



Simple optical nanomotion method for single-bacterium viability and antibiotic response testing

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Antibiotic resistance is nowadays a major public health issue. Rapid antimicrobial susceptibility tests (AST) are one of the options to fight this deadly threat. Performing AST with single-cell sensitivity that is rapid, cheap, and widely accessible, is challenging. Recent studies demonstrated that monitoring bacterial nanomotion by using atomic force microscopy (AFM) upon exposure to antibiotics constitutes a rapid and highly efficient AST. Here, we present a nanomotion detection method based on optical microscopy for testing bacterial viability. This novel technique only requires a very basic microfluidic analysis chamber, and an optical microscope equipped with a camera or a mobile phone. No attachment of the microorganisms is needed, nor are specific bacterial stains or markers. This single-cell technique was successfully tested to obtain AST for motile, nonmotile, gram-positive, and gram-negative bacteria. The simplicity and efficiency of the method make it a game-changer in the field of rapid AST.

nanomotion | bacteria | antibiotic | optical microscopy

The recent proliferation of bacterial resistance to antibiotics is among the most serious public health concerns (1). An efficient way to limit the proliferation of resistant bacteria consists in starting the therapeutic process with the most efficient drug (2), which is only possible if rapid antibiotic susceptibility testing (AST) methods are available. Unfortunately, traditional replication-based AST methods typically last 24 h, and even up to 1 month for slow-growing bacteria. Recently, some rapid AST techniques have been developed, such as whole genome sequencing, matrix-assisted laser desorption/ionization time-of-flight spectrometry, Fourier transform infrared spectroscopy, and various microfluidics-based technologies (3). However, these methods are complex and need sophisticated and costly equipment. A couple of years ago, we demonstrated that virtually all living organisms on Earth oscillate at a nanometric scale. These oscillations can be used to monitor their life/death state (4). We used this “signature of life” to develop nanomotion detectors that conduct AST in a timeframe of 1 to 2 h. The technique consists of attaching the organism of interest onto a cantilever and monitoring its oscillations as a function of time before and during the treatment with antibiotics (5). To simplify the technique, we recently demonstrated that traditional optical microscopes equipped with a camera can also detect nanomotions of cells such as yeast cells (*Saccharomyces cerevisiae*, *Candida albicans*, *Candida lusitaniae*, and *Candida glabrata*) (6). Their sensitivity or resistance to antifungals could be assessed in a simple and rapid way by monitoring cellular oscillations. Since individual cells are resolved, this technique, referred to as optical nanomotion detection (ONMD), is also single-cell sensitive.

In the present contribution, we modified and extended this detection method to bacterial AST. Clinical and research-relevant strains, such as *Escherichia coli* (gram-negative, motile), *Staphylococcus aureus* (gram-positive nonmotile), *Lactobacillus rhamnosus* (gram-positive, nonmotile), and *Mycobacterium smegmatis* (nonmotile and nonpathogenic bacterial model for tuberculosis) were analyzed. The experiments successfully demonstrated that ONMD monitors not only life–death transitions upon exposure to different chemicals but also variations in the bacterial metabolic level induced by different nutrient concentrations. We demonstrate that the technique only requires a basic traditional optical microscope, a camera/mobile phone, and dedicated software to achieve rapid, single-cell sensitive, attachment-, and label-free AST.

Results and Discussion

The organisms of interest were placed in a homemade analysis chamber and their displacements were recorded with a traditional optical microscope and a video camera/mobile phone (Fig. 1A). Cellular displacements were measured by tracking every single cell with a subpixel resolution (7) (Fig. 1B) or by highlighting in false colors the regions of the field of view that changed the most between consecutive frames. In the first set of experiments,

