Support Information

Direct Antimicrobial Susceptibility Testing on Clinical Urine Samples by Optical Tracking of Single Cell Division Events

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S1. Dual-channel LVSi system

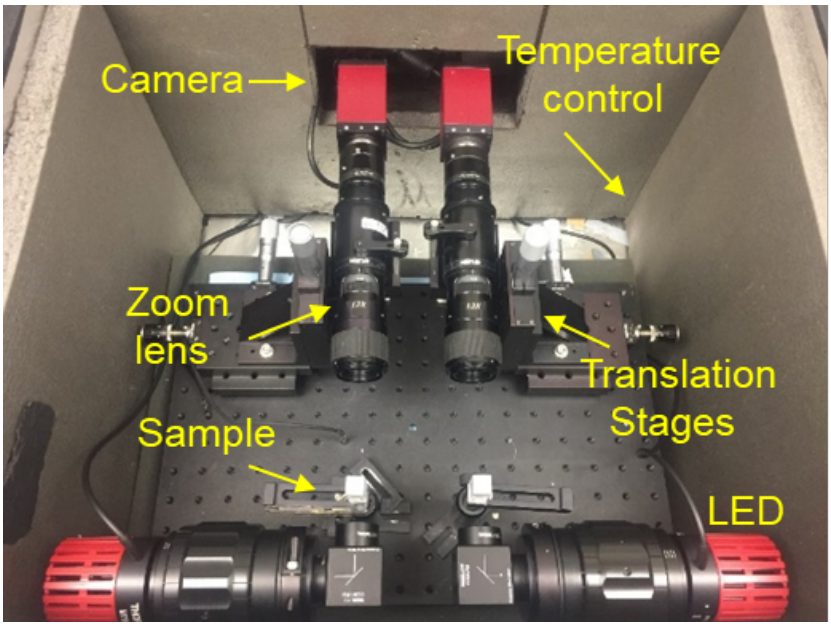

Fig S1. A photo of the dual-channel Large Volume Scattering imaging (LVSi) system for simultaneously imaging of the control and antibiotic treated samples. The two channels consist of identical components arranged in a mirror symmetrical design and mounted on an optical breadboard with passive antivibration isolation. Major components are labelled on the photo. The setup in enclosed in a thermally isolated enclosure for temperature control.

S2. Comparison of division tracking and traditional plating with pure *E.coli* cultures

To validate the results of division tracking, we performed simultaneous validation with traditional plating on LB agar and colony counting after overnight incubation at 37 ºC. The plating was performed in parallel during LVSi detection for every 10 min. The comparison of division tracking versus plate counting results is plotted in Fig. S2. Both division tracking and plating showed growth in 60 min detection and the growth trend was similar, which validated the division tracking accuracy.

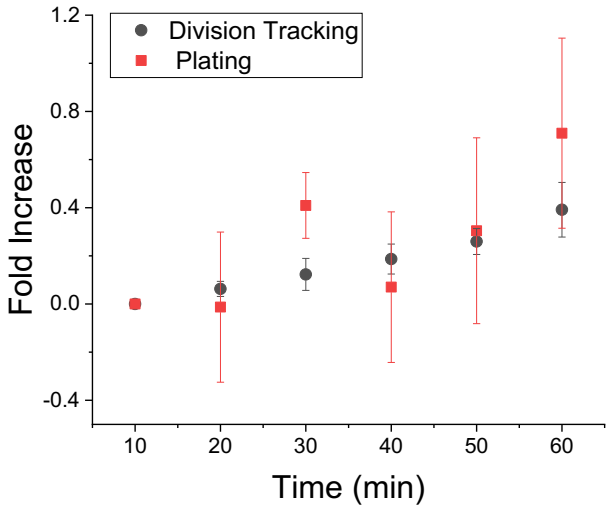

Fig. S2. The comparison results of division tracking and traditional plating. The error bars represent standard deviation of 3 tests.

