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Nanophotonic Array-Induced Dynamic Behavior for Label-Free Shape-Selective Bacteria Sieving

Yuzhi Shi¹, Haitao Zhao¹, Kim Truc Nguyen¹, Yi Zhang^{2*}, Lip Ket Chin¹,
Tongtong Zhu^{3,4}, Yefeng Yu⁵, Hong Cai⁶, Peng Huat Yap⁷, Patricia Yang Liu¹, Sha
Xiong⁸, Jingbo Zhang¹, Cheng-Wei Qiu³, Che Ting Chan⁹ and Ai Qun Liu^{1*}

¹ *School of Electrical and Electronic Engineering, Nanyang Technological University,
Singapore 639798, Singapore*

² *School of Mechanical and Aerospace Engineering, Nanyang Technological
University, Singapore 639798, Singapore*

³ *Department of Electrical and Computer Engineering, National University of
Singapore, Singapore 117583, Singapore*

⁴ *School of Optoelectronic Engineering and Instrumentation Science, Dalian
University of Technology, Dalian, 116024, China*

⁵ *School of Electronic and Optical Engineering, Nanjing University of Science and
Technology, Nanjing, Jiangsu 210094, China*

⁶ *Institute of Microelectronics, A*STAR (Agency for Science, Technology and
Research), 2 Fusionopolis Way, #08-02 Innovis Tower, Singapore 138634, Singapore*

⁷ *Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore
308232, Singapore*

⁸ *School of Information Science & Engineering, Central South University, Changsha
410083, China*

⁹ *Department of Physics and Institute for Advanced Study, The Hong Kong University
of Science and Technology, Hong Kong, China.*

**Corresponding Authors: yi_zhang@ntu.edu.sg & eaqliu@ntu.edu.sg*

ABSTRACT

Current particle sorting methods such as microfluidics, acoustics and optics focus on the exploiting differences in the mass, the size, the refractive index or fluorescence staining. However, there exists formidable challenges for them to sort label-free submicron particles with similar volume and refractive index yet distinct shapes. In this work, we report an optofluidic nanophotonic sawtooth array (ONSA) that generates sawtooth-like light fields through light coupling, paving the physical foundation for shape-selective sieving. Submicron particles interact with the coupled hotspots which impose different optical torques on the particles according to their shapes. Unstained *S. aureus* and *E. coli* are used as a model system to demonstrate this shape-selective sorting mechanism based on the torque-induced body dynamics, which was previously unattainable by other particle sorting technologies. More than 95% *S. aureus* are retained within ONSA while more than 97% of *E. coli* are removed. This nanophotonic chip offers a paradigm shift in shape-selective sorting of submicron particles and expands the boundary of optofluidics-based particle manipulation.

KEYWORDS: shape sorting, optical binding, optical torques, silicon photonics, near field, light-matter interactions

Optical tweezers, as versatile and non-invasive tools,¹ have raised enormous attentions and been awarded for the contributions to the atom manipulation² and biological applications^{3, 4} in 1997 and 2018, respectively. Viruses (diameter D 20 – 400 nm)^{5, 6} and DNAs^{7, 8} have been manipulated using highly focused laser spots either in free space⁵ or near field.^{8, 9} Using tailored beams such as the quasi-Bessel beam and with the assistance of other forces, such as the fluidic force and the photophoretic forces, sub-100 nm nanoparticles have been separated.¹⁰ Other techniques, such as microfluidics¹¹⁻¹³ and acoustics,¹⁴⁻¹⁶ are also efficient at separating nanoparticles and biomolecules¹⁷ with $D < 200$ nm. Examples include the use of centrifugal force to sort nanoparticles of 50–100 nm,¹¹ and the use of surface acoustic wave¹⁴ or nanoscale pillar array¹² to sort and isolate exosomes ($D < 200$ nm). These methods manipulate particles of different sizes at nano and submicron scale, but are unable to sort based on the shape. This is because the acoustic force F_{acoustic} only depends on the volume V of the particle but not the shape,¹⁸ and it is difficult to configure a 2D complex acoustic field for submicron particle sorting. One potential method of shape-selective sorting is based on hydrodynamic forces. For example, particles are sorted based on their rotational period, T_{orbit} , which depends on the aspect ratio of the particle (length/diameter ratio).^{17, 19} However, the hydrodynamic effect is only dominant at microscale (for instance, $D > 1.7 \mu\text{m}$, length $L > 5 \mu\text{m}$).¹⁹⁻²² Submicron particles, such as bacteria ($D = 400 - 800$ nm, $L \leq 2 \mu\text{m}$), may have similar size and refractive index (~ 1.38),^{23, 24} but different shapes.²⁵ Existing methods only exploit the difference in mass m , diameter D and refractive index RI to sort unstained bacteria, whereas other intriguing properties, such as the differences in torque M or moment of inertia J that bacteria experience in an optical field remain unexplored. These important parameters alter

the body dynamics of these submicron particles, which could be utilized to directly sort bacteria of similar size but different shapes without subjecting them to fluorescent staining. Even if the rod-shaped *E. coli* can deflect using the deterministic lateral displacement (DLD) technique, there were no experimental demonstrations of the separation of two label-free sub-micron particles based on the shape.¹⁷

Although optical forces, including the optical extinction and gradient forces,⁹ are shape-dependent,²⁶⁻²⁹ no strategy has been devised to exploit these forces to sort bioparticles based on the shapes.³⁰⁻³² That's because the force difference resulted from the shape is small in the conventional single-beam optical tweezer system, especially when the size of the particle $D < 1 \mu\text{m}$,³² as suggested by the Rayleigh and Mie theory. Another reason is that majority of the works based on the optical forces hitherto utilize only single laser beams^{10, 33, 34} or multiple independent laser spots^{29, 35} to manipulate particles. As a result, the intriguing behaviours of the rod-shaped particles in two coupled hotspots, which has a multitude of degree of freedom, have unfortunately been neglected. While, microbe usually have different shapes, with spherical and rod-shape the most common ones. For example, the Coccus (spherical bacteria) and Bacillus (rod-shaped bacteria) are two essential categories of bacteria, which exist widely in serum and water and cause many diseases in human beings. Sorting of those microbes with different shapes could facilitate the study of the pathogenesis. Despite of the critical importance, there lacks efficiency methods in microfluidics, optics and acoustics to sort label-free bacteria with different shapes as mentioned above and in **Figure S1**.

