Automated classification of single E. coli cells for high-throughput assays at 60x magnification

Introduction

The size, shape and other morphological characteristics of bacteria can be indicative of the cell health and viability [1], similar to eukaryotic cells. Morphological changes can be indicative of susceptibility or resistance to antibiotic treatment [2,3] and can be directly correlated with genetic variation for functional genetic studies [4 French]. As such, there is growing appeal applying real-time microscopy for development of antimicrobial susceptibility testing (AST) [5]. Moreover, morphology coupled with direct visualization of intra-bacterial structural may facilitate mechanism of action in bacteriotoxicity studies through direct visualization. Visualization of vancomycin, for instance, was directly demonstrated to bind sites of peptidoglycan synthesis [6] as it disrupts cell wall synthesis in Gram-positive bacteria.

The small size of bacterium, which are typically on a length scale of ~1 μm, place numerous demands on the imaging system used to acquire imaging data in an automated fashion. High magnification objectives of 40x – 60x is required. Due to the narrow depth of field characterizing such hardware requires that the autofocus reliably place the cells on the surface of the substrate in the objective’s focal plane to ensure a sharp image is generated. Lastly, screening studies involving time-lapse imaging to capture dynamics necessitate precise sample movement to image the same cells over time.

The Hermes® HCS imaging system was used here to acquire time lapse images at 60x magnification, as shown in Figure 1 and Figure 2. Image analysis was performed with Athena® software using the Cell Morphology application to count the number of E. coli cells present on the substrate surface and quantify their populations based on shape.

Assay Workflow

E. coli bacteria were grown to OD 0.6 in LB media to ensure they were in exponential growth phase. Small samples were extracted and mixed with FM 4-64 membrane staining dye, which labels the outer membrane of the E. coli cells. The labeled cells were further

E. coli cells exhibit morphology changes as the population grows, with the ensemble becoming more elliptical.
diluted and placed into 8-well Ibidi chamber slides. The slide sample was imaged at 60x with one acquisition every 5 minutes for two hours.

The resulting images were loaded into Athena® software for single-cell identification and counting. Because cell shape is indicative of viability and proliferative capacity, the built-in subpopulation tool within Athena® software enabled isolation of cells having smaller, more roundish shape using the morphological axial ratio and solidity attributes as the selection criteria. In this manner, the growth data of bacteria having different shape properties could readily be plotted and compared against one another.

**Results & Conclusion**

Elliptical, longer E. coli cells are representative of growing cells during the exponential phase, whereas they are shorter and more ovoid and compact during stationary phase [7]. Figure 2 depicts that although the bacteria population increases over time, the relative proportion of the population consisting of small and ovoid cells decreases over time (Fig 2C). Such population decrease is consistent with exponential growth and could potentially be applicable to the identification of novel compounds that induce stationary phase and prevent exponential growth.

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**References**

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