S3. Clinical ID results

Table S1. Single-cell division detection (D_{60}) of bacteriuria infection for 60 human patient samples

Sample ID	Color	D_{60}	LVSS-AST Call	Clinical Lab Call*	Plating Validation Call#
ATU090319_01	Yellow	0	Negative†	Positive	Positive
ATU090319_02	Yellow	264	Positive	Positive	Positive
ATU090319_04	Brown	21	Positive	Positive	Positive
ATU090319_06	Yellow	65	Positive	Positive	Positive
ATU090319_08	Yellow	100	Positive	Positive	Positive
ATU090319_10	Brown	93	Positive	Positive	Positive
ATU091019_01	Clear	8	Positive	Positive	Positive
ATU091019_03	Clear	62	Positive	Positive	Positive
ATU092619_01	Milky	393	Positive	Positive	Positive
ATU092619_02	Cloudy	249	Positive	Positive	Positive
ATU092619_06	Yellow	113	Positive	Positive	Positive
ATU101019_04	Cloudy	180	Positive	Positive	Positive
ATU103119_01	Milky	13	Positive	Positive	Positive
ATU103119_02	Clear	11	Positive	Positive	Positive
ATU103119_03	Clear	170	Positive	Positive	Positive
ATU103119_10	Milky	116	Positive	Positive	Positive
ATU111419_03	Cloudy	65	Positive	Positive	Positive
ATU111419_04	Clear	145	Positive	Positive	Positive
ATU120519_03	Milky	66	Positive	Positive	Positive
ATU120519_06	Yellow	99	Positive	Positive	Positive
ATU120519_07	Clear	30	Positive	Positive	Positive
ATU120519_09	Yellow	12	Positive	Positive	Positive
ATU120519_10	Yellow	31	Positive	Positive	Positive
ATU121219_02	Clear	68	Positive	Positive	Positive
ATU121219_04	Clear	0	Negative†	Positive	Positive
ATU121219_06	Cloudy	16	Positive	Positive	Positive
ATU121219_08	Cloudy	22	Positive	Positive	Positive
ATU121219_10	Milky	24	Positive	Positive	Positive
ATU010920_04	Cloudy	50	Positive	Positive	Positive
ATU010920_08	Cloudy	754	Positive	Positive	Positive
ATU011620_09	Yellow	217	Positive	Positive	Positive
ATU011620_10	Yellow	51	Positive	Positive	Positive
ATU090319_05	Clear	0	Negative	Negative	Negative
ATU090319_07	Clear	0	Negative	Negative	Negative
ATU090319_09	Clear	0	Negative	Negative	Negative
ATU091019_02	Clear	0	Negative	Negative	Negative
ATU091019_04	Clear	1	Negative	Negative	Negative
ATU091019_06	Clear	0	Negative	Negative	Negative
ATU092619_04	Clear	0	Negative	Negative	Negative
ATU092619_09	Clear	0	Negative	Negative	Negative
ATU092619_10	Cloudy	0	Negative	Negative	Negative
ATU103119_04	Yellow	0	Negative	Negative	Negative
ATU103119_05	Yellow	0	Negative	Negative	Negative
ATU111419_01	Clear	1	Negative	Negative	Negative
ATU111419_02	Clear	1	Negative	Negative	Negative
ATU111419_06	Clear	1	Negative	Negative	Negative
ATU111419_07	Clear	1	Negative	Negative	Negative
ATU111419_08	Clear	0	Negative	Negative	Negative
ATU120519_01	Yellow	0	Negative	Negative	Negative
ATU120519_02	Yellow	0	Negative	Negative	Negative
ATU120519_04	Clear	0	Negative	Negative	Negative
ATU120519_05	Yellow	0	Negative	Negative	Negative
ATU120519_08	Clear	0	Negative	Negative	Negative
ATU121219_01	Cloudy	1	Negative	Negative	Negative
ATU121219_03	Clear	0	Negative	Negative	Negative
ATU121219_05	Clear	0	Negative	Negative	Negative
ATU121219_07	Clear	1	Negative	Negative	Negative
ATU121219_09	Clear	1	Negative	Negative	Negative
ATU010920_09	Clear	0	Negative	Negative	Negative
ATU010920_10	Clear	0	Negative	Negative	Negative

* Reference method (Traditional plating followed by BD Phoenix™ automated AST) results generated by the Mayo Clinic microbiology lab.

