## Dynamic density shaping of photokinetic E. coli

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Many bacteria can move in response to environmental signals. This helps guide them towards better conditions for growth and survival. *Escherichia coli* is a bacterium that can swim quickly through liquid, using flagella that rotate many times per second. These 'propellers' are powered by a cellular motor, called the flagellar motor, which similarly to an electric motor, requires an energy source to drive movement. Proteorhodopsin, a protein originally isolated from free-swimming micro-organisms in the ocean, is a light-driven proton pump [1]. The protein is located at the cell membrane, where it acts like a solar panel and captures energy from light. Proteorhodopsin, the intensity of light from their environment determines their swimming speed: brighter light means faster movement, and less light, slower movement [2]. This is an efficient method that provides a way to control their movement remotely, using a light source. Swimming bacteria, much like cars in city traffic, are known to accumulate in areas where their speed decreases. By controlling swimming speed with proteorhodopsin, we can manipulate the local density of bacteria simply by projecting different patterns of light [3].

To study the factors influencing this phenomenon, we used genetically modified *E. coli* that could respond to light via proteorhodopsin to make layers of cells that could then have light patterns projected onto them [4]. The results showed that the bacteria responded slowly to these stimuli, which was the main factor limiting the resolution of the final pattern they formed. A simple feedback mechanism, which compared the pattern formed by the cells to the desired image and updated the projected light accordingly, was enough to solve this problem. This way, the layers of *E. coli* could be turned into a near-perfect copy of the original image (Fig. 1). Furthermore this system is dynamically reconfigurable. As a demonstration of this property we show the morphing of a bacterial layer from an Albert Enstein's to a Charles Darwin's portrait (Fig. 2). This method allows us to control the movement of millions of bacteria more precisely than ever before. This could be extremely valuable for building new microscopic devices. For example, bacteria could be made to surround a larger object such as a machine part or a drug carrier, and then used as living propellers to transport it where it is needed.

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Figure 1. Improved density control with a feedback loop. (a) Time evolution of the distance from the target normalized to the initial value (circles) before and after activating the feedback loop (gray area). The yellow and green bars indicate the time interval over which we average the density maps (shown in (b)) before and after feedback respectively. The full curve is a fit with a double exponential. (b) Comparison of the density map obtained by averaging for 2 min before (top) and after the feedback loop has been turned on (bottom) (see colored bars in (a)). (c) Time averaged density profile (6 min) with feedback on. Scale bars are 100 µm.

Figure 2. Reconfigurable density patterns. Starting from the stationary density modulation (a) we switch to a new light pattern at time 0 and record the density distribution of bacteria as it morphs through the intermediate state (b) and reaches the final state (c). (d) Time evolution of the normalized squared distances between instantaneous density maps and the initial (blue circles) and final (orange circles) targets. Curves are exponential fits.