Here, we present an optofluidic nanophotonic sawtooth array (ONSA) using a silicon photonic nano-waveguide array (**Figure 1a**). The coupling of light between two paired

waveguides generates an array of coupled hotspots. The design of the nano-waveguide array can be found in **Figure S2**. By controlling the width and the gap between the paired waveguides thus the coupling length between the hotspots, the spherical *Staphylococcus aureus* (*S. aureus*) with a diameter of 600 nm is stably trapped in the hotspots, whereas the rod-shaped *Escherichia coli* (*E. coli*, $D = 500$ nm, $L = 1500\text{--}2000$ nm) aligns in one hotspot, twists to the other, and eventually escapes from the ONSA. The two bacteria both have the same refractive indices of 1.38.^{23,24} And the volume V of the objects to be sorted, *i.e.* *S. aureus* and *E. coli*, are estimated to be $1.13 \times 10^{-19} \text{ m}^3$ and $2.62 \times 10^{-19} \text{ m}^3$, respectively. The equivalent radius difference $\Delta R = \sqrt[3]{3/4V/\pi}$ is only 97 nm. Even if two spherical nanoparticles with 97 nm size difference can be separated using existing techniques, it is still challenging to separate two particles with both little size difference and distinct shapes. Our ONSA, exploits the cooperation between the body dynamics of the bacteria and the coupled hotspots for shape-selective sorting of submicron bioparticles of similar size and same refractive index.

RESULTS AND DISCUSSION

Design of the ONSA. The coupling length between the hotspots, which decreases with the decreasing width (W) and gap (G) of the paired waveguide, is an important parameter for the particle manipulation in the ONSA as shown in **Figure 1b**. The type of optical particle manipulation in the ONSA is determined by parameters including the particle size and shape as well as the coupling length (C) between the hotspots. Multiple types of particle manipulation can be realized by adjusting these parameters. Let us use the rod-shaped *E. coli* and spherical *S. aureus* for example. When $W \geq 500$ nm and $G > 200$ nm (*e.g.* $W = 500$

nm, $G = 250$ nm), the coupling length C is greater than $50\text{ }\mu\text{m}$ as shown in **Figure 1b**. In this scenario, both *E. coli* and *S. Aureus* are trapped in the y -direction but not in the x -direction. They are eventually transported to the edge of the microchannel by the optical scattering force and flushed away by the flow stream as shown in **Figure S3a**. The energy profile of the potential well shows that there is no trapping position for both types of bacteria in either x - or y -directions. Here the optical scattering force is the dominant optical force because the light gradient is very low. When $W = 450$ nm and $G = 200$ nm, the coupling length C is around $25\text{ }\mu\text{m}$ as shown in **Figure S3b**. In this scenario, the influence of the hotspots on the bacteria are relatively independent in the upper and lower waveguides. Both bacteria are trapped stably in those hotspots by the optical gradient force. The potential wells exhibit a valley-like energy profile, indicating stable trapping as shown in **Figure S3b**. The upper and lower hotspots are relatively independent, and bioparticle inside one hotspot is unaffected by the other hotspot. As the width W of waveguide increases to 350 nm, the coupling length becomes $\sim 7.8\text{ }\mu\text{m}$, which is on the same length scale as the *E. coli* ($\sim 1.5\text{--}2\text{ }\mu\text{m}$). In this scenario, the interactions between two adjacent hotspots cause spherical *S. aureus* to be trapped in the hotspots as shown in **Figure 1c**. Because the *E. coli* is significantly longer than *S. aureus*, the *E.coli* that is temporarily trapped in the lower hotspot is under the influence of the upper hotspot, leading to an unstable trapping situation. The optical gradient force from the upper hotspot attracts the *E.coli* and makes it rotate by generating a torque M_z . The torque M_z can be evaluated by integrating Minkowski stress tensor on the surface of *E. coli*, which is expressed as $M_z = \oint \mathbf{r} \times (\bar{\mathbf{T}} \cdot \hat{\mathbf{n}}) dA$, where $\mathbf{T}$ is the Minkowski stress tensor. Detailed analysis can be found in **Methods**. Meanwhile, this slight rotation causes part of the *E.coli* that was temporarily

trapped in the lower hotspot to move into the upper hotspot. The *E. coli* eventually escapes because there is no stable trapping position in the upper waveguide. In addition, the *E. coli* in the upper waveguide is also attracted to the adjacent waveguide pair due to the small pitch of 1 μm between each waveguide pair. Therefore, even if the potential well for *E. coli* in the x -direction exhibit a valley-like energy profile, the potential well cannot trap the *E. coli* in the y -direction, leading to the *E. coli* escapements. It is noted that the sorting is only applicable to bacteria with a length ranging from 1 μm to 2 μm . When the size of bioparticles becomes smaller, *e.g.* $L < 1 \mu\text{m}$, both spherical and rod-shaped particles would be trapped in the hotspots as shown in **Figure 1d**, because particles in this size range are only under the influence of a single hotspot, and do not interact with the coupling hotspot. When the width and the gap of the waveguide become smaller, *e.g.* $W = 300 \text{ nm}$ and $G = 150 \text{ nm}$, the coupling length $C = 3.1 \mu\text{m}$. In this scenario, bacteria of different shapes can also be sorted with this design as shown in **Figure 1e** with the same mechanism discussed in **Figure 1c**. However, it is highly difficult to fabricate devices with such small features, especially with such small gaps between the paired waveguides. Meanwhile, large particles (L or $D > 3000 \text{ nm}$), regardless of their shape, would always escape from the waveguide because they are under the influence of multiple hotspots, which leads to the negative trapping as shown in **Figure 1f**. In Summary (**Table S1**), when the size (radius or length) of bacteria is small, *e.g.* R or $L < 500 \text{ nm}$, both spherical and rod-shaped would be trapped stably inside one hotspot. When $1000 \text{ nm} < R$ or $L < 2000 \text{ nm}$ and $C < 8 \mu\text{m}$, only spherical bacteria is trapped, and rod-shaped bacteria will escape from the ONSA. When $1000 \text{ nm} < R$ or $L < 2000 \text{ nm}$ and $10 \mu\text{m} < C < 40 \mu\text{m}$, both spherical and rod-shaped bacteria would be trapped. When $1000 \text{ nm} < R$ or $L < 2000 \text{ nm}$ and $C > 50 \mu\text{m}$, bacteria will be transported

to the edge of the microchannel and flushed away eventually by the fluid. When $3000 \text{ nm} < R$ or L , no bacteria would be trapped stably inside the ONSA because of the concurrent influence of multiple hotspots.