On-site validation results generated by overnight plating upon sample receiving.

† Disagreement between LVM-AST and reference method results.

S4. Normalized cumulative division curves of all susceptible and resistant samples

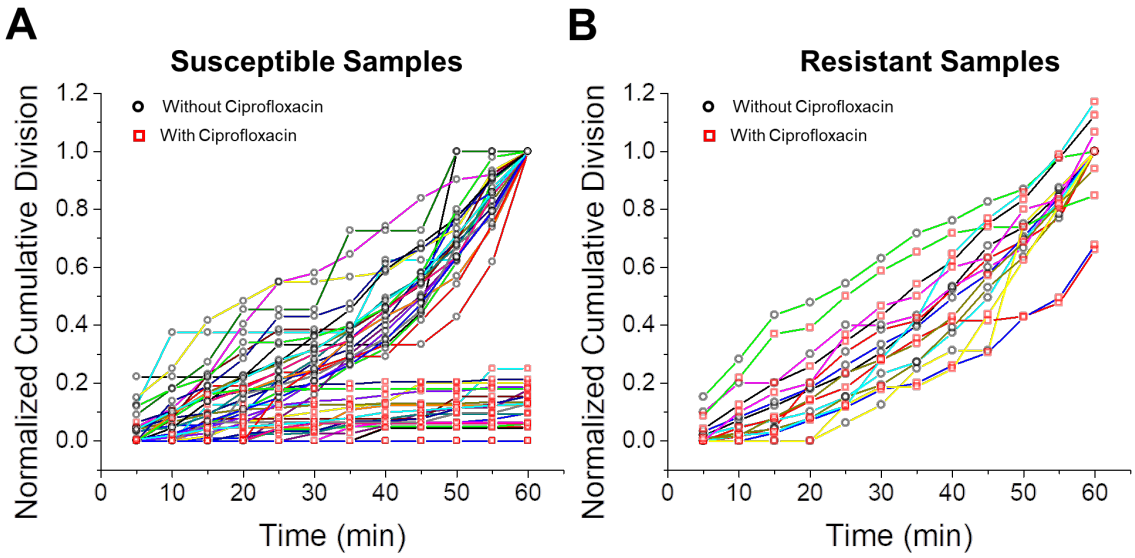

Fig S4. The normalized cumulative division result of all susceptible samples (A) and all resistant samples (B). Each color of the lines represents an individual sample. Circles are measured data points of control group, and squares are measured data points for ciprofloxacin treated group.

