Microbiology with ARCHIMEDES

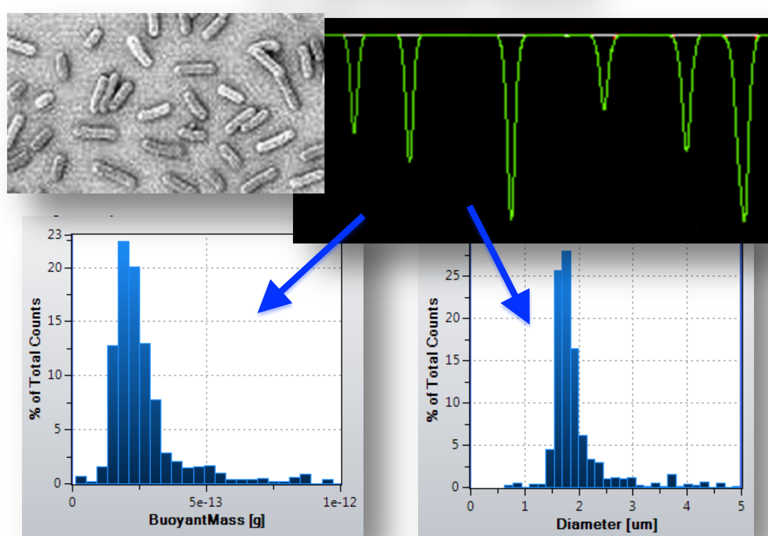
High-resolution measurements of whole bacteria *in vitro* can benefit a number of important applications in microbiology, including the study of bacterial "lifestyles" and their response to the environment; bacteria as model systems for understanding molecular processes and signaling pathways; and critical applications in infectious disease such as antibiotic discovery and resistance. However, most methods for measuring whole bacteria have been in use for decades and are fairly limited. For example, optical density methods can assess overall concentration in a culture, but give no direct information about individual bacteria or their physical properties; while the Coulter Counter's volume resolution is challenged by the small size of bacteria.

ARCHIMEDES brings, for the first time, the ability to measure the physical properties of whole bacteria with ultra-high resolution. The masses of large numbers of individual bacteria can be measured quickly and precisely, giving a clear and quantitative assessment of a culture's state. In addition, this profile can be tracked as it changes over time so as to monitor growth rate, mass and size distribution, transition between different phases (e.g., exponential growth *vs.* stationary phases), and response to environmental factors such as temperature, nutrient depletion, and antimicrobials. In addition to its unique mass measurement, ***ARCHIMEDES*** also integrates high-magnification video so that bacteria morphology and collective behavior can be observed directly. Together these capabilities can elucidate phenotypical behavior, help validate models of molecular pathways, support antimicrobial drug discovery, and provide a new approach to antibiotic susceptibility testing.

Mass Measurement of a Bacteria Culture

The figure at right shows the response of ***ARCHIMEDES'*** microchannel resonator to *Escherichia coli* ATCC 25922, a well-characterized "quality control" strain used to validate antibiotic susceptibility testing.[1] The culture was made by inoculating fresh Luria broth (LB) with stationary-phase bacteria and waiting for them to transition to the motile exponential growth phase. Each downward peak in the green curve reflects an individual bacterium transiting the microchannel resonator, causing a reduction in the sensor's resonant frequency in proportion to the bacterium's buoyant mass. ***ARCHIMEDES'*** sub-femtogram[2] mass resolution is less than 1% of a typical bacterium's mass. The associated histograms show the distributions of buoyant mass and size (in terms of the diameter of an equivalent sphere) for this culture, with mean values near 250 fg and 1.8 µm, respectively. The width of the primary mass distribution is about 100% of its mean value because the various bacteria are in random phases of their cell cycle, over which they double their mass.

***E. coli* ATCC 25922**



In addition to these mass measurements, ***ARCHIMEDES*** integrates high-magnification video that reveals the morphology and behavior of the individual bacteria as they enter the sensor inlet. A video demonstrating this capability can be seen at [*http://www.affinitybio.com/applications/microbiology.php*](http://www.affinitybio.com/applications/microbiology.php).

