3D visualization and quantification of bioplastic PHA in a living bacterial cell

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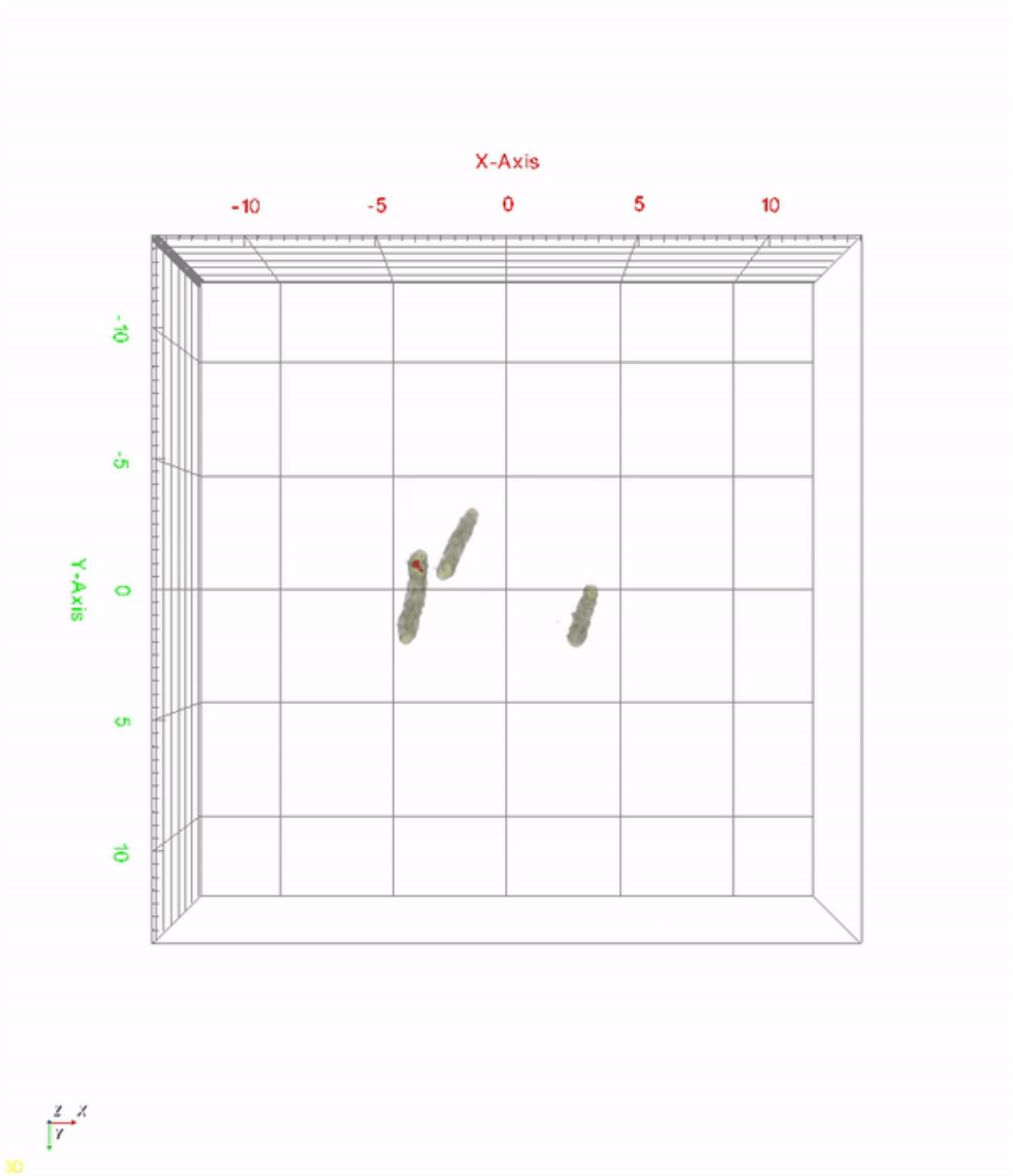


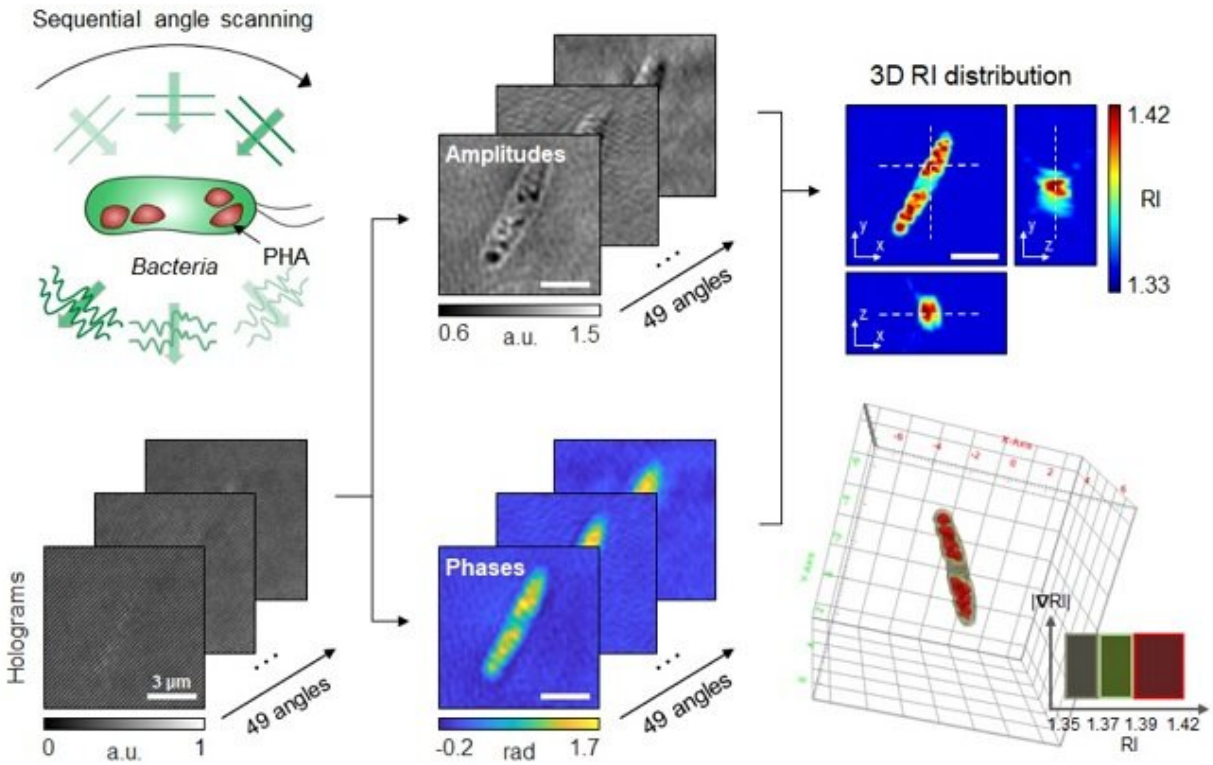
Time-lapse movie of recombinant E. coli cells for 520 minutes. The red color indicates in vivo PHA granules. The movie shows PHA granule formation and distribution during the cell division process. Credit: The Korea Advanced Institute of Science and Technology (KAIST)