S5. Clinical AST results

Table 2. LVSi-AST of 30 bacteriuria-positive human urine samples

Sample ID	Comments	D_{ABX}/D_C	LVSS-AST Call	Clinical Lab Call*	Plating Validation Call#
ATU090319_02	ESCHERICHIA COLI	1.125	R	R	R
ATU090319_04	ESCHERICHIA COLI	0.04762	S	S	S
ATU090319_06	ESCHERICHIA COLI	0.66154	R	R	R
ATU090319_08	ESCHERICHIA COLI	0.06	S	S	S
ATU090319_10	KLEBSIELLA AEROGENES	0.09677	S	S	S
ATU091019_01	ESCHERICHIA COLI	0.25	S	S	S
ATU091019_03	ESCHERICHIA COLI	0.06452	S	S	S
ATU092619_01	ESCHERICHIA COLI	0.13486	S	S	S
ATU092619_02	ESCHERICHIA COLI	0.21285	S	S	S
ATU092619_06	ENTEROBACTER CLOACAE	0.17699	S	S	S
ATU101019_04	ESCHERICHIA COLI	0.67778	R	R	R
ATU103119_01	ESCHERICHIA COLI	0.15385	S	S	S
ATU103119_02	ESCHERICHIA COLI	0	S	S	S
ATU103119_03	ESCHERICHIA COLI	0.09412	S	S	S
ATU103119_10	CITROBACTER FREUNDII COMPLEX	0.15517	S	S	S
ATU111419_03	ESCHERICHIA COLI	0.12308	S	S	S
ATU111419_04	KLEBSIELLA PNEUMONIAE	0.18621	S	S	S
ATU120519_03	ESCHERICHIA COLI	0.06061	S	S	S
ATU120519_06	ESCHERICHIA COLI	1.17172	R	R	R
ATU120519_07	ESCHERICHIA COLI	1.06667	R	R	R
ATU120519_09	ESCHERICHIA COLI	0.41667	S	S	S
ATU120519_10	KLEBSIELLA PNEUMONIAE	0.12903	S	S	S
ATU121219_02	ESCHERICHIA COLI	0.30882	S	S	S
ATU121219_06	ESCHERICHIA COLI	1	R	R	R
ATU121219_08	ESCHERICHIA COLI	0.04545	S	S	S
ATU121219_10	KLEBSIELLA PNEUMONIAE	0	S	S	S
ATU010920_04	ESCHERICHIA COLI	0.18	S	S	S
ATU010920_08	KLEBSIELLA PNEUMONIAE	0	S	S	S
ATU011620_09	ESCHERICHIA COLI	0.94009	R	R	R
ATU011620_10	ESCHERICHIA COLI	1.45098	R	R	R

***** Reference method (Traditional plating followed by BD Phoenix™ automated AST) results generated by the Mayo Clinic microbiology lab.

On-site validation results generated by overnight plating upon sample receiving.

S6. Comparison of division tracking and cell counting with pure *E. coli* samples

In pure *E. coli* samples, assuming all cells are viable, the cell division tracking should be similar to cell counting. To validate the division tracking result, we compared the cumulative division result with the corresponding cell counting result. One representative result is shown in Fig. S6, in which the cumulative division events are close to the increase in cell number. However, the division tracking is more accurate and can determine the AST in shorter time. For this sample,

division tracking can obtain reliable AST result in about 30 minutes, but counting needs about 45 minutes for reliable result.

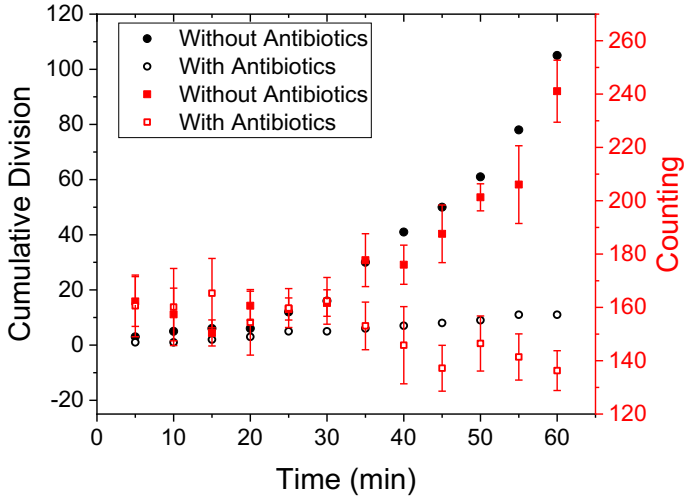

Fig. S6. Comparison LVSi result of division tracking and cell counting with pure *E. coli* samples. Error bar in counting represents the standard deviation of cell counts in each 5-min video, which is due to cell dynamic moving.

