



## Application Note

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

## Introduction

Size and shape of bacteria can be revealing of cellular health and viability [1], similar to eukaryotic cells. Morphological changes can also be indicative of susceptibility or resistance to antibiotic treatment [2,3] and can be directly correlated with genetic variation for functional genetic studies [4]. 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 structures may facilitate studies of mechanism of action in bacterial toxicity through direct visualization. Imaging of vancomycin, for instance, was directly demonstrated to bind sites of peptidoglycan synthesis [6] as it disrupts cell wall synthesis in Gram-positive bacteria.

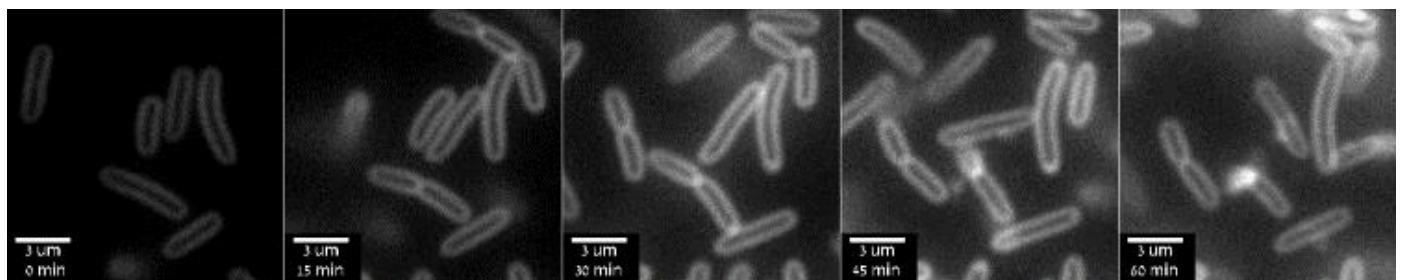
The small size of bacterium, which are typically on a length scale of  $\sim 1 \mu\text{m}$ , place numerous demands on the imaging system used to acquire imaging data in an automated fashion. High magnification objectives of 40x – 60x are required. The narrow depth of field

characterizing such optics requires that the autofocus reliably place the cells on the surface of the substrate in the objective's focal plane to ensure capture of a sharp, high contrast image. Lastly, screening studies involving time-lapse imaging to capture dynamics necessitate precise sample movement to image the same cells over time.

The **Hermes**<sup>®</sup> 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**<sup>®</sup> 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.

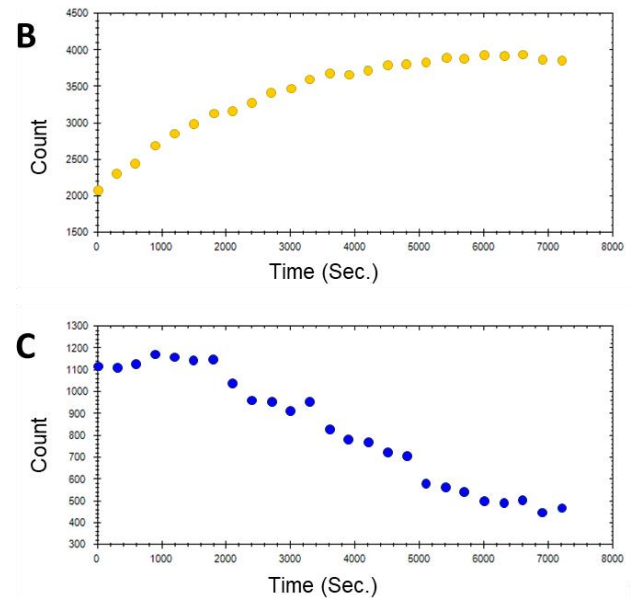
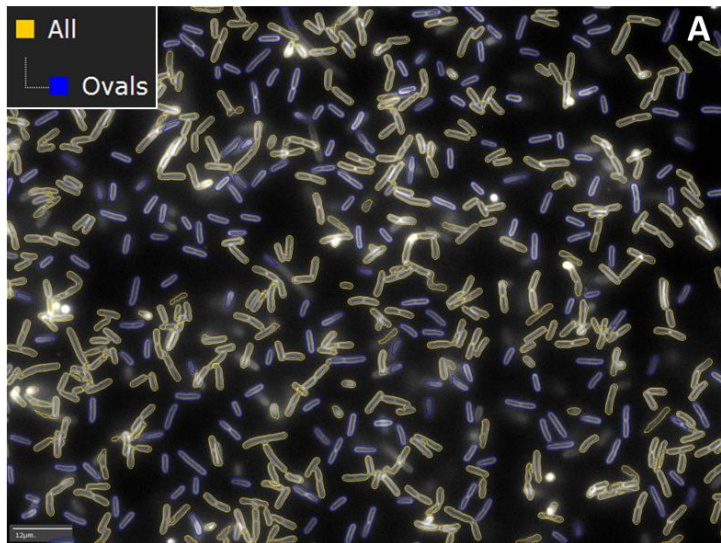
## Assay Workflow

*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 diluted and placed into several wells of an



**Figure 1 – Example time-lapse images (cropped) of *E. coli* cells at 60x in 15 minute intervals for one hour.**

*E. coli* cells exhibit morphology changes as the population grows, with the ensemble becoming more elongated.



**Figure 2 – Sub-population definition and growth-time curves.** **A)** The cells are imaged at 60x magnification, segmented for identification and separated into populations. Oval-shaped cells (blue) have high axial ratios and high solidity, longer and more elliptical cells are in yellow. **B&C)** The time-plots of the general population (B, yellow) and Oval subpopulation (C), which are generated automatically within the **Athena®**, depict population changes over time.

**8-well ibidi chamber** slide having a thin, glass bottom. 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. Cells having smaller, more roundish shape were isolated from the ensemble using **Athena's®** subpopulation tool with two morphological attributes: axial ratio and solidity. In this manner, the growth data of bacteria having different shapes could readily be plotted and compared against one another. Such analysis is of interest because cell shape and its prevalence within a population is indicative of overall viability and proliferative capacity.

## 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 alterations in its prevalence could potentially be applicable to the identification of novel compounds that induce stationary phase and prevent exponential growth.

## Acknowledgement

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## References

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