Force Analysis on two bacteria and optical binding. When the size of the bacteria is comparable to that of the hotspot, rigorous simulation based on Minkowski stress tensor is required to simulate the optical force on the particles,³⁶ which calculates the 2D force map to determine the trapping positions of these particles, such as *S. aureus*. Whether the longitudinal axis of *E. coli* is parallel or has a small angle with respect to the waveguide array plane initially, the bacterial cell would eventually be attracted to the surface of the waveguide due to the optical gradient force. Once sitting on the waveguide surface, the *E. coli* cell is always oriented in such a way that its longitudinal axis is parallel to the waveguide array plane. Therefore, we only need to simulate the optical force and torque on the bacteria on the plane. The 2D force map of F_x for *S. aureus* is shown in **Figure 2a**. F_x alternates between attractive force and repulsive forces in each waveguide along the direction of light propagation. The yellow region in the figure shows the force $F_x \sim 0$, and the black lines is the contour of $F_x = 0$. The force map of the total force in the y-direction F_y , which is the combination of optical force F_{y_opt} and the fluidic drag force F_{y_drag} , is shown in **Figure 2b**. The force map of the optical force alone in the y-direction F_{y_opt} is shown in **Figure S4**. The balance positions of $F_x = F_y = 0$ are near the edge of the waveguide, which is different from the single waveguide systems where particles are trapped at the balance positions along the central line of the waveguide. In order for bacteria to be trapped, the forces on both sides of the balance position must be restoring forces as illustrated in **Figure S5**. Based on this criterion, some points with $F_x = F_y = 0$ are unstable

balance positions. The stable balance positions of $F_x = F_{x_{opt}}$ equals 0 (black lines) and $F_y = F_{y_{opt}} + F_{y_{drag}}$ equals 0 (red lines) are plotted in **Figure 2c**. The two positions at the intersections of the black lines and the red lines (marked as the red dots) with a distance of 15.6 μm apart are the final trapping positions for *S. aureus*. The distance between the two trapping positions is twice of the coupling length. It seems that each coupling in one coupling length C creates one trapping positions for *S. aureus*. The overall force balance contour (including the stable and the unstable trapping positions in the x - and y -directions) is shown in **Figure S6**. However, when one *S. aureus* is trapped in position 1, the nearby light field would be redistributed, which induces the formation of additional trapping positions.²⁷ This effect is recognized as the “optical binding”. To investigate the optical binding in the ONSA, a *S. aureus* cell is pre-trapped in the first trapping position shown in **Figure 2c**. The forces in the x and y -directions are then calculated and mapped in **Figure 2d,e**, respectively. The additional stable trapping positions at $F_x = F_y = 0$ ($F_y = F_{y_{opt}} + F_{y_{drag}}$) with a pre-trapped particle are plotted in **Figure 2f**. Four additional trapping positions labelled as 3, 4, 5 and 6 are created when a single *S. aureus* is pre-trapped in position 1. The trapping position 2 is unaltered because it is located in another hotspot far enough from position 1. When additional *S. aureus* cells are trapped at position 3, 4, 5 or 6, they may further induce more additional trapping positions. Therefore, there could be more trapping positions in the ONSA than the ones shown in the initial force map in **Figure 2c**. Both force patterns in **Figure 2d,e** are more complex than **Figure 2a,b**, especially in the upper waveguide. This is because position 1 is close to the coupling region where light is guided from the lower waveguide to the upper waveguide. This is also the reason that the upper waveguide has more additional trapping positions. The enlarged view of the map

of F_y can also be found in **Figure S7**. In order to trap *S. aureus* on the nanowaveguide, the potential well is deep enough ($> 1 k_B T$) to confine the bacteria stably.^{34, 37} The simulated trapping force near the trapping position, the trapping stiffness and the potential well landscape are shown in Figure S8. It is shown that the measured average trapping stiffness with 0.5-mW and 0.8-mW output laser powers are 3.56 and 5.65 nN/m, respectively. The trapping stiffness measured with 0.8-mW output laser power (after the loss from the propagating and coupling) is equivalent to the average stiffness simulate with 20 mW laser power inside the waveguide. The minimum simulated potential barrier could reach $6.8 k_B T$ for 20-mW laser power inside the waveguide after optical binding, which is strong enough to trap *S. aureus*.

In contrary, *E. coli* does not have stable trapping positions in the ONSA. The detailed simulation of optical forces on the *E. coli* in the x - and y -directions at different locations are shown in **Figure S9**. We have compared the F_y at each position where $F_x = 0$. The F_x consists of only the optical force, while F_y consists of the optical force and the fluidic drag force $F_{y_opt} + F_{y_drag}$. The drag force for the rod-shaped *E. coli* is expressed as $F_{drag} = 8\pi\eta bv / (\ln(2b/a) + 0.5)$,³⁸ where η is the viscosity of the buffer, v is the velocity of the flow, and b and a are the half-length and the diameter of the rod, respectively. The optical force in the y -direction is shown in **Figure S10**. For the *E. coli* with a diameter of 500 nm and a length of 2 μm , the drag force perpendicular to the long axis of the bacterial cell is around 0.67 pN when the flow velocity is 5 $\mu\text{m/s}$. Comparing to **Figure S9**, two locations at $y = 0$ and 550 nm satisfy $F_x = F_y \sim 0$ and serve as the potential trapping position for *E. coli*. However, a closer investigation reveals that both are unstable trapping positions, which means that even if the *E. coli* is in force balance along the x -direction at these locations,

they can be easily taken away by forces along the y -direction. As a result, the ONSA is unable to trap rod-shaped *E. coli*. More plots near $y = 0$ and 550 nm can be found in **Figure S11**.