S7. Comparison of division tracking and cell counting with clinical samples

While cell counting works well with pure or cultured isolated bacterial samples, it is problematic for clinical urine samples where a large number of impurity particles are present, which often overwhelm the smaller number of bacterial cells. Many urine samples contain so many impurity particles that particles appear to precipitate and fall out of the imaging volume during the test, which lead to false detection of bacterial cell growth. A representative example is shown in in Fig. S7. The counting number decreases in the first 20 min, thus obscuring the increase in the last 20

minutes (40-60 min) after the particles have settled out. Tracking of the division events of single bacterial cells is more sensitive and more robust in the presence of a large number of impurity particles, which improves the accuracy and shortens the detection time. For this sample, division tracking can obtain reliable AST result in about 30 minutes, but counting results is not reliable at 60 minutes.

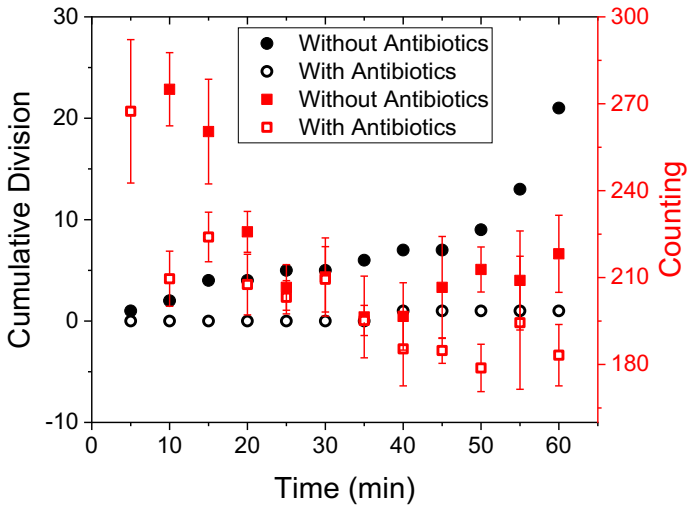

Fig. S7 Comparison of division tracking and cell counting with a representative clinical urine sample (ATU090319_04). Error bar in counting represents the standard deviation of cell counts in each 5-min video, which is due to cell dynamic moving.

S8. On-site validating method

On-site validation is performed with sample plating and colony counting. Upon sample receiving, all urine samples were subjected to agar plating for initial sample validation. Furthermore, parallel plating validation was performed along with LVSi detection to test the diluted samples. Specifically, within 5 minutes of the urine sample preparation (filtration through 5μm filter and

dilution in LB), 3 control replicates are serially diluted 1:3 in sterile saline and plated in duplicate on LB agar. Within 5 minutes of the end of the detection, replicates are again serially diluted 1:3 in sterile saline and plated in duplicate on LB agar. Plates are incubated at 37°C for 18-24 h before colonies are enumerated. For samples preparations containing antibiotics, 7µl of each replicate is plated at both the start and end of the imaging. Positive growth and/or an increase in the number of colonies from the start of imaging is considered resistant to ciprofloxacin at 2µg/ml.

S9. Clinical reference method

The results of clinical reference method are provided by the Mayo Clinic Microbiology Laboratory, which served as the ground truth for LVSi results. In the Microbiology Laboratory, a urine sample collected from a symptomatic patient is plated to at least two types of media, incubated for 18-24 hours, and assessed for growth, quantity, and morphology of bacteria present. If a particular species is in excess of 10^4 CFU/mL, it is then sub-cultured to assure purity. Identification is primarily determined by MALDI-TOF mass spectrometry (Bruker Daltonics, Billerica, MA). The routine culturing steps usually take 48-72 hours for sample-to-result time. Susceptibility testing in the Mayo Clinic Laboratory was determined by use of the automated BD Phoenix™ system (Becton Dickinson, Franklin Lakes, NJ), according to manufacturer's instructions. The system uses broth micro-dilution to measure growth of bacterial isolates in dilutions of different drugs for determination of a minimum inhibitory concentration (MIC).

