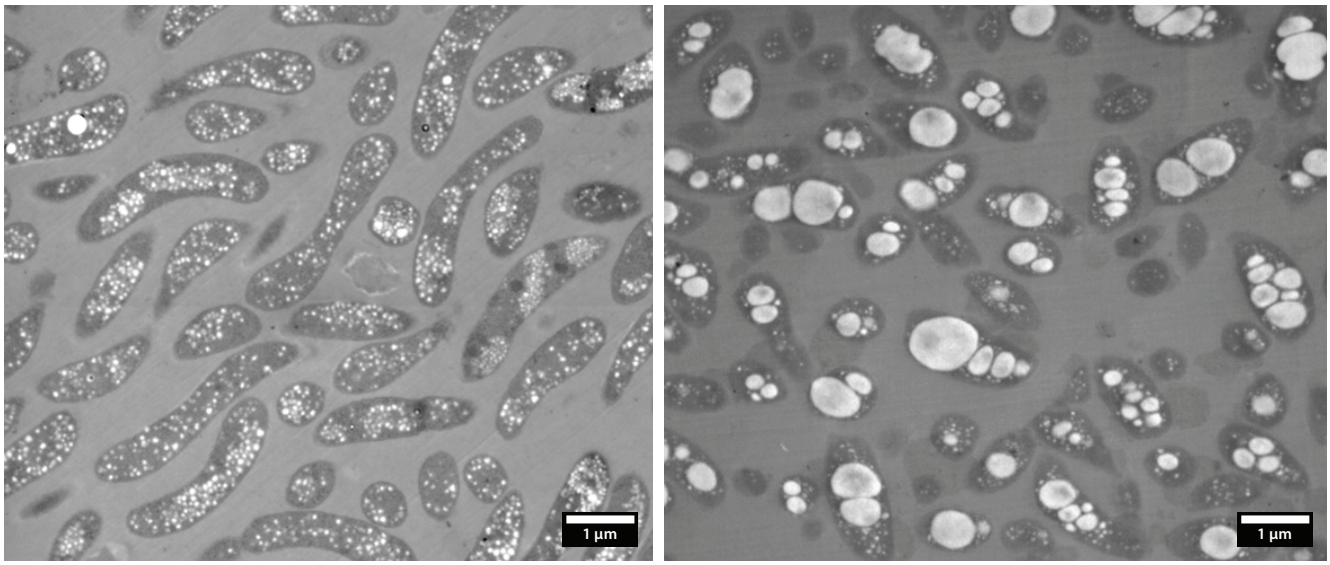


*Figure 2: Bacteria cells with PHB granules in TEM mode. LVEM allows direct visualization of PHB granules in bacterial cells and shows the internal structure and distribution of the polymer.*

## Polyhydroxyalkanoates

Biopolymers, in general, are natural, large molecular chains produced by living organisms, including proteins, nucleic acids, polysaccharides, and polyesters. They play a vital role in biological processes, providing structural support, storing genetic information, and facilitating biochemical reactions. Due to their biodegradability and sustainability, biopolymers are also widely used in biomedical, food packaging, and environmental applications [1].

Among the various biopolymers, polyhydroxyalkanoates (PHAs) stand out for their unique properties and applications. PHAs are a group of biodegradable polyesters synthesized by microorganisms as intracellular granules for energy storage (see Fig. 2). The most common representative of PHAs is polyhydroxybutyrate (PHB), produced through the fermentation of organic carbon sources such as saccharides or oils. PHAs are valued for their biocompatibility and biodegradability, making them suitable for use in applications such as biodegradable plastics, medical devices, and packaging. They offer an environmentally friendly alternative to petroleum-based plastics [2].

### Reduced need for staining

As discussed, high-voltage electron microscopes require sample treatment with toxic stains such as uranyl acetate and osmium tetroxide. These stains require special handling procedures and pose risks to health and the environment. LVEM minimizes or eliminates the need for such staining, offering a safer and more sustainable imaging method.

A recent study by Mrazová et al. [3] demonstrated that LVEM could effectively image the intracellular structures of cyanobacterial cells such as *Synechocystis* sp. PCC 6803 containing PHA granules. Unlike high-voltage TEM, LVEM provides clear ultrastructural details without requiring staining agents (see Fig. 3). The study also showed that electron beams accelerated to 25 kV in TEM mode are sufficient to penetrate standard 70 nm thick sections, ensuring high contrast and preserving sample integrity. This demonstrates that there are no limitations on the thickness of biological samples; rather, the system is designed to work without problems with standard sample thicknesses without any limitations.