A research team at KAIST has observed how bioplastic granule is being accumulated in living bacteria cells through 3D holographic microscopy. Their 3D imaging and quantitative analysis of the bioplastic 'polyhydroxyalkanoate' (PHA) via optical diffraction tomography provides insights into biosynthesizing sustainable substitutes for petroleum-based plastics.

The bio-degradable polyester polyhydroxyalkanoate (PHA) is being touted as an eco-friendly bioplastic to replace existing synthetic plastics. While carrying similar properties to general-purpose plastics such as polyethylene and polypropylene, PHA can be used in various industrial applications such as container packaging and disposable products.

PHA is synthesized by numerous bacteria as an energy and carbon storage material under unbalanced growth conditions in the presence of excess carbon sources. PHA exists in the form of insoluble granules in the cytoplasm. Previous studies on investigating in vivo PHA granules have been performed by using fluorescence microscopy, transmission electron microscopy (TEM), and electron cryotomography.

These techniques have generally relied on the statistical analysis of multiple 2D snapshots of fixed cells or the short-time monitoring of the cells. For the TEM analysis, cells need to be fixed and sectioned, and thus the investigation of living cells was not possible. Fluorescence-based techniques require fluorescence labeling or dye staining. Thus, indirect imaging with the use of reporter proteins cannot show the native state of PHAs or cells, and invasive exogenous dyes can affect the physiology and viability of the cells. Therefore, it was difficult to fully understand the formation of PHA granules in cells due to the technical limitations, and thus several mechanism models based on the observations have been only proposed.



Schematic process of 3D optical diffraction tomography for the bacterial cell accumulating bioplastic polyhydroxyalkanoate (PHA). A cell sample is illuminated at multiple sequential illumination angles (Left, Top). From the raw holograms recorded at individual angles (Left, Bottom), quantitative amplitude and phase information (Middle) is retrieved and the 3D refractive index distribution (Right, Top) is reconstructed. The 3D rendering image of the sample is then obtained (Right, Bottom). Credit: The Korea Advanced Institute of Science and Technology (KAIST)

The team of metabolic engineering researchers led by Distinguished Professor Sang Yup Lee and Physics Professor YongKeun Park, who established the startup Tomocube with his 3D holographic microscopy, reported the results of 3D quantitative label-free analysis of PHA granules in individual live bacterial cells by measuring the refractive

index distributions using optical diffraction tomography. The formation and growth of PHA granules in the cells of Cupriavidus necator, the most-studied native PHA (specifically, poly(3-hydroxybutyrate), also known as PHB) producer, and recombinant Escherichia coli harboring C. necator PHB biosynthesis pathway were comparatively examined.

From the reconstructed 3D refractive index distribution of the cells, the team succeeded in the 3D visualization and quantitative analysis of cells and intracellular PHA granules at a single-cell level. In particular, the team newly presented the concept of "in vivo PHA granule density." Through the statistical analysis of hundreds of single cells accumulating PHA granules, the distinctive differences of density and localization of PHA granules in the two micro-organisms were found. Furthermore, the team identified the key protein that plays a major role in making the difference that enabled the characteristics of PHA granules in the recombinant E. coli to become similar to those of C. necator.

The research team also presented 3D time-lapse movies showing the actual processes of PHA granule formation combined with cell growth and division. Movies showing the living cells synthesizing and accumulating PHA granules in their native state had never been reported before.

Professor Lee says that "this study provides insights into the morphological and physical characteristics of in vivo PHA as well as the unique mechanisms of PHA granule formation that undergo the phase transition from soluble monomers into the insoluble polymer, followed by granule formation. Through this study, a deeper understanding of PHA granule formation within the bacterial cells is now possible, which has great significance in that a convergence study of biology and physics was achieved. This study will help develop various bioplastics production processes in the future."

More information: So Young Choi et al, Three-dimensional label-free visualization and quantification of polyhydroxyalkanoates in individual bacterial cell in its native state, *Proceedings of the National Academy of Sciences* (2021). DOI: 10.1073/pnas.2103956118

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