

Stealthy microscopy method visualizes *E. coli* sub-cellular structure in 3-D

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A sub-cellular world has been opened up for scientists to study *E. coli* and other tissues in new ways, thanks to a microscopy method that stealthily provides three-dimensional, high-quality images of the internal structure of cells without disturbing the specimen.

By combining a novel algorithm with a recently-developed add-on technique for commercial microscopes, researchers at the University of Illinois have created a fast, non-invasive 3D method for visualizing, quantifying, and studying cells without the use of fluorescence or contrast agents.

In a paper published online today in the journal [PLoS ONE](#), the researchers who developed the technique reported that they were able to use it to visualize the *E. coli* bacteria with a combination of speed, scale, and resolution unparalleled for a label-free method.

The method is based on a broadband interferometric technique known as Spatial [Light Interference](#) Microscopy (SLIM) that was designed by Beckman Institute researcher Gabriel Popescu as an add-on module to a commercial phase contrast microscope. SLIM is extremely fast and sensitive at multiple scales (from 200 nm and up) but, as a linear optical system, its resolution is limited by diffraction.

By applying a novel deconvolution algorithm to retrieve sub-diffraction limited resolution information from the fields measured by SLIM, Popescu and his fellow researchers were able to render tomographic images with a resolution beyond SLIM's diffraction limits. They used the sparse reconstruction method to render 3D reconstructed images of *E. coli* cells, enabling label-free visualization of the specimens at sub-cellular scales.

Last year the researchers successfully

demonstrated a new optical technique that provides 3D measures of complex fields called Spatial Light Interference Tomography (SLIT) on live neurons and photonic crystal structures. In this project they developed a novel algorithm to further extend the three-dimensional capabilities by performing deconvolution on the measured 3D field, based on modeling the image using sparsity principles. This microscopy capability, called dSLIT, was used to visualize coiled sub-cellular structures in *E. coli* cells.

The researchers said that these structures have only been observed using specialized strains and plasmids and fluorescence techniques, and usually on non-living cells. These new methods provide a practical way for non-invasive study of such structures.

Mustafa Mir is first author on the paper and member of Popescu's Quantitative Light Imaging Laboratory at Beckman. Mir said that studying and understanding the three-dimensional internal structure of living cells is essential for furthering our understanding of biological function.

"Visualizing them is extremely challenging due to their small size and transparent nature," Mir said. "This new method, however, provides a way to take advantage of the intrinsic properties of these very small, transparent cells non-invasively and without the use of fluorescence techniques and contrast agents.

"Previous studies have thus used extrinsic contrast such as fluorescence and specialized strains in combination with complex superresolution techniques for such studies. This will allow biologists to study sub-cellular structures while minimally perturbing the cell from its natural state."

The researchers wrote that the method addresses two major issues in cell microscopy: lack of contrast, due to the thin and optically transparent

nature of cells, and diffraction limited resolution.

"Although several such structures have been previously identified, little is known about their function and behavior due to the practical difficulties involved in imaging them," they concluded. "The results presented here indicate that dSLIT can be used to characterize and study such sub-cellular structure in a practical and non-invasive manner, opening the door for a more in depth understanding of the biology."

Provided by University of Illinois at Urbana-Champaign

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