Additionally, using STEM mode further enhanced image sharpness by minimizing the effect of chromatic aberration by scanning a focused electron beam across the sample and detecting the passed electrons at different scattering angles. Unlike TEM, which suffers from inelastic scattering that causes energy loss and image blurring, STEM is less affected by these distortions, especially for thicker samples. As a result, it produces sharper images with better image contrast (see Fig. 4).

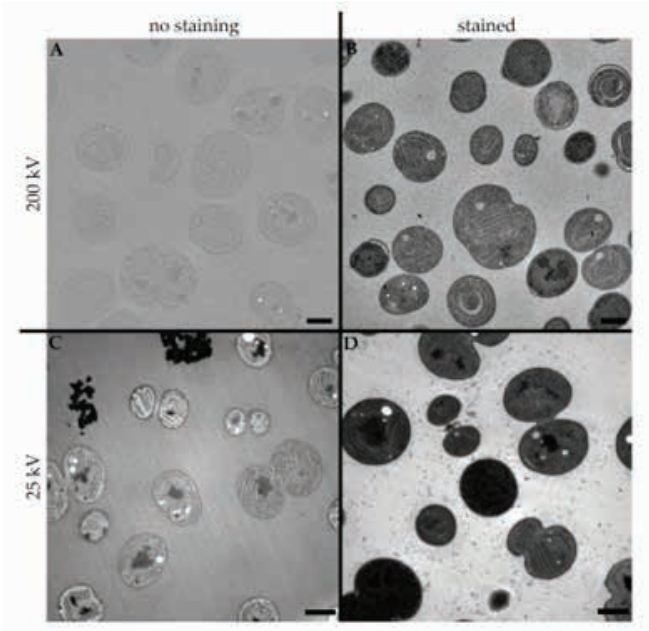


*Figure 3: Comparison of TEM images of cyanobacterial cells under different sample preparation protocols and electron beam energies: (A) 200 kV beam, no staining; (B) 200 kV beam, stained with uranyl acetate and lead citrate; (C) 25 kV beam, no staining; (D) 25 kV beam, stained with uranyl acetate and lead citrate. Scale bar: 1 µm [3].*

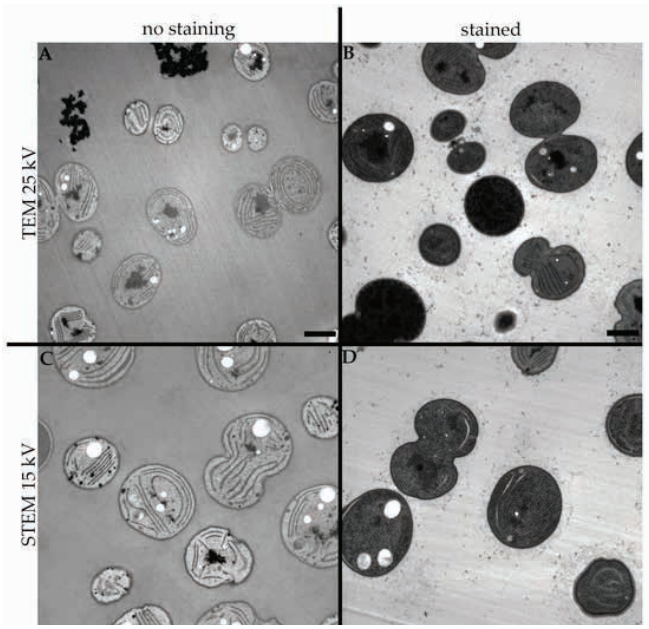


*Figure 4: Comparison of TEM and STEM images of cyanobacterial cells under different sample preparation protocols and electron beam energies: (A) TEM, 25 kV beam, no staining; (B) TEM, 25 kV beam, stained with uranyl acetate and lead citrate; (C) STEM, 15 kV beam, no staining; (D) STEM, 15 kV beam, stained with uranyl acetate and lead citrate. Scale bar: 1 µm [3].*

