Supporting Information for

High-Throughput Antimicrobial Susceptibility Testing of *Escherichia coli* by Wide-Field Mid-Infrared Photothermal Imaging of Protein Synthesis

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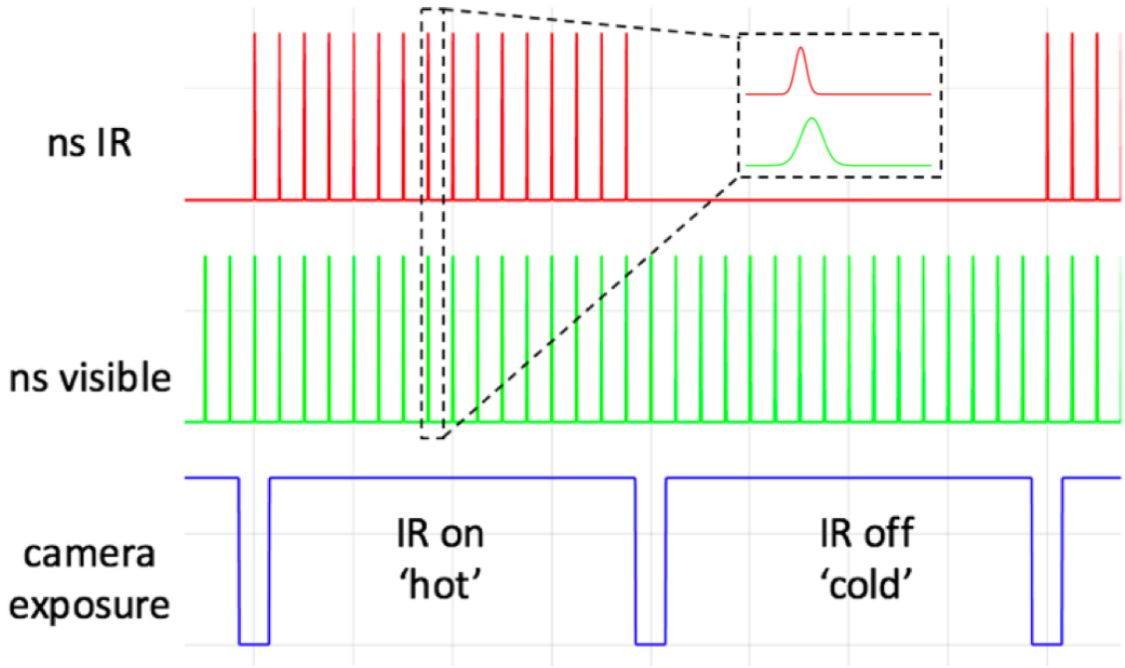
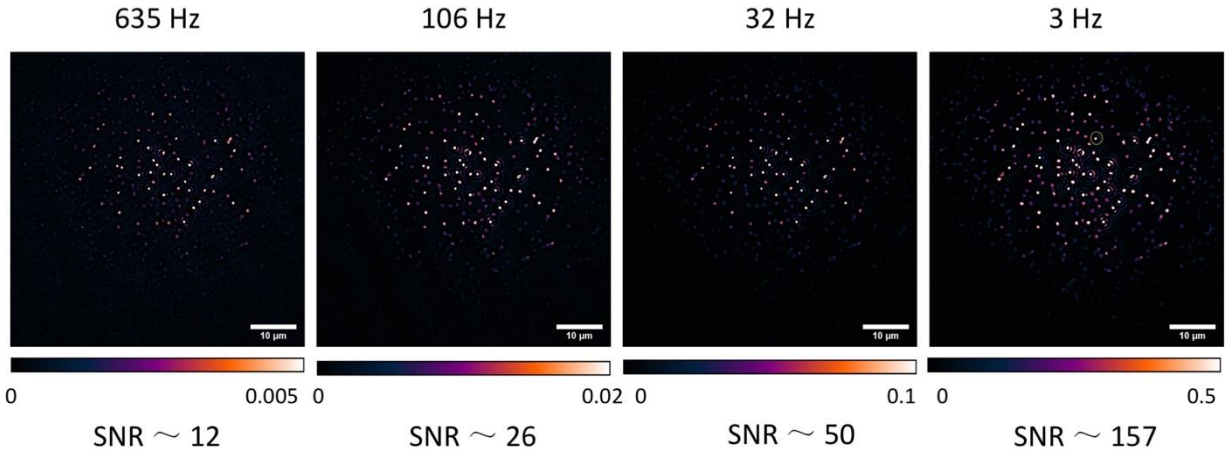
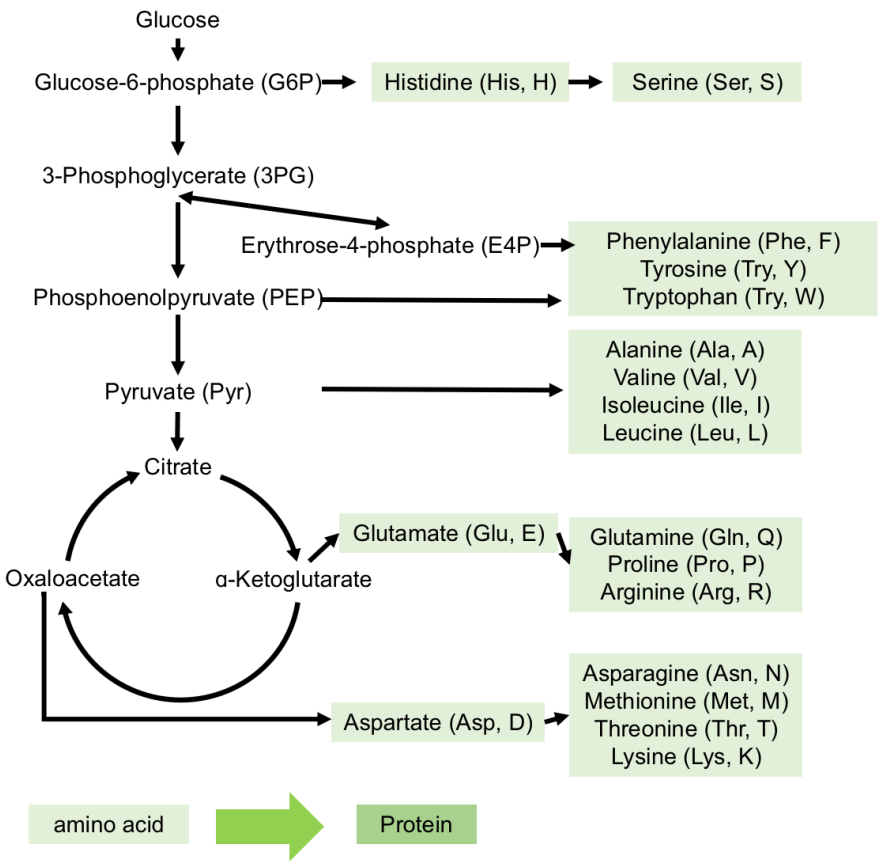