Separation of *S. aureus* and *E. coli* by optical torque. The random Brownian motion, bacterial locomotion and optical forces from adjacent potential wells cause *E. coli* to rotate inside the ONSA. We investigate the rotation-induced force F_y and the optical torque M_z by placing *E. coli* at different locations as shown in **Figure 3a,b**. When the coordinate x is small, *e.g.* $x < 2 \mu\text{m}$, the rotational force F_y is positive when the rotational angle θ (*i.e.* the angle between the long axis of *E. coli* and the waveguide) is negative, and *vice versa*. This means that the *E. coli* tends to be aligned in the hotspot along the light propagating direction. It is similar to the behaviour of the *E. coli* in a single waveguide. When $x \geq 2 \mu\text{m}$ and $\theta < 0$, F_y is always positive because the upper hotspot does not affect the *E. coli*. However, when $x \geq 2 \mu\text{m}$ and $\theta > 0$, the *E. coli* starts to step into the region of influence of the upper hotspot. Hence, a rotation with a small angle would generate a very large optical force pointing to the centre of the upper hotspot as shown in **Figure 3a**. When $\theta > 60^\circ$, a large portion of the *E. coli* overlaps with the upper hotspot. As a result, the forces on the upper and lower part of the *E. coli* begin to cancel each other, resulting in a smaller overall optical force. That is why F_y is maximum at $\theta = 20^\circ$ and decreases gradually to near 0 with increasing θ . The large F_y at the small θ causes *E. coli* to rotate easily from the lower hotspot to the upper hotspot because $F_y > 0$. This rotation leads to the release of the *E. coli* from the ONSA, which is the second reason why the ONSA is unable to trap particles with a large aspect ratio, such as *E. coli*. Light also exerts a torque M_z on the rod-shaped *E. coli* and induces a rotation around the z -direction (short axis of the *E. coli*). The simulation

results of the torque M_z are shown in **Figure 3b**. The working principle of the optical force on the *E. coli* is illustrated in **Figure S10**. When an *E. coli* is in the light field, the distributed force on the surface emerges and can be evaluated using the Minkowski stress tensor. The distributed force dF will induce a torque M_z by cross product with the vector r . The surface integral of the distributed force results in a net force F_{sum} on the edge of the *E. coli* as shown in **Figure S12**. The equivalent arm of force L can be obtained by $L = \oint_S dM_z / F_{sum}$. The net effect of the F_{sum} acting on the edge of the *E. coli* is equivalent to the effect of a force with the same magnitude acting at the center of mass combined with an additional torque M_z . Therefore, the dynamics of the *E. coli* in the complex light field can be decomposed into a translation of the center of mass under the force F_{sum} and a self-rotation under the torque M_z . The behaviour of the *E. coli* in the twisted light (light coupled between the paired waveguides) can also be modelled using two potential wells (PWs) and three dipole particles along the *E. coli* cell as shown in **Figure 3c**. In the first scenario, particle 1 (P1) and P2 are in PW1, while P3 is in PW2 which has a weak energy and force. P3 would be attracted to PW1 by the traction of P2. When $-90^\circ < \theta \leq -40^\circ$, the M_z is negative (clockwise) because only one end of *E. coli* experiences a positive optical scattering force. In the second scenario, M_z becomes positive when $-40^\circ < \theta \leq 0^\circ$ as shown in **Figure 3d**. Because the *E. coli* now experiences an optical gradient force which attracts and aligns it to the waveguide. In this case, P1, P2 and P3 are all in PW1. In the third scenario, M_z becomes complex when $\theta > 0^\circ$. When $x \geq 2 \mu\text{m}$ and $0^\circ < \theta \leq 30^\circ$, the attracting optical gradient force from the upper waveguide causes the *E. coli* to rotate anti-clockwise as shown in **Figure 3e**. In this scenario, P2 and P3 are in PW2, and both dipoles pull P1 from PW1 to PW2. When $x \geq 2 \mu\text{m}$ and $\theta > 30^\circ$, the *E. coli* rotates clockwise, which is

caused by the pushing force of the lower waveguide and the attracting force of upper waveguide as shown in **Figure 3b**. In the fourth scenario, the *E. coli* would pass through the gaps between the coupling hotspots, both F_y and M_z are very small at these locations as shown in **Figure 3a,b,f**. In this scenario, only F_x is non-zero at various angles as shown in **Figure S13**. However, F_x is one order of magnitude smaller than F_y as shown in **Figure 3c** and **Figure S13**. The energy of PW1 and PW2 are both weak, hence P1, P2 and P3 would be flushed away by the fluidic drag force. The detailed expression about the simulation of the optical force and the torque can be found in **Methods**.

There are two ways that the *E. coli* can escape from the ONSA. In the first scenario, the rotation of the *E. coli* causes it not only to hop from one waveguide to another in one paired waveguide, but also hop from one paired waveguide to the adjacent one because the gap between each paired waveguide is only 1 μm , and the length of the *E. coli* is around 2 μm as shown in **Figure 3g**. The *E. coli* cell is initially aligned along the waveguide as shown in at $t = 20$ ms, but it would rotate on its own due to the Brownian motion and the bacterial locomotion. The self-rotation brings one end of the bacterial cell in touch with the adjacent waveguide as shown at $t = 40$ ms. The end of the *E. coli* cell then experiences a strong optical gradient force. The entire *E. coli* cell is eventually steered to the second waveguide as shown in at $t = 60$ ms. The *E. coli* could also rotate and step from waveguide pair 1 (WP1) to waveguide pair 2 (WP2) from $t = 80$ ms – 100 ms because the optical force from WP2 is sufficient to attract *E. coli* to the surface of WP2. Because the optical scattering force along the light propagating direction is much smaller than the optical gradient force caused by the gradient of the light field (see **Figure S9**). The *E. coli* will escape due to the twisting before being pushed to the right side. In the second scenario, the

rod-shaped *E. coli* cut through the ONSA along a path between the hotspots. In this case, the *E.coli* moves along a path perpendicular to the waveguide and goes through the weak optical field between the coupled hotspots as shown in **Figure 3h**.

