Multiparametric quantification of bacterial cells using digital holographic microscopy

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The ability to perform rapid, high-throughput classification of bacterial cells is a critical requirement across numerous domains, including food safety, biomedical research, clinical diagnostics, and the surveillance of antimicrobial resistance. Within this context, bacterial infections are especially concerning, given their potential to escalate swiftly into life-threatening sepsis [1].

Bacterial dry mass, defined as the total weight of non-aqueous cellular components—including proteins, nucleic acids, carbohydrates, and lipids—has attracted considerable attention over the past decade [2]. Alongside morphological characteristics, dry mass represents a highly informative parameter of bacterial physiology. Given that proteins constitute approximately half of the total dry mass and are central to virtually all cellular functions, quantifying this metric offers critical insights into the biosynthetic and degradative pathways operating within the cell.

Within this framework, quantitative phase imaging (QPI) has emerged over the past decades as a powerful, label-free, non-invasive technique capable of real-time application in biological research [3]. By capturing optical phase variations, QPI yields precise measurements of cellular dry mass distribution. Recent advances in QPI have enable single-bacterium resolution [4] while maintaining high-throughput capabilities. Digital holographic microscopy (DHM), a subclass of QPI, is emerging as a valuable technique for bacterial identification classification. In this study, transmission-mode DHM, figure 1a), to quantify the morphological features mass and Staphylococcus epidermidis (S.epidermis) and Escherichia coli (E.coli), figure 1b). To achieve this, we developed an image-processing workflow with background correction, Gaussian filtering, and adaptive masking, enabling reliable extraction of both cellular dry mass and morphology, figure 1c). This multiparametric approach provides a richer basis for bacterial characterization

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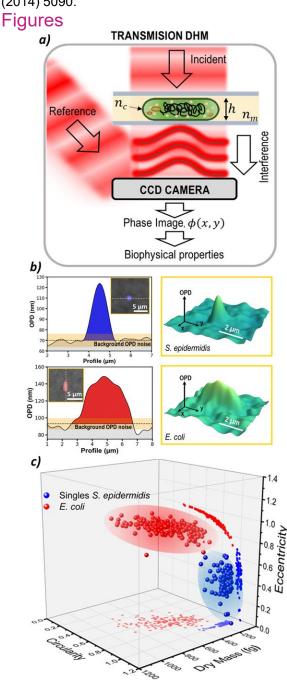


Figure 1. a) Schematics of the DHM measurement. b) OPD profiles and 3D representations of S.epidermis and E.coli bacteria. c) Multiparametric classification of both bacterial strands.