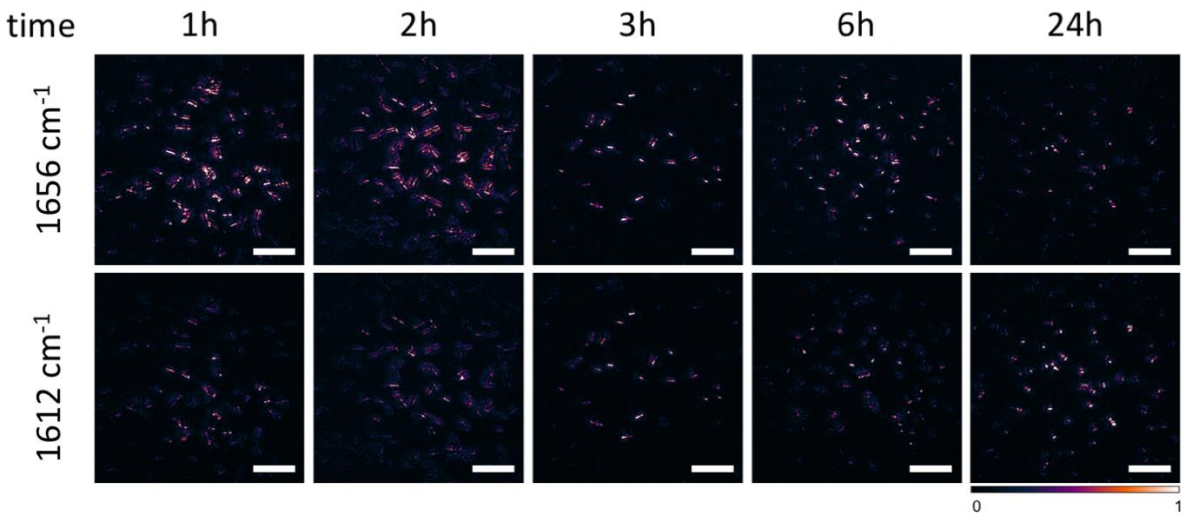
Supplement Figure 1. Illustration for the synchronization of the nanosecond probed wide-field MIP system. The output signal from the nanosecond IR laser will be used as the external trigger for the delay pulse generator. The IR pulses and visible pulses were monitored on the oscilloscope by a Mercury-Cadmium-Telluride detector and a photodiode respectively. The delay between the nanosecond IR and nanosecond visible pulses was carefully minimized to achieve maximum MIP contrast. The IR pulses were modulated by the optical chopper to a 50% duty cycle and the delay of the camera trigger signal was carefully adjusted to capture IR on ('hot') frames and IR off ('cold') frames. The MIP contrast was generated by the subtraction of 'hot' and 'cold' frames and the overall speed was determined by the number of 'hot'-'cold' pairs averaged.