Multiple trapping of fluorescence and unstained *S. aureus* in the ONSA. To experimentally demonstrate the sorting of live *S. aureus* and *E. coli*, we first test the trapping efficiency of *S. aureus* with and without the fluorescence staining. When the measured laser power was increased to 0.8 mW, nearly all fluorescent *S. aureus* stained with DAPI dye were trapped as shown in **Figure 4a-d** and **Video S1**. The white rectangle is the region of the ONSA. The number of observed trapped *S. aureus* did not increase linearly with time due to photobleaching. Next, we increased the flow velocity to 20 $\mu\text{m/s}$, and almost all *S. Aureus* began to escape from the ONSA. The surface treatment was applied to prevent the bacterial cells from adhering to the microfluidic channel. The trapping results of unstained *S. aureus* is presented in **Figure 4e-h** and **Video S2**. A few tens of *S. aureus* were continuously trapped in the ONSA in 50 s. Only a few *S. aureus* were found to escape from the ONSA. The escapement may be caused by the irregular shape of a small number of *S. aureus* and the aggregation of bacterial cells as shown in **Figure 4f**. When the velocity was increased from 2 $\mu\text{m/s}$ to 10 $\mu\text{m/s}$ and the measured laser power in each waveguide was 0.5 mW (after the loss of the system), the trapped *S. aureus* escaped from the ONSA. The quantity of trapped *S. Aureus* increased linearly with time as shown in **Figure 4h**. Close to 40 *S. aureus* cells were trapped within 2 minutes when the bacterial concentration was around $10^7/\text{ml}$. The number may vary with different concentrations and flow velocities. For instance, the number of trapped *S. aureus* can increase significantly with > 80 cells/min when the flow velocity for the trapping is

increased to 10 $\mu\text{m/s}$ with an output laser of 2 mW. It is noted that our chip targets on the low sample concentration with tens to hundreds of cells in a few microliters.

Experimental demonstration of the sorting of *S. aureus* and *E. coli*. The number of trapped bacteria against time is shown in **Figure 5a**. The number of *S. aureus* initially increased linearly with time until it reached a plateau (No. around 26) at $t = 150$ s. Meanwhile, the number of the *E. coli* trapped in the ONSA was less than 4 at any given moment. The trapping of the *E.coli* was temporary, and all *E. coli* would eventually escape from the ONSA. We also investigated the trapping efficiency of *S. Aureus* at various flow velocities when the measured laser powers were kept as 1 and 2 mW as shown in **Figure 5b**. When the velocity was less than 6 $\mu\text{m/s}$, the average trapping efficiency of *S. aureus* was more than 95% at 1mW output power. The velocity can be increased to 14 $\mu\text{m/s}$ to obtain a trapping efficiency of more than 90% for *S.aures* at 2 mW. In contrast, less than 3% of *E. coli* was trapped at low velocities, and less than 1% trapping of *E. coli* was observed when the velocity reached 7 $\mu\text{m/s}$ for 1 mW. Even if the increased laser power from 1-2 mW may increase the potential trapping ability of *E. coli* in one nanowaveguide, the optical gradient force from the other waveguide is also increased, which still triggers the twisting and releasing of *E. coli* from the nanowaveguide pair easily. The throughput could be increased by increasing the laser power. We also include the experimental results of trapping of *S. aureus* using 2-mW output power in **Figure S14**. These results demonstrated ONSA's exceptional capability of shape-selective bioparticle sorting with a high shape selectivity. The experimental demonstration of the negative trapping of unstained *E. coli* is also shown in **Figure S15** and **Video S3**.

The experimental demonstration of the sorting of unstained *S. aureus* and *E. coli* is also shown in **Figure 5c-f** and **Video S4**. The spherical *S. aureus* were caught immediately when they reached the ONSA. While, the rod-shaped *E. coli* aligned and rotated along the waveguides, and eventually escaped from the ONSA by the work of optical and fluidic forces. To clearly distinguish *S. aureus* and *E. coli* in a mixture, the *S. Aureus* was stained with DAPI which appeared blue and the *E. coli* was stained with SYTOX Green which appeared green under the fluorescent microscope as shown in **Figure 5g-j** and **Video S5**. Initially, some *S. aureus* had already been trapped in the ONSA at $t = 0$ when two *E. coli* (green circle) and one *S. aureus* (blue circle) were moving towards ONSA as shown in **Figure 5g**. Later, the *S. aureus* was caught by the ONSA at $t = 16$ s, whereas one *E. coli* had already passed through the ONSA and the other one was about to (**Figure 5h**). The two *E.coli* eventually left the ONSA (**Figure 5i**). Both the unstained and fluorescence-stained bacteria showed a high shape selectivity of the ONSA. It is noted that the staining was only to visualize both bacteria clearly. The sorting progress did not depend on the staining to work.

Currently, flow cytometry has a high throughput, yet they have limited resolution of imaging and sorting of label-free nanoparticles and are unable to accomplish shape-selective sorting of label-free submicron particles.³⁹ FACS could separate cells of different shapes with a high throughput based on different fluorescent labelling.⁴⁰ However, the labelling process would hinder the downstream functional analysis. Manual sorting is possible for mammalian cells whose typical sizes are around 10 microns.⁴¹ Manual sorting of submicron bacterial cells is extremely challenging.

Our platform could assist the diagnostics of polymicrobial infections. Polymicrobial infection is a condition defined as the disease caused by mixed infection of two or more microorganisms. Compared to monomicrobial infection which only involves one microorganism, polymicrobial infection is more challenging to diagnose because multiple microorganisms need to be identified. Existing diagnostic technologies are optimized to detect a single microbial species at a time and have limited accuracy in identifying multiple microorganisms at the same time in the event of polymicrobial infection.⁴² Our platform could potentially separate different microorganisms in polymicrobial infection samples based on their morphologies. The individual microorganisms could be identified separately with a higher accuracy in the downstream analyses, which would improve the reliability of diagnosis and lead to better clinical outcomes.