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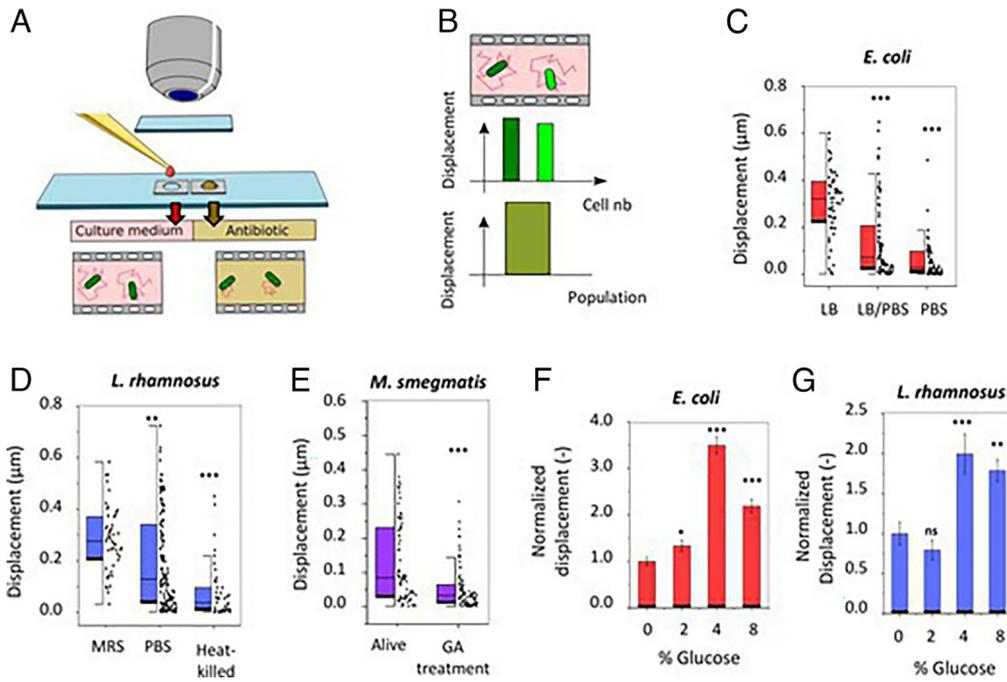


Fig. 1. Optical nanomotion detection of bacteria. (A) Sample preparation method. (B) The data analysis software calculates the displacements of single cells (two green columns) as well as the mean displacement of a full cellular population (one large green column). Effect of the nutrient concentration on (C) *E. coli* ($n > 50$) and (D) *L. rhamnosus* ($n > 41$), heating on (D) *L. rhamnosus* ($n > 41$), 5% GA incubation on (E) *M. smegmatis* ($n > 75$), and the glucose concentration on (F) *E. coli* ($n > 286$) and on (G) *L. rhamnosus* ($n > 144$). Student's *t* test: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. ♦: single-cell displacement.

we investigated the effect of the dilution or the replacement of the bacterial growth medium with phosphate-buffered saline. The lack of nutrients induced a dramatic decrease in the displacements of motile *E. coli*, and nonmotile *L. rhamnosus* (Fig. 1 C and D). In these experiments, we also explored the ability of ONMD to monitor life-dead states in these bacterial species. Differences in nanomotion between living and glutaraldehyde (GA)-killed *M. smegmatis* were highlighted by this technique (Fig. 1E). *M. smegmatis* is used as a model organism for *Mycobacterium tuberculosis* whose traditional (replication rate-based) antibiotic sensitivity tests last several weeks.

Next, we assessed the effect of the carbon source, glucose, on bacterial nanomotion. We incubated *E. coli* and *L. rhamnosus* cells in their respective culture medium with different glucose concentrations. By analyzing the cellular nanomotions, we

noticed that increasing the glucose concentration up to 4% (m/v) increased the *E. coli* nanomotion by 350% whereas a further increase in glucose concentration induced a drop in the bacterial movements (Fig. 1F). This phenomenon can be explained by the inhibitory effect of high glucose concentrations on *E. coli* flagella (8). The results are fully consistent with previously published data obtained by AFM-based nanomotion detection (9). The effect of the glucose on *L. rhamnosus* also induced a significant increase in its nanomotion (Fig. 1G).

Finally, we explored the capacity of the ONMD technique to monitor bacterial sensitivity to antibiotics in their exponential phase. For this purpose, we incubated various bacterial species with different concentrations of chemicals and monitored their nanomotion after exposure to the drugs. The sensitive *E. coli* strain showed a statistically significant decrease in its average nanomotion after exposure for 2 h