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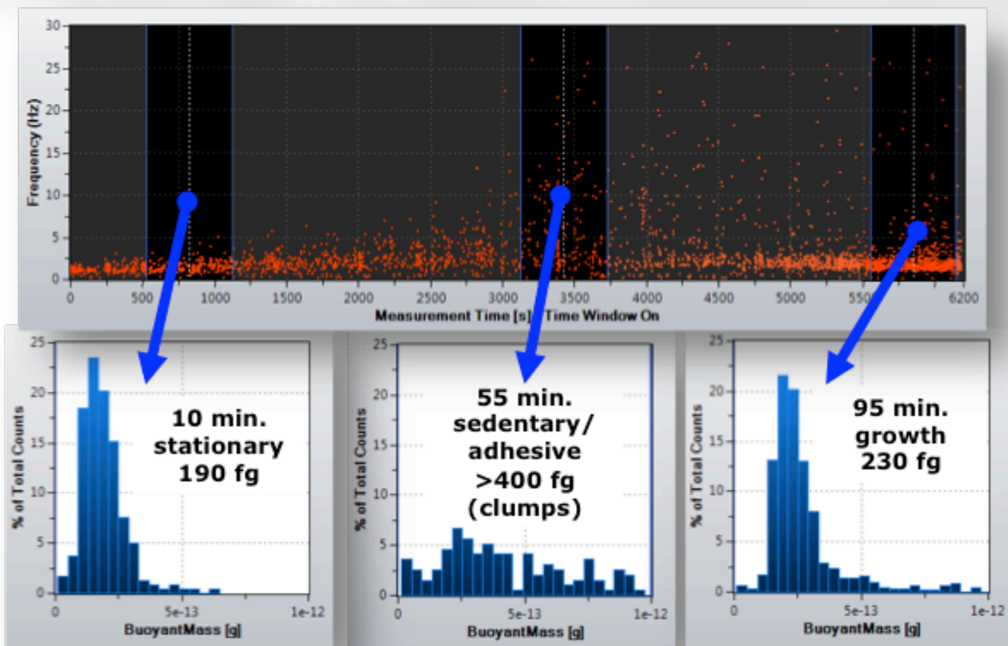
[1] American Type Culture Collection, Escherichia coli (Migula) Castellani and Chalmers, FDA strain Seattle 1946 [DSM 1103, NCIB 12210].

[2] One femtogram (fg) = 10^{-15} g.

Bacteriograms: Tracking the Development of a Culture Over Time

The full power of ***ARCHIMEDES*** lies in extending this simple mass measurement to monitor changes in a population over time. To create the "bacteriogram" below, *E. coli* ATCC 25922 were allowed to deplete most of their nutrients in the environment, upon which they entered a "stationary" phase of low metabolism and no mitosis. This population was then used to inoculate fresh LB at 37 deg C. From this starting point, the fluidics automatically re-sampled the bacteria culture every four minutes, and ***ARCHIMEDES*** then measured the buoyant masses of many individual bacteria in each sub-sample. In the bacteriogram (upper plot), the red points indicate the buoyant mass of individual *E. coli* vs. their time of measurement. The associated histograms show "snapshots" of the culture's mass distribution as the culture matures.