CONCLUSIONS

The ONSA provides an intriguing nanophotonic method of direct sorting unstained bacteria with different shapes. Multiple *S. aureus* ($D = 600$ nm) can be trapped in each single hotspot due to the optical binding. Two mechanisms have been observed for the negative trapping of *E. coli* ($D = 600$ nm, $L = 2000$ nm). In one mechanism, the force balance position of *E. coli* in one hotspot in the x -direction is overpowered by the force in the y -direction from the other coupled hotspot, causing the trapping instability. In another mechanism, the Brownian motion- and the bacterial locomotion-induced rotation cause bacterial cells to twist into the region of other hotspots, which generates strong optical force and torque M_z to pull the bacterial cells away from the original hotspot. Occasionally, the

rod-shaped *E. coli* can also cut through the weak light field between the coupling of two waveguide, resulting in a quick escape. The ONSA has demonstrated a high trapping efficiency and a high shape selectivity, capable of retaining over 95% of *S. aureus* but less than 3% of *E. coli*. Our study provides the missing piece of optofluidic particle manipulation by enabling the shape-selective sorting at submicron scales. It offers an in-depth insight into the interactions of coupled hotspots and their collective influence on the particles according to their shapes. This work could inspire future effort in designing multifunctional particle manipulation optofluidic chips. The ONSA also have many other potential applications such as direct trapping and sorting unstained bioparticles for drug screening, toxicity study and single cell analysis, which could have significant potential in developing disease diagnostics and therapeutics.

METHODS

***Escherichia coli* and *Staphylococcus aureus* culture and staining.** *Escherichia coli* (*E. coli*) (ATCC® 25922) and *Staphylococcus aureus* (*S. aureus*) (ATCC ® 25923) were cultured in the Luria-Bertani broth (LB) (Sigma) at 37°C for 18 hours. The number of bacteria were counted by the flow cytometry (ImageStream X MarkII, Merck) after the culture. The bacteria suspension that was diluted to 10^9 cells per mL was centrifuged at the speed of 3500 rpm and re-dispersed in 1 mL of 1× Phosphate-buffered saline (PBS) buffer (Gibco) which contained 0.1% trypsin-EDTA (Gibco) to prevent the bacteria agglomeration.

For fluorescent staining, 2 mL of bacteria with a concentration of 10^9 cells per mL was first incubated in 75% ethyl alcohol (EtOH) (v/v) (Merck) for 1 min. The fixed bacteria

sample was centrifuged at 3500 rpm and washed twice with 1× PBS buffer. The bacterial cells were then re-dispersed in 1× PBS which containing DAPI (Invitrogen) (1:1000 dilution) for *S. aureus* and SYTOX Green (Invitrogen) (1:500 dilution) for *E. coli* and stained for 30 minutes in dark. Then the stained bacteria sample was centrifuged at 3500 rpm and washed twice with 1× PBS buffer again. The stained *E. coli* (dimension of 0.5×2 μm) and *S. aureus* (600 nm in diameter) stock were later diluted 10 times to a concentration of 10⁸ cells per mL in 1× PBS solution for sorting experiment in the ONSA.

Optofluidic chip fabrication. The optofluidic chip consisted of a microfluidic channel with two inlets and three outlets. The microchannel had dimensions of $L = 1000\ \mu\text{m}$, $W = 100\ \mu\text{m}$ and $H = 1.5\ \mu\text{m}$. The shallow depth (1.5 μm) of the microchannel was fabricated by etching into the deposited silicon oxide.⁴³⁻⁴⁵ The glass wafer with a 1-mm thickness was coated with a thin layer (1 μm) of PMDS for the bounding and writing of hole patterns.⁴⁶ The microfluidic network is patterned into the thin PDMS layer using laser ablation (VLS2.30, Universal Laser Systems, USA). Holes for the inlet and outlet were drilled through the glass wafer using the sonic drilling machine (SOM-121, Drill Master, Japan) with a drill head diameter of 0.9 mm. The drilled glass wafer was cleaned with IPA and sonication to remove the residual dust from drilling. The glass wafer was then blow-dried with an air gun and placed in the oven at 75°C for 10 mins. The surface of the fabricated silicon chip together with PDMS glass wafer was treated with oxygen plasma using the plasma cleaner (PDC-002, Harrick Plasma, USA) for 10 mins to activate the surface. The chip was bond to the glass wafer by attaching the two active surfaces together and pressed still for 5 mins. The assembled chip was secured in the chip sample holder and connected to the inlet and outlet tubing. Samples were injected into the chip by two syringe pumps

(SPM 200C, SIMTech, Singapore), which can inject the liquid precisely in pL/min. Detailed fabrication process is shown in **Figure S16**. A single-mode 1550 nm fibre was used to couple the light into the waveguide using a nano-precision alignment stage. The laser power in the waveguide was measured using a multi-mode infrared fibre attached to a power meter (1918-R, Newport). The optical images and videos were captured using a Nikon camera with a 40× chromatic aberration correction lens. A white light LED light source attached to a UV filter (band wavelength: 350 ± 25 nm) was used for fluorescence excitation. The fluorescent emission is observed with a 435-nm long pass filter. The silicon surface of the nano-waveguide was blocked with the PEG-silane (PG1-SL-2k, Nanocs, USA) followed by the Startingblock (Thermo Fisher Scientific) solution to prevent the bacteria adhesion. The diluted StartingBlock solution (1:1 in PBS) was injected with a flow rate of 2 μ L/min for 1 hour. Then the optofluidic chip was ready for experiment with bacteria suspended in in PBS.

