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Automated Multiple ROI Labeling for Intracellular Dynamics-Resolved Scattering (IDRS) in Single Bacterial Analysis

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Real-time monitoring of metabolism at the single-bacterial level is essential for microbiology and antibiotic research. Conventional fluorescence labeling can perturb cellular metabolism or limit long-term observation, highlighting the need for label-free techniques such as Intracellular Dynamics-Resolved Scattering (IDRS). IDRS enables tracking of spatiotemporal scattering fluctuations within bacteria, providing metabolic state-dependent patterns and potential markers of antibiotic response. In this study, we developed a MATLAB-based automated analysis pipeline to address the limitations of manual Region of Interest (ROI) annotation in IDRS images. Manual labeling is subject to user bias and may lead to inconsistent classification of bacterial morphology. The proposed algorithm consists of blob detection, cluster identification, Principle component analysis (PCA)-based ellipse fitting, and minor-axis adjustment to maintain biological relevance. The algorithm first removes low-intensity pixels (intensity < 40) to eliminate noise from background signals or perturbations, then identifies blobs exceeding the threshold. Blobs are filtered based on pixel area (area > 20) to exclude small noise, and adjacent blobs are clustered to define individual bacteria. Cluster ellipses are fitted using PCA to capture principal components, with minor-axis adjustment ensuring biologically accurate morphology representation. Validation using E. coli time-lapse microscopy demonstrated stable multi-ROI labeling, effective separation of closely spaced cells, and real-time processing capability. The proposed method significantly reduces manual annotation time while preserving accurate bacterial morphology. This automated ROI labeling framework enables large-scale dataset generation for deep learning and can be extended to object detection model training and time-series data acquisition across multiple bacterial species, facilitating bacterial classification and

antibiotic susceptibility prediction.