***E. Coli* ATCC 25922 from Stationary to Exponential Growth Phase**



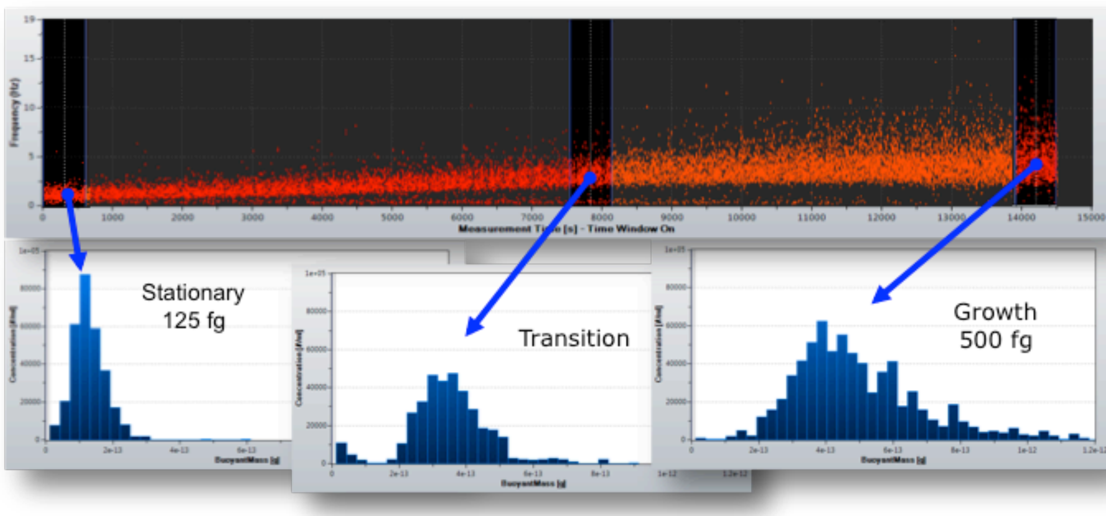
Soon after inoculation the bacteria have a fairly narrow mass distribution near 190 fg. After about 45 minutes, the *E. coli* show signs of clumping as indicated by widely scattered masses. The clumping is most likely due to generation of *curli fimbriae*, proteinaceous appendages that mediate adhesion of *E. coli*,[3] as indicated by the onset of a very wide mass distribution that includes large aggregates of *E. coli*. About 65 minutes from the start the clumping behavior transitions to the growth phase, in which the *E. coli* are mostly flagella-driven swimmers that undergo mitosis with a doubling time near 20 minutes. In this state the mass distribution narrows, centered at 230 fg. The "clumpy" *vs.* motile behaviors are easily observed optically with ***ARCHIMEDES*** (again, please see video at http://www.affinitybio.com/applications/microbiology.php).

[3] *Inverse* regulatory *coordination of motility and curli-mediated adhesion in Escherichia coli*, C. Pesavento, G. Becker, N. Sommerfeldt, A. Possling, N. Tschowri, A. Mehlis, and R. Hengge; Genes and Development, http://genesdev.cshlp.org/content/22/17/2434.full

Comparing Different Strains

ARCHIMEDES' quantitative analysis provides a wealth of information that can be used to compare the behavior of different strains in great detail. The bacteriogram for the strain *E. coli* K12 is shown below under the same conditions as the first bacteriogram (stationary phase inoculated into fresh LB). As compared to *E. coli* ATCC 25922, the mean mass of the initial stationary phase is lower, 125 fg *vs.* 190 fg. In addition, during the transition to the growth phase the mass distribution remains fairly narrow and there is no noticeable clumping, indicating that this strain does not exhibit adhesive behavior under these conditions. And, in the growth phase they are considerably more plump, 500 fg vs. 230 fg for *E. coli* ATCC 25922.