Simulation of light field and optical forces. The light patterns were simulated using Finite Element Method (FEM) with the commercial software COMSOL. The optical force maps in the x - and y -directions were simulated using the finite-difference time-domain (FDTD) method with the commercial software Lumerical to save computer memory. The rotational force and torque on *E. coli* were calculated using Lumerical and Matlab. First, $\mathbf{E}_x$, $\mathbf{E}_y$, $\mathbf{E}_z$, $\mathbf{H}_x$, $\mathbf{H}_y$ and $\mathbf{H}_z$ in a monitor box containing the *E. coli* were simulated in Lumerical. The *E. coli* was placed with a 10 nm gap above the surface of the waveguide. The maximum mesh in this monitor box covering the *E. coli* was set to 3 nm to ensure that the mesh in the gap was fine enough for the correct and convergent force calculation. Second, $\mathbf{E}_x$, $\mathbf{E}_y$, $\mathbf{E}_z$, $\mathbf{H}_x$, $\mathbf{H}_y$ and $\mathbf{H}_z$ on a rod surface 5 nm away from an *E. coli* were obtained

in Matlab. Hence, the optical force and the torque on the *E. coli* were calculated by integrating Minkowski stress tensors on the extracted rod surface in Matlab. Their time average expressions for the optical force was given as^{27, 36}

$$\langle F \rangle = \oint_s \langle \vec{T} \rangle \cdot \hat{n} dA \quad (1)$$

And for the torque,

$$\langle M \rangle = \oint_s r \times (\langle \vec{T} \rangle \cdot \hat{n}) dA \quad (2)$$

where the stress tensor using here is Minkowski form and is defined as

$$\langle T_{ij} \rangle = \frac{1}{2} Re \left[D_i E_j^* + B_i H_j^* - \frac{1}{2} (D \cdot E^* + B \cdot H^*) \delta_{ij} \right] \quad (3)$$

where $\langle \cdot \rangle$ means the time average operation over an oscillation period. $\hat{n}$ is the unit vector normal to the enclosing surface.

ASSOCIATE CONTENT

The Supporting Information is available free of charge on the ACS Publications website at DOI: .

Comparison of ONSA with current particle manipulation methods, chip fabrication and SEM images of the chip, extra force analysis for sorting mechanisms, illustrations of optical torques in the ONSA, and the extra experimental results for the sorting of *S. aureus* and *E. coli* (PDF)

The authors declare no competing financial interest.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: eaqliu@ntu.edu.sg.

*E-mail: yi_zhang@ntu.edu.sg.

Author Contributions

Y.Z.S. and A.Q.L. jointly conceived the idea. Y.Z.S., A.Q.L, Y.F.Y, T.T.Z, C.-W.Q. and C.T.C. performed the numerical simulations and theoretical analysis. Y.Z.S., H.T.Z., K.T.N., L.K.C. and H.C. did the fabrication and experiments. Y.Z.S., K.T.N., H.T.Z., L.K.C., S. X., H.C., T.T.Z., J.B.Z., P.H.Y., Y.Z., C.-W.Q. and C.T.C. involved in the discussion and data analysis. Y.Z.S., A.Q.L., Y.Z., K.T.N and P.Y.L wrote the manuscript. Y. Z. and A.Q.L. supervised and coordinated all the work. All authors commented on the manuscript.

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ToC graph

FIGURE CAPTIONS:

Figure 1. Design of the ONSA for the manipulation of spherical (*S. aureus*) and rod-shaped (*E. coli*) bacteria. (a) Illustration of sorting and dynamics of bacteria in the ONSA. (b) Diagram of multi-functions of optical manipulation with different coupling length by changing the width and gap of two waveguides. Illustration of different behaviors and potential wells of spherical and rod bacteria in different designs: (c) Shape sorting of normal bioparticles (width 350 nm, gap 200 nm); (d) Non-selective trapping of small bioparticles (width 350 nm, gap 200 nm); (e) Shape sorting of normal particles (width 300 nm, gap 150 nm); (f) Negative trapping of large bioparticles (width 300 nm, gap 150 nm).

Figure 2. Analysis of force and trapping positions of *S. aureus*. (a) Map of optical force in the x -direction. Black contour denotes $F_x = 0$. (b) Map of the force (including the optical and drag forces) in the y -direction. Black contour denotes $F_y = 0$. (c) Intersections of contours of real trapping positions in the x - (black) and y -directions (red) without *S. aureus* pre-trapped. (d) Map of optical force in the x -direction with one *S. aureus* pre-trapped in position 1. (e) Map of the force (including the optical and drag forces) in the y -direction with one *S. aureus* pre-trapped in position 1. (f) Intersections of contours of real trapping positions in the x - (black) and y -directions (red) with one *S. aureus* pre-trapped in position 1. The pre-trapped *S. aureus* induces more trapping positions 3-6.

Figure 3. Dynamics of *E. coli* in the ONSA. (a) Simulated optical force in the y -direction F_{y_opt} with different angles and in different positions. (b) Simulated torque on the z -axis

M_z with different angles and in different positions. (c)-(f) Illustration of optical force F_x and F_y and torque M_z on *E. coli* in different locations of the spot and with different rotation angles. PW: potential well. (g) Experimental observation of *E. coli* torquing between two waveguide and waveguide arrays. WP: waveguide pair. (h) Experimental observation of *E. coli* cutting through the ONSA. Scale bars in (g) & (h) equal to 2 μm .

Figure 4. Multiple trapping of *S. aureus* in the ONSA. (a) & (b) *S. aureus* labelled with DAPI were continuously trapped in the ONSA with a trapping efficiency of 100%. (c) & (d) Almost all *S. aureus* were released from the waveguide when the flow velocity is increased from 5 $\mu\text{m/s}$ to 20 $\mu\text{m/s}$. The measured laser power from the power meter and the applied velocity were 0.8 mW and 5 $\mu\text{m/s}$, respectively. Scale bar equals 20 μm . (e)-(g) Trapping of non-fluorescence *S. aureus*. (e) Most of *S. aureus* are continuously trapped in the ONSA. Only few *S. aureus* including the clump escaped from the waveguide in (f). (g) Almost all *S. aureus* were released from the waveguide when the flow velocity is increased from 2 $\mu\text{m/s}$ to 10 $\mu\text{m/s}$. The measured laser power from the power meter and the applied velocity were 0.5 mW and 2 $\mu\text{m/s}$, respectively. Scale bar equals 20 μm . (h) Quantity of trapped *S. aureus* with time using 0.5 and 2 mW output laser powers. The solid lines are the average values. The results are obtained from a 16-waveguide-pair chip. The flow velocities are 2 $\mu\text{m/s}$ and 10 $\mu\text{m/s}$ in 0.5 mW and 2 mW, respectively.