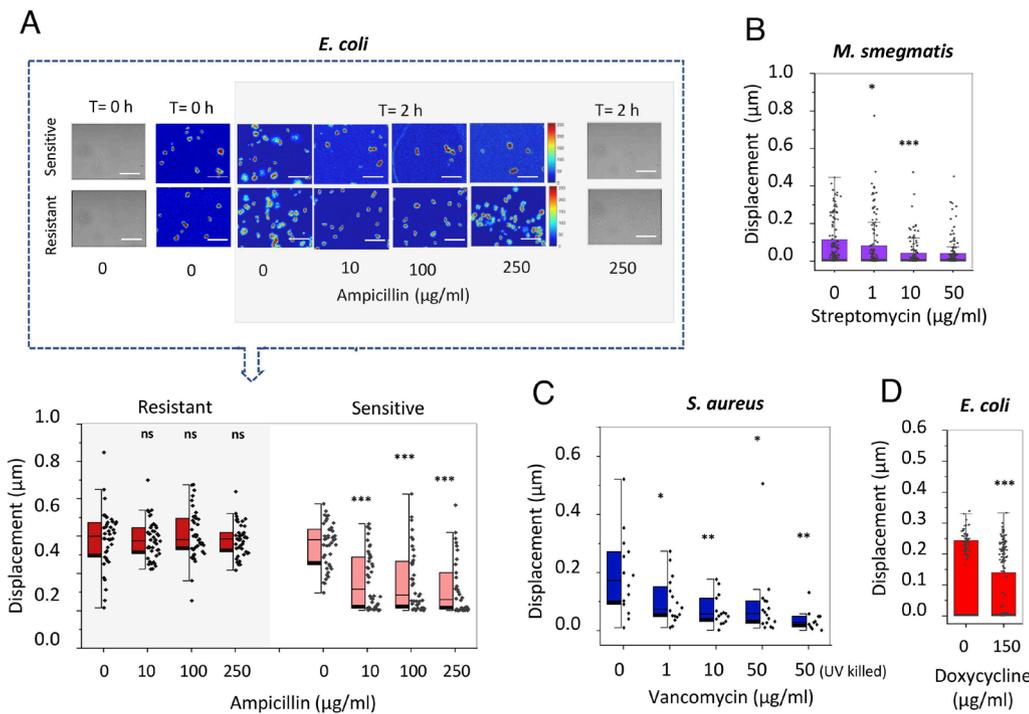


Fig. 2. Nanomotion of bacteria upon exposure to antibiotics. (A) Optical, changing pixels images and cell displacement of sensitive ($n > 34$) and resistant ($n = 42$) *E. coli* after 2 h ampicillin incubation; Scale bar: 10 μm . (B) Nanomotion of *M. smegmatis* exposed to increasing concentration of streptomycin during 5 h ($n > 100$). (C) Whole population nanomotion of *S. aureus* after 2 h vancomycin exposure and UV-killed cells as dead population control ($n > 11$). (D) Nanomotion of *E. coli* exposed to doxycycline during 2 h ($n > 50$). Student's *t* test: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns: not significant. ♦: single-cell (A, B, and D) or single population (C) displacement.

to ampicillin (Fig. 2A) whereas the resistant strain was unaffected. Similarly, a reduction in the average nanomotion was revealed for *M. smegmatis* after 5 h incubation with streptomycin (Fig. 2B). The ONMD analysis of a bacterial populations (i.e., displacement analysis of the full field of view) highlighted the response of *S. aureus* to different concentrations of vancomycin (Fig. 2C). In this case too, the total displacement of the whole cellular population was significantly reduced. Finally, *E. coli* exposed to doxycycline for 2 h, decreased their nanomotion by 40% demonstrating that the technique is sensitive to bactericidal as well bacteriostatic antibiotics (Fig. 2D). In all previously described experiments, we considered dead bacteria's displacements close to purely Brownian motion to which living cells' nanomotion was compared.

Conclusion

Rapid identification of the most appropriate antibiotic is key in limiting the spread of multi-resistant bacteria. The present technique is rapid, extremely simple to set up, possesses a single-cell sensitivity, is attachment- and label-free, and significantly faster and cheaper than other existing fast AST methods (10). Importantly, it is independent of the bacterial replication rate, the structure of their cell wall, or motility. This technique allowed us to study bacteria viability and assess nanomotion under different nutrient and antibiotics conditions. Its simplicity opens novel avenues to fast AST, specifically in developing countries. Additional application domains could include the search for living organisms in extreme environments

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in a chemistry-independent manner, such as on some planets in the Universe.

Materials and Methods

A 0.6 to 1 μL droplet of the bacteria solution is used to fill a microfluidic analysis chamber that is eventually placed under a traditional optical microscope. Alternatively, to limit bacteria adhesion to the analysis chamber, the bacteria suspensions are mixed before the measurement in oil. Typically, the organisms are observed with an optical microscope and 10-s movies are recorded (30 fps). A homemade MATLAB (<https://www.mathworks.com>) software eventually detects every single-cell displacement during 200 frames and analyzes the distance traveled by the cells. Detailed descriptions are provided in *SI Appendix*.

Data, Materials, and Software Availability. Data have been deposited in the BioStudies database (EMBL-EBI), accession: [S-BSST1065](https://www.ebi.ac.uk/biostudies/studies/S-BSST1065) (11). The code is available in GitHub at: <https://github.com/BacteriaNanomotion/Bacteria-Nanomotion.git> (12).

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