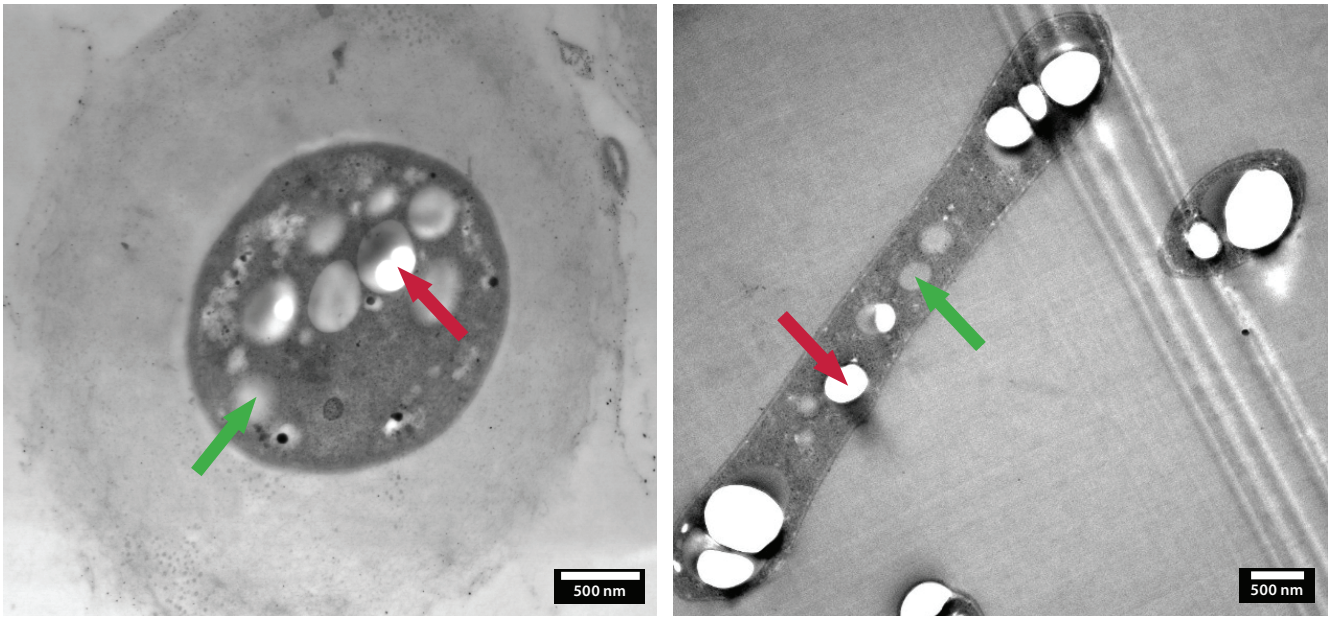


*Figure 5: Distinguishing PHA granules from holes in Azotobacter vinelandii (left) and Aneurinibacillus thermoaerophilus (right). Red arrow = hole, green arrow = PHA granules.*

Another challenge in biopolymer imaging is the composition and structural properties of biopolymers themselves. Because biopolymers are composed mainly of light elements – carbon, hydrogen, oxygen, and nitrogen – they often show low contrast in high-voltage electron microscopy. LVEM improves contrast, allowing clearer visualization of structures such as PHA granules in bacterial cells. For example, when imaging bacterial strains *Azotobacter vinelandii* and *Aneurinibacillus thermoaerophilus*, LVEM effectively distinguished PHA granules from empty spaces (holes) and revealed the morphology and spatial distribution of the granules (see Fig. 5).

## Conclusion

The LVEM 25E offers several advantages over high-voltage TEM imaging of PHA granules in microorganism cells. At an accelerating voltage of 25 kV, it provides high-contrast images even without sample treatment with toxic heavy metal-based staining agents, simplifying sample preparation while reducing health and environmental risks. The lower energy of the electron beam increases the scattering contrast, allowing a clear distinction between PHA granules and empty spaces (holes) in cells, which is often challenging with high-voltage TEM. The STEM mode of the LVEM 25E further improves image sharpness by minimizing the effect of chromatic aberration, allowing detailed visualization of intracellular structures without additional staining. In addition, the ability to work with standard sample thicknesses, rather than requiring ultrathin sections, makes this approach a practical tool for routine imaging of biological and material samples.

## References:

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