Supplement Figure 2. Ultrafast chemical imaging of 500-nm PMMA beads up to 635 Hz using our highly sensitive wide-field MIP microscope. The samples were 500 nm in diameter PMMA beads on the silicon substrate. The mid-IR laser was tuned to 1728 cm^{-1} with a power of about 35 mW before the microscope. The visible laser was a 532 nm nanosecond laser with about 1mW before the microscope and a pulse duration of shorter than 1 ns. The objective was 50× 0.8 NA. The total acquisition time was 0.3 s. The 635 Hz ultrafast imaging with an SNR ~12 corresponded to single 'hot'-'cold' frame subtraction. MIP images with different averaging times were individually contrasted and the color bars were shown at the bottom. Scale bars: 10 µm.



Supplement Figure 3. Glucose is widely used in protein synthesis by synthesizing various amino acids in bacteria. An overview of amino acid biosynthesis shows that glucose is widely used to synthesize various amino acids via the pentose phosphate pathway, glycolysis, and citric acid cycle. These amino acids will then be used to synthesize the proteins.



Supplement Figure 4. Representative MIP images for *E. coli* with different ^{13}C-glucose incubation times. The top row shows MIP images at the original amide I band (1656 cm^{-1}) and the bottom row shows MIP images at the shifted amide I band (1612 cm^{-1}) for ^{13}C-glucose incubation time of 1, 2, 3, 6, and 24 h. The MIP intensities at the shifted amide I band slowly increased with increasing incubation time. Near full replacement was reached with 24 hours incubation time, resulting in a significant peak shift, and reversed MIP contrasts. Scale bars, 10 μm.

antibiotics	Mechanism of action	MIC by broth microdilution (μg/mL)	Clinical breakpoint concentration (μg/mL)	Susceptibility at breakpoint concentration by broth microdilution	Susceptibility at breakpoint concentration by our method
gentamicin	Inhibit protein synthesis	1	2	susceptible	susceptible
ampicillin	Inhibit cell wall synthesis	32	8	resistant	resistant
trimethoprim	Inhibit folate synthesis	0.25	4	susceptible	susceptible
erythromycin	Does not inhibit *E. coli*	>128	/ (4 was used)	resistant	resistant

Supplement Table 1. Susceptibility of *E. coli* to antibiotics with diverse mechanisms of action validated by standard broth microdilution assay. MIC: minimal inhibitory concentration. Clinical breakpoint concentrations were determined from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint tables v10.0 and the clinical and laboratory standards institute (CLSI) M100 Performance Standards for Antimicrobial Susceptibility Testing 28th edition.