Figure 5. Experimental measured trapping efficiency of *S. aureus* and *E. coli*. (a) Number of bacteria trapped with time. The number of *S. aureus* initially increased linearly with time in 150 s. Meanwhile, the number of *E. coli* trapped in the ONSA was less than 4 at any given moment. Data are obtained from an 8-waveguide-pair chip. The measured laser power is 1 mW. (b) Trapping efficiency of *S. aureus* and *E. coli* with different velocities. *S. aureus* has a high trapping efficiency (> 95%) when the velocity is smaller than 6 $\mu\text{m/s}$ at 1 mW and 14 $\mu\text{m/s}$ at 2 mW. While, *E. coli* remains a very low trapping efficiency (< 3%) among almost all velocities. Each dot is recorded from a 2-minute event. (c)-(f) Sorting with the mix of non-fluorescent *S. aureus* (blue circles) and *E. coli* (green circles). (c) and (d) All *S. aureus* were trapped in the ONSA. (e) While the *E. coli* slowed down for

a while, aligned and rotated between waveguides, and (f) eventually escaped from the ONSA. (g)-(j) Sorting with the mix of fluorescent *S. aureus* (blue color) and *E. coli* (green color). The *E. coli* with green fluorescence looked brighter and larger than *S. Aureus*. Two *E. coli* passed the ONSA. While, almost all *S. aureus* remained trapped.

Figure 1. Design of the ONSA for the manipulation of spherical (*S. aureus*) and rod-shaped (*E. coli*) bacteria. (a) Illustration of sorting and dynamics of bacteria in the ONSA. (b) Diagram of multi-functions of optical manipulation with different coupling length by changing the width and gap of two waveguides. Illustration of different behaviors and potential wells of spherical and rod bacteria in different designs: (c) Shape sorting of normal bioparticles (width 350 nm, gap 200 nm); (d) Non-selective trapping of small bioparticles (width 350 nm, gap 200 nm); (e) Shape sorting of normal particles (width 300 nm, gap 150 nm); (f) Negative trapping of large bioparticles (width 300 nm, gap 150 nm).

Figure 2. Analysis of force and trapping positions of *S. aureus*. (a) Map of optical force in the x -direction. Black contour denotes $F_x = 0$. (b) Map of the force (including the optical and drag forces) in the y -direction. Black contour denotes $F_y = 0$. (c) Intersections of contours of real trapping positions in the x - (black) and y -directions (red) without *S. aureus* pre-trapped. (d) Map of optical force in the x -direction with one *S. aureus* pre-trapped in position 1. (e) Map of the force (including the optical and drag forces) in the y -direction with one *S. aureus* pre-trapped in position 1. (f) Intersections of contours of real trapping positions in the x - (black) and y -directions (red) with one *S. aureus* pre-trapped in position 1. The pre-trapped *S. aureus* induces more trapping positions 3-6.

Figure 3. Dynamics of *E. coli* in the ONSA. (a) Simulated optical force in the y-direction F_{y_opt} with different angles and in different positions. (b) Simulated torque on the z-axis M_z with different angles and in different positions. (c)-(f) Illustration of optical force F_x and F_y and torque M_z on *E. coli* in different locations of the spot and with different rotation angles. PW: potential well. (g) Experimental observation of *E. coli* torqueing between two waveguide and waveguide arrays. WP: waveguide pair. (h) Experimental observation of *E. coli* cutting through the ONSA. Scale bars in (g) & (h) equal to 2 μm .

Figure 4. Multiple trapping of *S. aureus* in the ONSA. (a) & (b) *S. aureus* labelled with DAPI were continuously trapped in the ONSA with a trapping efficiency of 100%. (c) & (d) Almost all *S. aureus* were released from the waveguide when the flow velocity is increased from $5 \mu\text{m/s}$ to $20 \mu\text{m/s}$. The measured laser power from the power meter and the applied velocity were 0.8 mW and $5 \mu\text{m/s}$, respectively. Scale bar equals $20 \mu\text{m}$. (e)-(g) Trapping of non-fluorescence *S. aureus*. (e) Most of *S. aureus* are continuously trapped in the ONSA. Only few *S. aureus* including the clump escaped from the waveguide in (f). (g) Almost all *S. aureus* were released from the waveguide when the flow velocity is increased from $2 \mu\text{m/s}$ to $10 \mu\text{m/s}$. The measured laser power from the power meter and the applied velocity were 0.5 mW and $2 \mu\text{m/s}$, respectively. Scale bar equals $20 \mu\text{m}$. (h) Quantity of trapped *S. aureus* with time using 0.5 and 2 mW output laser powers. The solid lines are the average values. The results are obtained from a 16-waveguide-pair chip. The flow velocities are $2 \mu\text{m/s}$ and $10 \mu\text{m/s}$ in 0.5 mW and 2 mW , respectively.

Figure 5. Experimental measured trapping efficiency of *S. aureus* and *E. coli*. (a)

Number of bacteria trapped with time. The number of *S. aureus* initially increased linearly with time in 150 s. Meanwhile, the number of *E. coli* trapped in the ONSA was less than 4 at any given moment. Data are obtained from an 8-waveguide-pair chip. The measured laser power is 1 mW. (b) Trapping efficiency of *S. aureus* and *E. coli* with different velocities. *S. aureus* has a high trapping efficiency ($> 95\%$) when the velocity is smaller than $6 \mu\text{m/s}$ at 1 mW and $14 \mu\text{m/s}$ at 2 mW. While, *E. coli* remains a very low trapping efficiency ($< 3\%$) among almost all velocities. Each dot is recorded from a 2-minute event. (c)-(f) Sorting with the mix of non-fluorescent *S. aureus* (blue circles) and *E. coli* (green circles). (c) and (d) All *S. aureus* were trapped in the ONSA. (e) While the *E. coli* slowed down for a while, aligned and rotated between waveguides, and (f) eventually escaped from the ONSA. (g)-(j) Sorting with the mix of fluorescent *S. aureus* (blue color) and *E. coli* (green color). The *E. coli* with green fluorescence looked brighter and larger than *S. Aureus*. Two *E. coli* passed the ONSA. While, almost all *S. aureus* remained trapped.