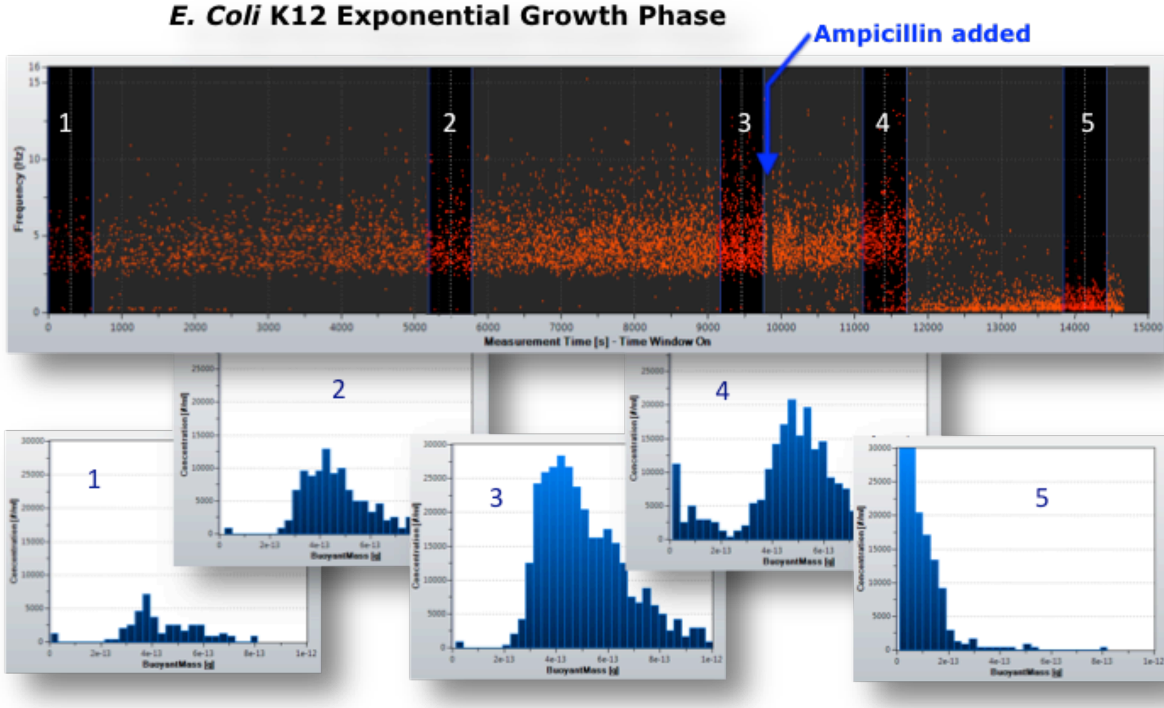
***E. Coli* K12 from Stationary to Exponential Growth Phase**



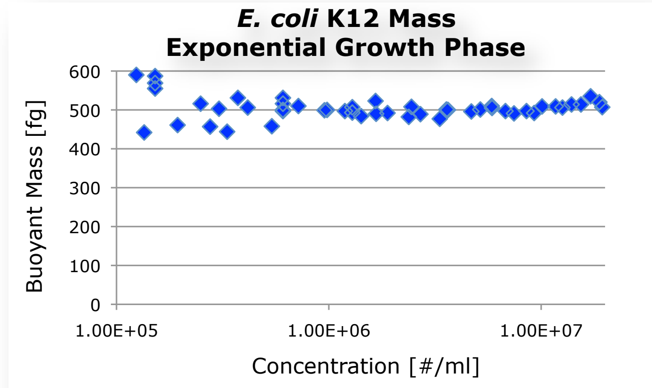
Monitoring Response to the Environment

For the bacteriogram below, a culture was started by inoculating fresh Luria broth with a small number of *E. coli* K12 taken from a culture already in an exponential growth phase. The increase in mean concentration over a three-hour period as the *E. coli* double through their cell cycle multiple times is clearly visible.