S10. Division tracking flowchart

To track the real division events, each spot in the video is connected in time to form single-cell trajectories. For each trajectory, we can extract all temporal and spatial information, including trajectory start time, trajectory duration, trajectory end time, spot location (x, y) in each frame, spot mean intensity in each frame, and so on. With all of this information, we filtered out the division events as the flow chart illustrated (Fig. S10). First of all, the daughter cell candidate trajectory has to be a newly appeared one, and the possible parent trajectory is required to appear earlier than the daughter cell and be located close to the new trajectory in both time and position. At the start time point of the new trajectory, we checked for splitting from a nearby old trajectory, in other words for one old trajectory to split into two new trajectories. If no splitting is detected, this new trajectory is categorized as an appeared trajectory, which may originate from z direction. For the trajectory with nearby splitting, we continue check for a merging event with the old trajectory before splitting. If yes, this merging-splitting is considered as overlapping/crossing event. If not, we will further check the intensity profile of the daughter cell candidate trajectory and parent candidate trajectory, ensuring the two daughter cell trajectories have similar intensity profiles and the parent trajectory has a higher intensity profile. If the intensity of two daughter cell trajectories are very different, it is very likely to be two attached cells or a visual merging event that occurred out of the image view. If the detected splitting satisfies all intensity criteria, we determine it as a valid division event.

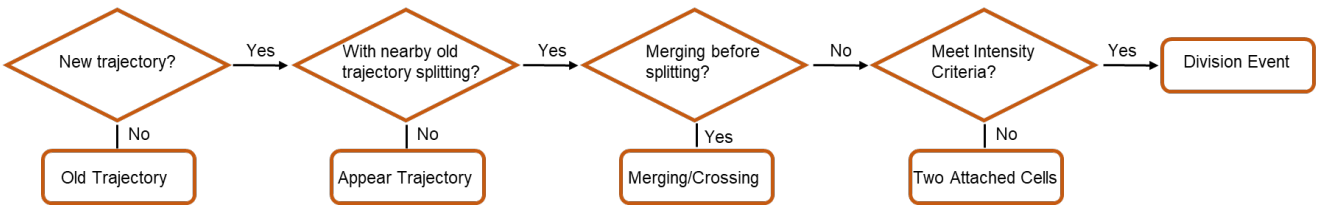

Fig. S10. Flow chart for division event filter.

S11. Calibration of the division over-counting

To estimate the single-cell division tracking accuracy, we performed the calibration experiment with the heat-deactivated *E. coli* cells, in which there should be no real growth associated division event. The division events tracked in this experiment is called division over-counting for final division calibration. With different number of *E. coli* cells, the division over-counting result is shown in Fig. S11, which increases with increasing cell number in the image. When the cell number in view is below 500 (*~1 x 10^5 CFU/mL*), the division over-counting is small. When the cell number increases, the possibilities of overlapping/crossing increases. If two similar cells merged together outside of the image view and split inside the view, we will not be able to filter it out. Therefore, to remove the division over-counting effects, the final division results are calibrated by subtracting the division over-counting number.

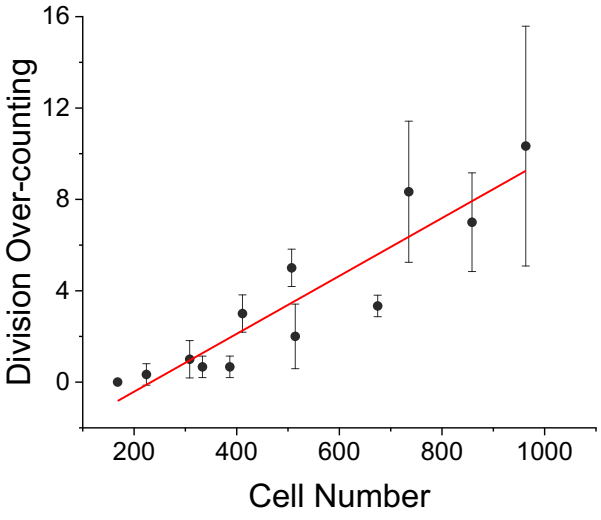

Fig. S11. Division over-counting with different cell number (n=3). Error bars represent standard deviation of the 3 replications.