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HoloV3C: unveiling eukaryotic flagella in 3D with holographic microscopy

The flagellum is pivotal in the survival mechanisms of eukaryotic cells and measuring its three-dimensional shape is essential to understanding these key mechanisms. However, accurately assessing the intricate structure of flagella has been challenging due to the lack of a reliable method for determining the 3D position of individual points. Our digital holographic microscopy (DHM) method overcomes this hurdle, enabling dynamic 3D tracking of eukaryotic cell flagella shape and motility. We harness holographic microscopy benefits, including high-speed imaging of large sample volumes, for 4D tracking (X, Y, Z, and time) of microorganisms and their flagella. This technique offers precise 3D localization of nanometer-sized, unlabeled structures and is robust against changes in reconstruction parameters. We reconstructed for the first time the shape of a 200 nm diameter *Chrysochromulina simplex* flagellum and measured mouse sperm flagella over time, capturing approximately 800 points along a single flagellum. Our proposed method unleashes the full potential of digital holography, enabling high-speed and precise 3D tracking of microorganisms and their flagella at the nanoscale across depths that are beyond the reach of other existing techniques. This opens new avenues for studying flagella's roles in cellular functions and survival strategies.

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