***E. Coli* K12 Exponential Growth Phase**

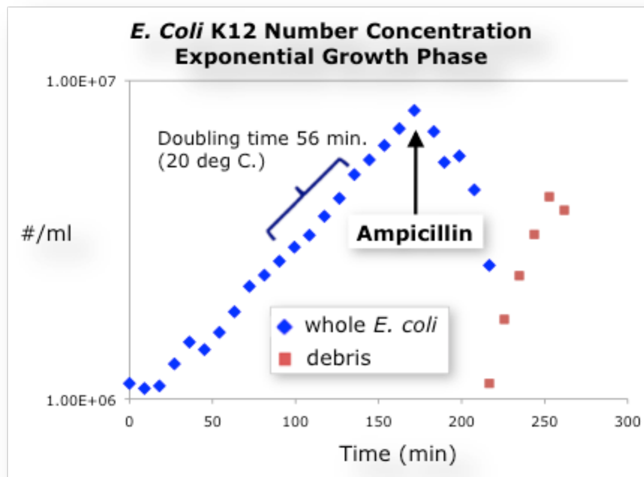


In the growth phase, it is thought that the intrinsic parameters of the bacteria stay constant, consistent with the media composition remaining unchanged within the cells' detection limits. Thus the mean volume, density, and macromolecular composition of the cells stay constant, and the number of bacteria increase exponentially over time with a well-defined doubling period. The plot at right shows the mean mass of the bacteria vs. their number concentration, as extracted from the above bacteriogram data. It shows that they are indeed in a steady state, consistent with previous, lower-resolution measurements in which optical density is taken as a surrogate for total cell mass in the culture.[4]



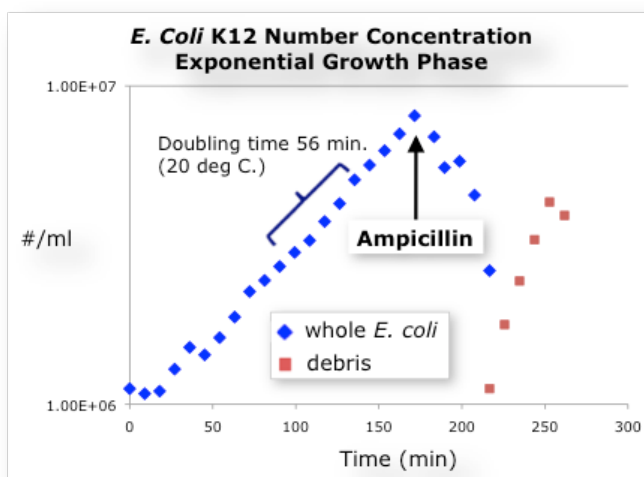
Response to Antibiotics

At about 2¾ hours from the start of the above bacteriogram, ampicillin was added to the broth at a concentration of 100 µg/ml, about 10 times the minimum needed to inhibit growth.[5] The population growth ceased within one four-minute resampling interval (right), showing that antibiotic efficacy can be evaluated on very short time scales. In addition, the number of whole bacteria began to decrease, and the population completely vanished in about 40 minutes. Because the ampicillin interferes with cell wall growth and is bactericidal, the bacteria simply fall apart. This can be seen in last histogram in the above bacteriogram, which shows a large amount of "low mass" debris. Resonant mass measurement has also been used to determine antibiotic resistance based on their response to osmotic shock.[6]



Effect of Temperature

As another illustration of effects of environment, the plot at right compares the growth rate of *E. coli* K12 at 20 deg C and at 36 deg C. The dashed lines represent doubling times of 56 minutes (blue) and 20 minutes (red), which are consistent with previous measurements using optical density.



In sum, ***ARCHIMEDES*** combines ultra-high resolution measurements of the mass of individual bacteria with precise concentration measurements, high-magnification video, and time-course analysis to bring unprecedented capabilities to the microbiologist.

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[4] *Escherichia coli Physiology in Luria-Bertani Broth, G. Sezonov, D. Joseleau-Petit, and R. D'Ari, JOURNAL OF BACTERIOLOGY, 189 no.23 (2007) 8746.*

[5] *R Factor-Mediated and Chromosomal Resistance to Ampicillin in Escherichia coli, A. Roupas and J. S. Pitton, ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, 5, No. 2, (1974) 186.*

[6] *Determination of Bacterial Antibiotic Resistance Based on Osmotic Shock Response, S.M. Knudsen, M.G. von Muhlen, D.B. Schauer*, and S.R. Manalis, ANALYTICAL CHEMISTRY, **81** No. 16, (2009) 7087; DOI: 10.1021/ac900968r.